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RESEARCH ARTICLE

Identification and characterization of *Phaseolus vulgaris CHS* genes in response to salt and drought stress

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Abstract Common beans hold significant importance in sustainable agriculture and their critical role in human nutrition cannot be overstated. Factors such as climate change underscore the necessity for expanding genetic diversity and delineating the attributes of local bean cultivars. Among the various abiotic stressors, drought emerges as a formidable constraint limiting bean cultivation. While diverse set of strategies are employed to mitigate the impacts of drought stress, the ultimate and enduring solution lies in the development of drought-resistant bean cultivars, and it can be stated that the same situation is valid for salinity stress. Flavonoid biosynthesis is very important for plant growth and development and flavonoids are known to be involved in many pathways including stress response. This study aimed to comprehensively identify and characterize the *CHS* gene family within different bean cultivars exposed to drought and salt stress, utilizing genome-wide analysis, and assessing gene expression levels. Employing a spectrum of in silico methodologies, 14 *CHS* genes were identified in the common bean genome. These genes exhibited molecular weights ranging from 37.38 to 43.34 kDa and consisted of 341–393 amino acid residues. Remarkably, all *Pvul-CHS* genes shared a common structure comprising two exons. Phylogenetic analyses conducted across *Phaseolus vulgaris*, *Arabidopsis thaliana*, and *Glycine max* revealed that the *Pvul-CHS* gene family could be classified into three primary clusters. The expression profiles of *Pvul-CHS* genes unveiled their varied tissue-specific expressions and their pivotal roles in responding to diverse abiotic stresses. Furthermore, we conducted in silico assessments of the chromosomal positions of *CHS* gene family members in beans, their orthologous associations with related genomes, and *cis*-acting element analyses. The outcomes of this study hold the potential to significantly contribute to the breeding of beans endowed with enhanced resilience against salt and drought conditions.

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Introduction

Rapidly changing conditions, such as population growth and climate change, are presenting significant challenges to our agricultural systems (Lobell et al. 2008). In the search for a more sustainable agricultural model, legumes like common bean (*Phaseolus vulgaris*) play a pivotal role. The benefits of nitrogen-fixing bacteria, which are live as nodules in the roots and effective in the growth and development of beans, include reducing the use of fertilizers in agriculture and as a result, reducing the use of chemicals, preventing soil pollution and more economical agriculture (Herridge et al. 2008; Thilakarathna and Raizada 2018; Reinprecht et al. 2020). In addition, beans are rich sources of minerals, carbohydrates, vitamins, fiber, and notably, proteins (Doria et al. 2012; Campos-Vega et al. 2013; Ganesan and Xu 2017). Therefore, as an environmentally friendly alternate, beans offer an excellent substitute for more costly animal-derived proteins (Pimentel and Pimentel 2003; Nikbakht Nasrabadi et al. 2021).

Larcher (2003) observed that plants continually engage in both positive and negative interactions with their environment, categorizing these interactions into biotic and abiotic factors. Biotic factors encompass interactions such as pest attacks, microorganisms (e.g., bacteria, viruses, and fungi), and other anthropogenic stressors (Larcher 2003). Abiotic factors, conversely, involve environmental conditions altered by various stressors, including ultraviolet rays, radiation, temperature fluctuations, drought, and salinity (Hirayama and Shinozaki 2010). Among abiotic stressors, drought and salinity stress stand out as significant contributors to reduced plant yield and development.

Salinity stress, as an abiotic stressor, diminishes the available water content through osmotic effects while elevating ion concentrations to toxic levels via its ionic effect (Çulha and Çakırlar 2011). Approximately 6% (800 million hectares) of the total cultivated areas worldwide contend with salinity issues (Munns 2002; Zurnacı 2019). Drought, another major abiotic stressor, significantly impairs plant growth and development, severely limiting agricultural productivity. Reduced soil water intake causes electron leakage from mitochondria, which raises reactive oxygen species (ROS) concentrations within cells and weakens the antioxidant capability of those cells. This is the immediate consequence of drought-induced stress (Choudhury et al. 2013; Sachdev et al. 2021). In order to successfully deal with this abiotic stress, plants can scavenge free oxygen radicals. Moreover, earlier research has demonstrated the efficacy of flavonoids in reducing reactive oxygen species generated during abiotic stress, hence enhancing a plant's stress tolerance. (Ma et al. 2014; Naing and Kim 2021).

Flavonoids are essential for growth and development, responses to stresses both abiotic and biotic, and adaptation to the environment (Petrussa et al. 2013). Furthermore, flavonoids possess a wide range of biological roles, such as regulating floral pigmentation, UV radiation protection, auxin transport, defense against pathogens, and pollen fertility (Winkel-Shirley 2002; Buer et al. 2010; Falcone Ferreyra et al. 2012; Raza et al. 2023). Chalcone synthase (CHS) represents a key enzyme initiating the flavonoid biosynthesis pathway, closely linked with various physiological and biological processes in plants, examples include anthocyanin production in *Matthiola incana* (L.) wild type (Hemleben et al. 2004) and disease resistance in sorghum (Cui et al. 1996). Chalcone synthase (CHS) plays a pivotal role in the flavonoid biosynthesis in plants, catalyzing the chalcone formation, such as naringenin chalcone, which serves as a precursor for various flavonoids. This process represents the initial and stable step in the phenylpropanoid pathway for flavonoid biosynthesis in plants (Ferrer et al. 1999; Lijuan et al. 2015).

In our research, we determined 14 *CHS* genes in the genome of *Phaseolus vulgaris*, a plant renowned for its nutritional value. We employed various bioinformatics tools to perform a comprehensive genome-wide characterization of the identified *P. vulgaris CHS* genes (*Pvul-CHS*). This characterization included their gene structures, chromosomal locations, cellular localizations, evolutionary analysis, and gene ontology analysis. Additionally, we conducted a comparative phylogenetic analysis and synteny study, involving *A. thaliana* and *G. max CHS* genes, to gain deeper insights into the evolutionary processes of *Pvul-CHS* genes. qRT-PCR was performed to analyze the expression patterns of *Pvul-CHS* genes in *P.*

vulgaris cultivars subjected to salt and drought stress. This research provides a comprehensive characterization of *CHS* genes in *P. vulgaris*, serving as a valuable resource for future functional and molecular biology research.

Materials and methods

Detection of *CHS* in the common bean genome

Sequences belonging to the *CHS* gene family in the *P. vulgaris* genome were retrieved from the Phytozome v13 database (https://phytozome-next.jgi.doe.gov/ 16.11.2022) using Pfam Accession Numbers Chal_sti_synt_C (Chalcone/stilbene synthase, C-terminal-PF00195) and Chal_sti_synt_N (Chalcone/stilbene synthase, N-terminal-PF02797) domains obtained from the Pfam database (http://pfam.xfam.org/ 18.11.2022). To comprehensively identify all potential CHS proteins within the genomes of *P. vulgaris* (Schmutz et al. 2014), *G. max* (Valliyodan et al. 2019), and *A. thaliana* (Lamesch et al. 2012), blastp analysis was performed and conducted a hidden Markov model (HMM) search within the Phytozome v13 database with default parameters. The presence of the CHS domain in the retrieved sequences was assessed with the help of the HMMER database (http://www.ebi.ac.uk 17.11.2022). Using "ProtParam" tool, some information about CHS proteins were clarified (Gasteiger et al. 2005).

Physical locations, structures, conserved motifs and gene duplications of *Pvul-CHS* genes

The exon intron structures of *Pvul-CHS* genes were generated by Gene Structure Display server (GSDS v. 2.0) (https://gsds.gao-lab.org/ 14.11.2022) with the information obtained from the Phytozome v13 database as summarized in the earlier work conducted by Muslu et al. (2023), and the physical locations of *Pvul-CHS* genes on chromosomes were marked with TBtools program (Hu et al. 2015; Chen et al. 2020). In addition, gene duplication events between *P. vulgaris*, *A. thaliana* and *G. max* were determined using the MCScanX tool as described by Aygören et al. (2022), and non-homolog (Ka), homolog (Ks) values and Ka/Ks ratios were calculated to determine how long ago these genes diverged from each other as described by Aygören et al. (2022) and Isıyel et al. (2022). The number of million years ago (MYA) the genes diverged was determined by the λformula (Yang and Nielsen 2000).

The "MEME- Multiple Em for Motif Elicitation" (https://meme-suite.org/meme/tools/meme 13.10.2023) tool was used to identify conserved motifs of *Pvul*-CHS proteins and then the InterPro database was used to screen the identified motifs, in accordance with previous research (Quevillon et al. 2005; Bailey et al. 2006; Muslu et al. 2023). Motif regions were defined within the range of 2–300 which is default. The distribution of motifs was set default as well. The determined motifs were subsequently analyzed using the InterPro database (Quevillon et al. 2005).

Sequence alignment and phylogenetic analyses

The amino acid sequences of the *Pvul*-CHS proteins were aligned utilizing the ClustalW algorithm (Thompson et al. 1997). Phylogenetic analyses were conducted using the MEGA v11 software (Tamura et al. 2011) following the Neighbor-Joining (NJ) method with a bootstrap value of 1,000 replicates, resulting in the construction of a phylogenetic tree. The phylogenetic tree was subsequently visualized utilizing the Interactive Tree of Life (iTOL) web interface (Letunic and Bork 2011).

Syntenic relationship of CHSs

Characterizing the evolutionary relationships between genomes through the identification of syntenic regions, gene blocks, evolutionarily conserved markers, and chromosomal rearrangements is a fundamental objective in comparative genomics (Sinha and Meller 2007). A synteny map of the *CHS* genes located within the genomes of *P. vulgaris*, *G. max*, and *A. thaliana* was constructed using TBtools (Chen et al. 2020).

Analysis of the promoter region of the *P. vulgaris CHS* genes

This stage of the investigation was conducted in a comparable method to that outlined and published in previous research (Oner et al. 2022). The PlantCARE database was utilized to investigate the 2 kb area

upstream for the identification of *cis*-acting elements (Lescot et al. 2002). The final data was visualized with TBtools software.

Homology modeling and intracellular localization prediction of CHS proteins in *P. vulgaris*

Utilizing the previously identified CHS protein sequences, we conducted homology modeling of the proteins with the assistance of the Phyre2 database (Kelley et al. 2015). During the validation process, visualizations were prepared by focusing on models with 100% confidence values and selecting the 3D image with the best match. The intracellular localization was determined with the assistance of the WoLF PSORT database (Horton et al. 2007).

CHS gene ontology analysis and interactions of proteins

Protein–protein interactions (PPI) have a crucial role in various biological processes, including cellular regulation, metabolic development, and intercellular communication (Braun and Gingras 2012). To identify physical and functional interactions among proteins, we employed the STRING database (Szklarczyk et al. 2023). The collected data were categorized and integrated, providing confidence scores for all protein–protein interactions. In the realm of plant biotechnology research, the use of functional genomics approaches is essential for annotating new sequence data. To access functional information regarding Pvul-CHS proteins, ontology data was utilized from the DAVID Bioinformatics Resources database (Sherman et al. 2022). The Gene Ontology (GO) database was used to collect protein ontology data (Aleksander et al. 2023).

In silico gene expression analysis

The expression patterns of *P. vulgaris CHS* genes were analyzed using Illumina RNA-seq data. The data were obtained from the Sequence Read Archive (SRA) data bank stored within the NCBI database. In order to determine the appropriate RNA-seq datasets, the Bioproject accession codes PRJNA216981 for salt stress and PRJNA508605 for drought stress were employed. The accession numbers used for leaf tissues of *P. vulgaris* were SRR957668 (subjected to salt stress), SRR958469 (control for salt stress) (Hiz et al. 2014), SRR8284481 (subjected to drought stress), and SRR8284480 (control for drought stress) (Gregorio Jorge et al. 2020). To standardize gene expression data, the Reads per kb of the Exon per Million Mapped Reads (RPKM) technique was utilized, as described by previous study (Mortazavi et al. 2008). Afterwards, the RPKM data were converted to the $\log_2$ format, and a heatmap was created using the CIMMiner algorithm (https://discover.nci.nih.gov/cimminer/home.do). Furthermore, the analysis also involved the examination of tissue-specific expression level values obtained from the Phytozome database, which were measured in FPKM (Fragments Per Kilobase Million) units. Based on these results, the expression patterns of *Pvul-CHS* genes in different bean tissues were identified.

Cultivation and stress applications

The stress treatments were conducted following the procedures and methodologies outlined in the previous study (Aygören et al. 2023). In brief, Serra and Elkoca-05 cultivars were selected as the plant materials and sourced from Erzurum Technical University, Department of Molecular Biology and Genetics. According to a prior study, the Elkoca-05 cultivar exhibited comparatively higher resistance than the Serra cultivar (Aygören et al. 2023). The seeds were surface-sterilized in a 1% NaOCl solution for 5 min and then washed with distilled water. Subsequently, the sterilized seeds were planted in perlite for pre-germination, with 100 seeds per pot, and were watered. Seedlings at the same developmental stage were then selected from the germinated seeds and cultivated hydroponically in pots containing 200 mL of a modified 1/10 Hoagland's basal salt solution. These seedlings were maintained under controlled conditions in a plant growth room with a 70% humidity level, 25 °C temperature, 250 mmol $m^{-2}s^{-1}$ light intensity, and a 16/8-h photoperiod until they reached the first trifoliate stage (Muslu et al. 2023). Once the seedlings reached the first trifoliate stage, they were subjected to stress treatments. Salt stress was induced by treating the plants with Hoagland solution containing 0 mM NaCl (control) or 150 mM NaCl (salt treatment). To assess the ionic effects of salt stress, leaf tissue samples were collected from the cultivars on the 9th day of the treatment. Drought stress

was applied to other cultivars grown under similar conditions, using Hoagland solution containing 0% PEG6000 (control) or 20% PEG6000 (drought stress treatment) for 24 h. Following the stress treatments, leaf tissue samples from the cultivars were collected and immediately frozen in liquid nitrogen, then stored at −80 °C until further analysis. The cultivars have been planted in triplicate, and qRT-PCR investigations were conducted with three biological replicates.

cDNA synthesis and determination of expression profiles of some *Pvul-CHS* genes

RNA isolation, complementary DNA (cDNA) synthesis and qRT-PCR research were performed as described in the previous study (Rakhimzhanova et al. 2023). RNA isolation to acquire cDNAs was performed using TRIzol® reagent (Invitrogen Life Technologies, USA). For this procedure, the manufacturer's instructions were followed, and RNA isolation was performed. The concentration of isolated RNAs was measured using a Multiskan GO instrument (Thermo Fisher Scientific, Massachusetts, USA) and then the concentrations were fixed at 1 µg/µL and RNA samples were imaged using a 1.5% agarose gel. SensiFAST™ cDNA Synthesis Kit (Cat. No.: Bio-65053, Meridian Bioscience, Tennessee, USA) was used to do cDNA synthesis, following the manufacturer's procedure. According to in silico analysis data, 6 *CHS* genes were identified as candidates for qRT-PCR study and primers were designed for these 6 genes (primer sequence information was given in Supplementary File 1). The qRT-PCR experiments were performed using the RotorGene Q RealTime PCR System (Qiage, Hilden, Germany) and the ABT SYBER Green (Cat. No.: Q03-02-01, ATLAS Biyoteknoloji, Ankara, Turkiye) master mix. The qRT-PCR reaction mixture was prepared with a total volume of 20 µL. It contained 200 ng of cDNA, 0.4 µL of both forward and reverse primers, and 2 µL of ABT SYBER Green master mix. Total volume was adjusted to 20 µL using ddH_2O. The thermal cycling technique comprised an initial denaturation phase at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The *P. vulgaris β-actin* gene was employed as the reference gene for normalization purposes. The $2^{-\Delta\Delta Ct}$ approach (Livak and Schmittgen 2001) was utilized to normalize the qRT-PCR data for quantitative measurements. One-way ANOVA statistical model was used for statistical tests with GraphPad Prism 9 software (GraphPad, California, USA). Significance level was evaluated at the 5% significance level using Dunnett's test (Rakhimzhanova et al. 2023).

Results

Characteristics of the *CHS* genes identified in the *P. vulgaris* genome

A total of 14 chalcone synthase genes were identified in the *P. vulgaris* genome through a search in Phytozome v13 database, using the identifiers PF00195 and PF02797 obtained from the Pfam database, as well as the E.C. 2.3.1.74 enzyme commission number. These genes were designated as *Pvul-CHS* (the numbers beside these gene names were assigned to indicate the order of occurrence of the identified genes on the chromosomes). The literature study revealed the presence of *CHS* gene family members in various plants.

Table 1 provides details on the locations, start and end points, lengths of amino acid chains, isoelectric points, molecular weights, and instability indexes of the *Pvul-CHS* genes and their products. The polypeptide lengths of CHS proteins identified in common bean ranged from 341 to 393 amino acids, with molecular weights ranging from 37.38 to 43.34 kDa, which are *Pvul*-CHS-10 and *Pvul*-CHS-14 respectively. The isoelectric points were found to be in the acidic region, ranging from 5.82, which is *Pvul*-CHS-1, to 6.40, which is *Pvul*-CHS-10. With all CHS genes except *Pvul*-CHS-3 being stable for ProtParam.

Chromosomal distribution and duplication event analysis of *Pvul-CHS* genes

The Phytozome database was employed to ascertain the positional information and chromosomal locations of *Pvul-CHS* genes in *P. vulgaris* (Supplementary File 2). The chromosomal distribution of *CHS* genes in common bean were demonstrated using TBtools software (Chen et al. 2020). As illustrated in Fig. 1, the chromosomal distribution of *CHS* genes was observed on chromosomes 1, 2, and 9. Specifically, there were three genes located on chromosome

Table 1 Information about the discovered 14 *Pvul*-CHS genes. Phytozome ID, chromosome locations and number (Chr), gene direction, amino acid (aa) sequence length, molecular weight (MW), theoretical isoelectric point (Pi) and stability index of *Pvul*-CHS proteins

Gene Name	Phytozome ID	Chromosome locations (bp)		Direction	Chr	aa Length	MW (kDa)	pI	Stability index
		Start	End						
Pvul-CHS-1	Phvul.001G067800	8,612,565	8,613,862	R	Chr01	389	43.04	5.82	Stable
Pvul-CHS-2	Phvul.001G067950	8,657,704	8,659,078	R	Chr01	389	43.11	6.39	Stable
Pvul-CHS-3	Phvul.001G083000	12,575,232	12,577,364	F	Chr01	391	43.04	6.24	Unstable
Pvul-CHS-4	Phvul.002G038600	3,704,298	3,705,927	R	Chr02	389	42.68	6.10	Stable
Pvul-CHS-5	Phvul.002G038700	3,710,852	3,712,431	R	Chr02	389	42.72	5.82	Stable
Pvul-CHS-6	Phvul.002G038800	3,716,703	3,718,257	R	Chr02	389	42.69	6.27	Stable
Pvul-CHS-7	Phvul.002G038900	3,726,333	3,727,795	R	Chr02	389	42.73	5.96	Stable
Pvul-CHS-8	Phvul.002G039000	3,734,660	3,736,221	R	Chr02	389	42.70	6.10	Stable
Pvul-CHS-9	Phvul.002G039100	3,744,175	3,745,756	F	Chr02	389	42.68	6.27	Stable
Pvul-CHS-10	Phvul.002G039166	3,749,576	3,751,066	F	Chr02	341	37.38	6.40	Stable
Pvul-CHS-11	Phvul.002G039232	3,756,479	3,757,928	F	Chr02	389	42.70	6.10	Stable
Pvul-CHS-12	Phvul.002G039300	3,760,668	3,762,142	R	Chr02	389	42.73	6.27	Stable
Pvul-CHS-13	Phvul.002G184300	34,495,833	34,497,370	R	Chr02	389	42.68	6.11	Stable
Pvul-CHS-14	Phvul.009G131000	19,791,491	19,792,969	R	Chr09	393	43.34	5.88	Stable

1, ten genes on chromosome 2, and only one gene on chromosome 9.

Gene duplications serve as valuable indicators of the presence of *CHS* gene families across different plant species and play a vital role in elucidating relationships among genetically similar genes. They are recognized as one of the most significant mechanisms contributing to the expansion and diversification of gene families, facilitating the identification of novel protein functions. Gene duplications are categorized into two primary types: segmental and tandem duplications. Tandem duplication is the term used when duplicated genes are located within a single chromosome. Conversely, if different alleles of the same gene are located on distinct chromosomes, it is classified as segmental duplication (Clancy and Shaw 2008). Gene duplication events within the *P. vulgaris* genome have been accomplished using MCScanX (Wang et al. 2012). Homologous (Ks) and non-homologous (Ka) values of tandemly duplicated *Pvul-CHS* gene sequences, aligned with ClustalW, were determined using the Ka/Ks calculation tool in TBtools (Chen et al. 2020) (Table 2). The duplication time (in MYA) (Schmutz et al. 2014) and the divergence for each *Pvul-CHS* gene are detailed in Table 2. In the gene duplication analysis of *CHS* genes, eight tandem duplications were identified, while no segmental duplications were detected.

In the course of evolution, gene duplication events enable proteins to gain novel functional attributes (Kondrashov et al. 2002). The evolution of gene families, driven by Darwinian positive selection, gene divergence, and duplication, has been a subject of investigation. To interpret these relationships, non-homologous (Ka) and homologous (Ks) exchange ratios (Ka/Ks) were computed for gene-to-gene involved in both tandem and segmental gene duplications, as well as for orthologous gene pairs. Significantly, a Ka/Ks ratio more than 1 shows the presence of positive selection throughout the progression of evolution. Conversely, a ratio below 1 denotes purifying selection, while a ratio equal to 1 signifies natural selection (Juretic et al. 2005; Ilhan 2018a, 2018b; İlhan et al. 2023; Kasapoğlu et al. 2020; Kizilkaya et al. 2020). As evident from Table 2, the analysis revealed that all duplicated genes within the *CHS* gene family of the *P. vulgaris* genome were subjected to purifying selection. To examine the evolutionary dynamics within the *CHS* gene family, the non-homologous (Ka), homologous (Ks), and Ka/Ks exchange rates of tandem duplicated genes identified in the bean genome were calculated.

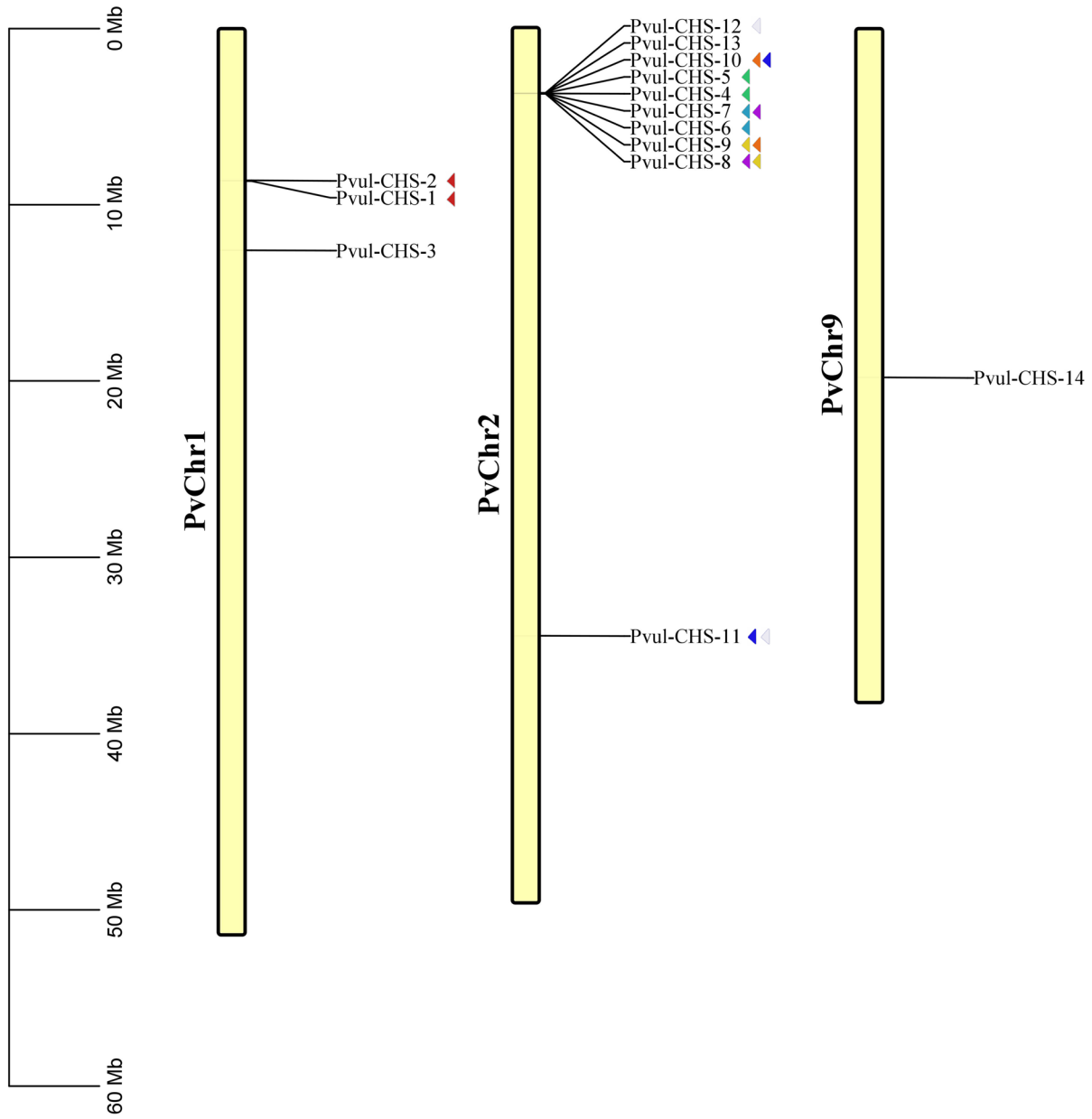

Fig. 1 Location of 14 *CHS* genes on common bean (*P. vulgaris*) chromosomes (*Pvul-CHS*). The chromosome number is shown as PvChr and colored arrowhead icons next to gene names indicate duplications between genes, the same colors indicate that those genes are duplicated

The analysis revealed that the first gene divergence occurred between *Pvul-CHS-4* and *Pvul-CHS-5* genes, estimated to be around 8.63 MYA, while the last divergence took place between *Pvul-CHS-1* and *Pvul-CHS-2* genes, estimated to be approximately 1.55 MYA.

Exon and intron regions of *Pvul-CHS* genes

The diversity of exons and introns across members of a gene family, which is essential for the evolution of various gene families, primarily occurs through three different processes: the gain or loss of exons/introns,

Table 2 Ka/Ks analysis and estimate of the absolute dates (MYA) for the duplication events between the duplicated *Pvul-CHS* genes

Gene pairs		Ka	Ks	Ka/Ks	MYA	Selection	Duplicated type
Pvul-CHS-1	*Pvul-CHS-2*	0.02	0.03	0.60	1.55	Purifying	Tandem
Pvul-CHS-4	*Pvul-CHS-5*	0.00	0.15	0.02	8.63	Purifying	Tandem
Pvul-CHS-6	*Pvul-CHS-7*	0.01	0.08	0.07	4.62	Purifying	Tandem
Pvul-CHS-7	*Pvul-CHS-8*	0.01	0.06	0.12	3.40	Purifying	Tandem
Pvul-CHS-8	*Pvul-CHS-9*	0.00	0.05	0.08	3.17	Purifying	Tandem
Pvul-CHS-9	*Pvul-CHS-10*	0.00	0.07	0.04	3.96	Purifying	Tandem
Pvul-CHS-10	*Pvul-CHS-11*	0.01	0.10	0.07	5.63	Purifying	Tandem
Pvul-CHS-11	*Pvul-CHS-12*	0.00	0.06	0.07	3.65	Purifying	Tandem

exonization (the conversion of an intronic or intergenic sequence into an exonic state), and pseudoexonization (the reverse transformation of an area from exonic to intronic) (Xu et al. 2012; Kasapoğlu et al. 2020). GSDS v2.0 database was utilized to determine the exon–intron structures of *Pvul-CHS* genes. As illustrated in Fig. 2, the size (in base pairs) and number of introns in *Pvul-CHS* genes were determined. The analysis revealed that *Pvul-CHS* genes contain a total of 14 introns and 28 exons. Notably, the intron/exon composition was evenly distributed across all genes, with each *Pvul-CHS* gene consisting of 1 intron and 2 exons.

Interspecies phylogenetic analysis of CHS

To elucidate the evolutionary relationships among CHS proteins and to predict their potential functions, CHS protein sequences from *P. vulgaris*, *G. max*, and *A. thaliana* species were acquired from the Phytozome database. In this study, CHS proteins were categorized into three major groups: I, II, and III, and a phylogenetic tree was constructed and visually presented using the neighbor-joining method given in Fig. 3. Group I exclusively include CHS from the *P. vulgaris*, while Group III consists solely from the *G. max*. Similarly, Yang et al. (2023) showed in their phylogenetic analysis that *Oryza sativa* members were not found in Group II in the phylogenetic tree divided into 3 groups. In contrast to Group I and III, Group II reveals orthologous relationships among *P. vulgaris*, *G. max*, and *A. thaliana*. Similar observations were made in previous research involving different species, such as *C. nankingense* (Zhu et al. 2022), which was categorized into four different groups, and *Solanum melongena* L. (Wu et al. 2020), which was divided into ten different groups, which where group 3, 4, 5, 6 include no *SmCHS* member.

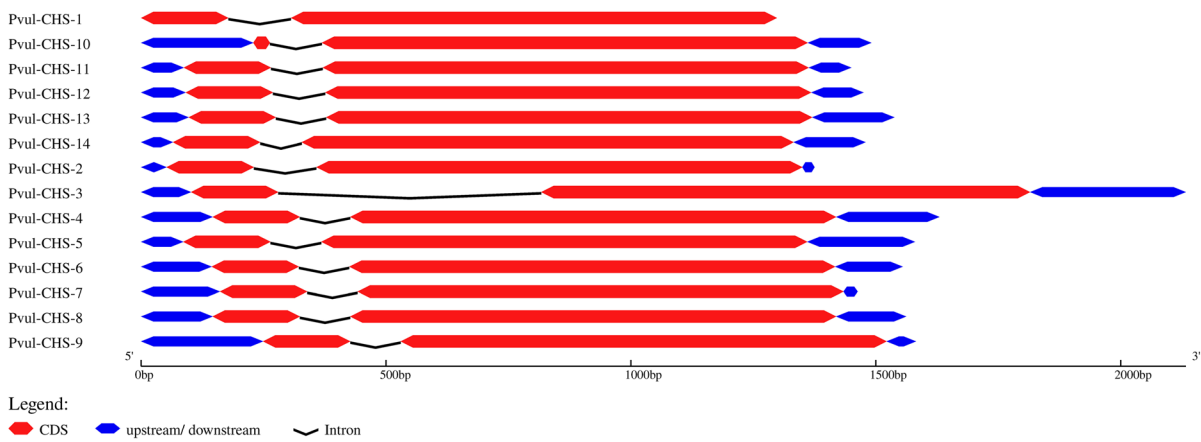

Fig. 2 Exon and intron regions of 14 *Pvul-CHS* genes explored using GSDS (together with the upstream and downstream regions, genes are generally represented in the image to be up to 1500 bp long. The CDS region, shown in red, represents exons as an abbreviation for coding DNA sequence words)

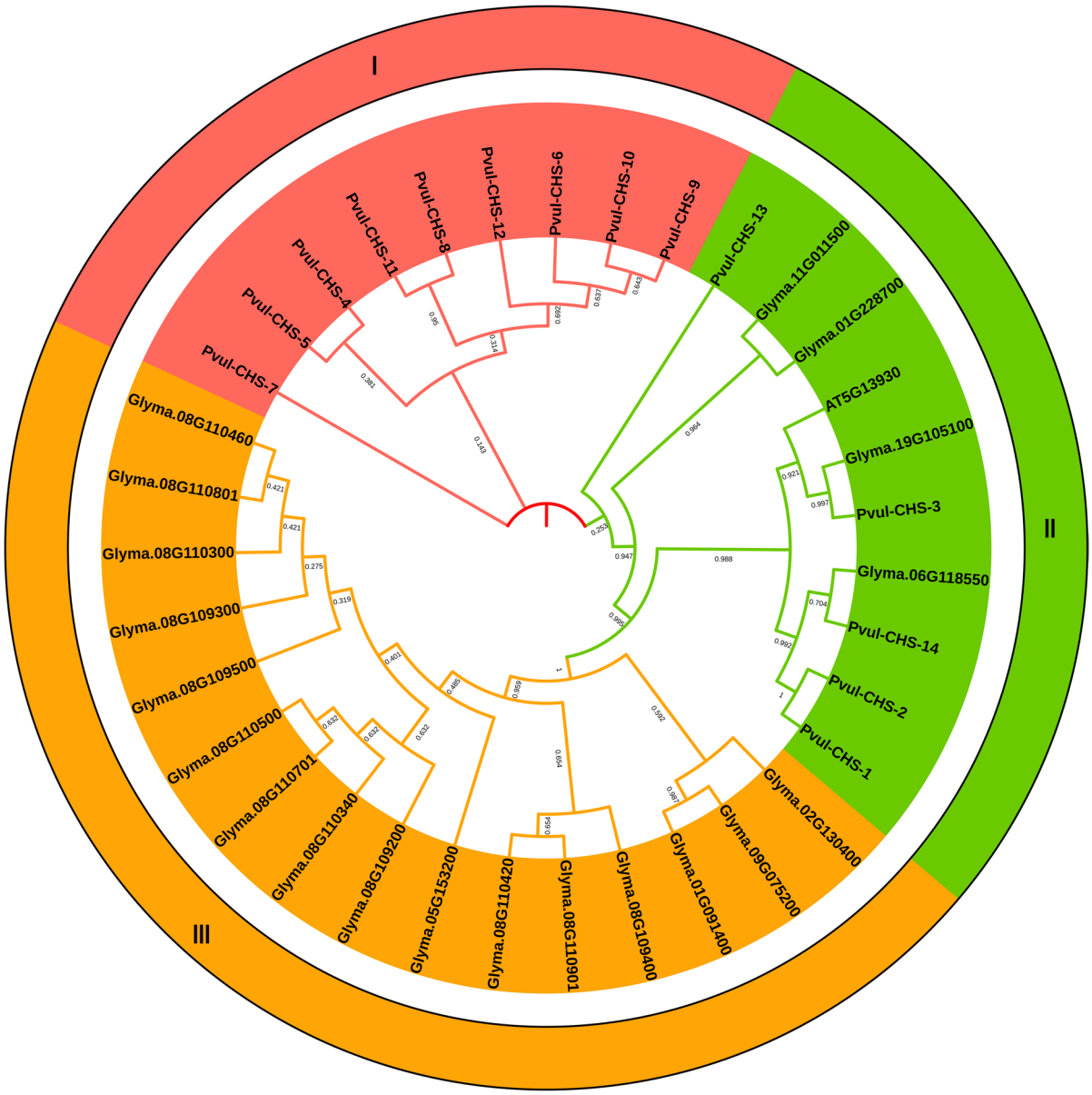

Fig. 3 Phylogenetic tree of CHS protein sequence from three different plant species. The phylogenetic tree of CHS proteins from three distinct plant species (*Phaseolus vulgaris*, *Glycine max*, and *Arabidopsis thaliana*) was constructed using the neighbor-joining (NJ) method, relying on amino acid similarity, and created with the MEGA program.

Syntenic analysis of the Pvul-CHS gene family

The analysis revealed syntenic relationships among *P. vulgaris*, *A. thaliana*, and *G. max* species, as depicted in Fig. 4. It was observed that an orthologous relationship existed between the *Pvul-CHS-4* gene and the *Glyma.01G228700.1* and *Glyma.11G011500.1* genes, as well as between the *Pvul-CHS-3* gene and the *AT5G13930.1* and *Glyma.19G105100.1* genes.

Conserved motif analysis of Pvul-CHS proteins

The presence of conserved regions within the peptide sequences of *Pvul*-CHS proteins confirms the findings from the phylogenetic analysis. In the analysis of

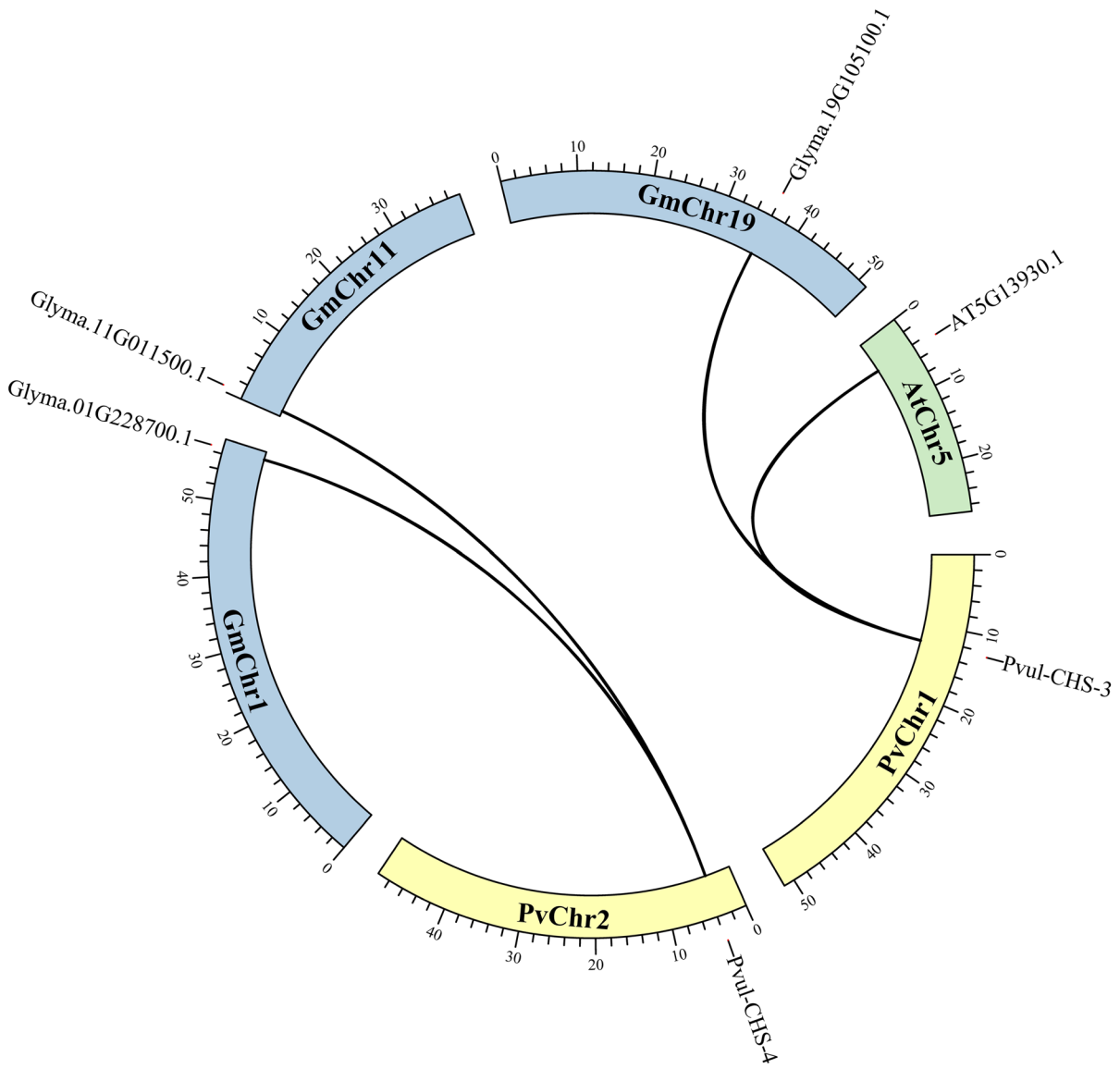

Fig. 4 Comparative map of *CHS* genes in the *Phaseolus vulgaris*, *Arabiopsis thaliana*, and *Glycine max* genomes. In this figure, black lines are represented the syntenic relationships between species and the colors are represented different species' chromosomes

conserved motifs within the 14 CHS proteins identified in beans, a total of 8 distinct motifs were recognized (Fig. 5). Similar motif groupings aid in determining structural similarities among CHS proteins. The sequences of the motifs specific to *Pvul*-CHS proteins, along with their respective locations, are detailed in Fig. 5. All proteins except *Pvul*-CHS-3 were found to contain the first 5 motifs. Motif 6 was found in all proteins except *Pvul*-CHS-1, while Motif 7 was found only in *Pvul*-CHS-1 and 14, and Motif 8 was found only in *Pvul*-CHS-3 and 14. According to the results obtained in this context, it was determined that only *Pvul*-CHS-14 contains all the identified motifs. Furthermore, the conserved motif sequences of CHS proteins were screened using the InterProScan web interface. This analysis revealed that Motif 2 contains the Chal-sti-synt_N domain and Motif 3 contains the Chal-sti-synt_C domain, while the other motifs contain no specific domain or any domain (Supplementary File 3). Motif 2 and

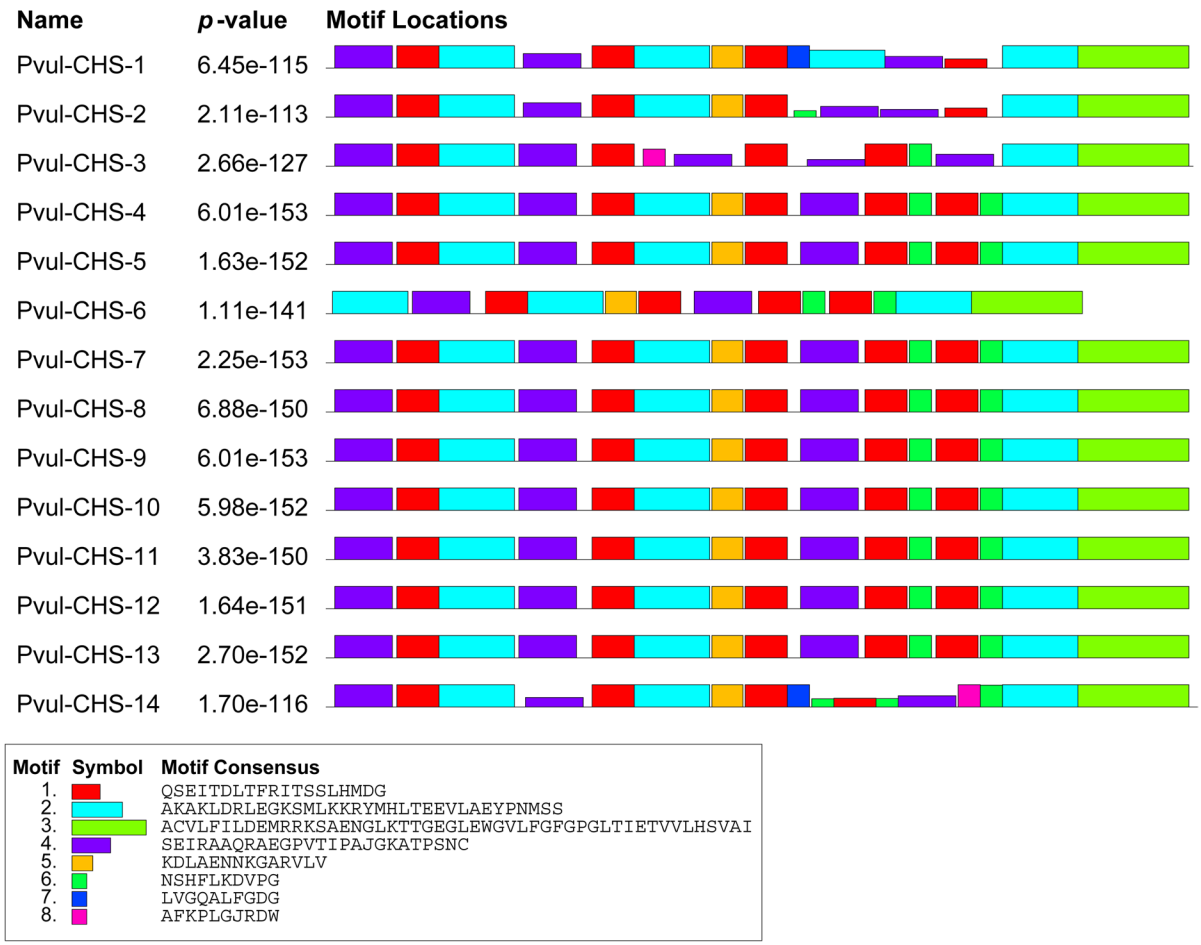

Fig. 5 Schematic representation of eight conserved motifs found in *Pvul*-CHS proteins through MEME suite tool online (each motif is numbered as shown in the legend and colors are attributed to these numbers. The arrangement of motifs in protein sequences is visualized and detailed)

Motif 3 are shown in colors cyan and long-box-green, respectively.

Promoter analysis of *Pvul-CHS* genes

Promoter regions are vital segments of DNA located near the transcription start site of genes, and they serve as key regulatory elements that directly manage gene expression. These promoters are controlled by specific *cis*-regulatory elements that modulate gene expression in response to environmental factors. In various plant species, several well-characterized *cis*- regulatory elements are responsive to abiotic stresses, such as ABRE (ABA-responsive element), which responds to abscisic acid (ABA), and DRE/CRT (dehydration-responsive element/C-repeat), which is sensitive to high salinity, dehydration, low temperature, and drought (Yamaguchi-Shinozaki and Shinozaki 2005). Additionally a recent study has shown MYB and Myb regulatory elements involving various stressors (Muslu et al. 2023). In this research, the sequences obtained from 2 kb upstream of the 5′ region of all *CHS* genes were analyzed, revealing that the promoter regions of *CHS* genes play roles in plant development, adaptation to changing environmental conditions, and molecular responses to abiotic stresses (Supplementary File 4).

As a result of the scans, 11 different elements, which interact directly or indirectly with stress factors, were found to be present in the upstream region

of the genes a total of 1870 times (all cis-regulatory elements found 2 kb upstream region were given in Supplementary File 5). *Pvul-CHS-7* contained the least number of elements (88) and *Pvul-CHS-3* the most (189). The most common cis elements found in the promoter region of Pvul-CHS genes are TATA-box and CCAT-box, which are repeated 1009 times and 449 times, respectively. The least common cis elements are LTR and TC-rich repeats. These elements serve various functions, including sensitivity to light, responses to environmental stress, promotion of gene expression, regulation by hormones, and further details about these functions can be found in Fig. 6. The reaction of plants to abiotic stresses is predominantly regulated through the interplay between *cis*- regulatory elements and transcription factors (Liu et al. 2014). Transcription factors serve as components of regulatory systems that facilitate the regulation of developmental processes, response to biotic and abiotic stressors, and control of gene expression in plants (Mitsuda and Ohme-Takagi 2009). The analysis revealed that *Pvul-CHS* genes contain ABRE, ARE, DRE, and MYB *cis* elements associated with abiotic stress factors. Notably, ABRE and MYB elements were identified in all *CHS* genes, while the ARE element was present in all genes except for *Pvul-CHS-3*, *-8*, and *-11*. The DRE element was detected in the *Pvul-CHS-1* and *-2* genes.

Ontology analysis of *Pvul-CHS* genes

Based on the analysis conducted using the DAVID Bioinformatics Resources database, Chalcone synthase (CHS) was found to be associated with the flavonoid biosynthesis pathway within the cell. Its molecular function was identified as catalyzing Naringenin-chalcone synthase activity (Table 3). All *Pvul-CHS* genes were found to be involved in the

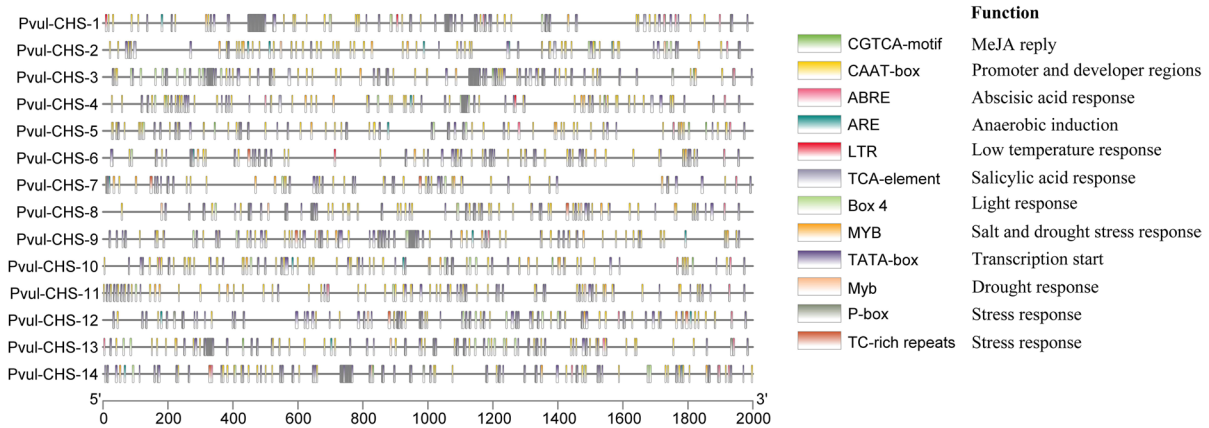

Fig. 6 *Cis*-regulatory elements map of the 2 kb upstream region of *Pvul-CHS* genes (all the elements given in the image are shown in different colors and the functions of the elements are also acquired through PlantCARE)

Table 3 List of *Pvul*-CHS proteins, which are exhibiting Naringenin-chalcone synthase activity, as a result of DAVID bioinformatics resource database analysis

Phytozome ID	Gene name	NCBI reference sequence	GO ID	Function
Phvul.001G067800.1	*Pvul-CHS-1*	XP_007161427.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.001G083000.1	*Pvul-CHS-3*	XP_007161599.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.002G038600.1	*Pvul-CHS-4*	XP_007157042.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.002G038700.1	*Pvul-CHS-5*	XP_007157043.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.002G039300.1	*Pvul-CHS-12*	XP_007157053.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.002G184300.1	*Pvul-CHS-13*	XP_007158815.1	GO:0016210	Naringenin-chalcone synthase activity
Phvul.009G131000.1	*Pvul-CHS-14*	XP_007137487.1	GO:0016210	Naringenin-chalcone synthase activity

same biological processes in the organism. After GO analysis, 5 different biological processes were identified: transferase activity (transferring acyl groups-other than amino-acyl groups), biosynthetic process, catalytic activity, lipid biosynthetic process and metabolic process. Seven of the 14 *Pvul-CHS* genes are involved in specifically the naringenin-chalcone synthase pathway as shown in Table 3.

Homology modeling and intracellular localization of identified Pvul-CHSs

The CHS proteins available in the Protein Data Bank (PDB) were subjected to screening through blastp, and 3D homology models of these proteins were generated in a visually interpretable manner using the Phyre2 (Protein Homology/Analogy Recognition Engine v2.0) database. Figure 7 illustrates the 3D homology models of the identified proteins. Upon analysis of the 3D structures of these proteins, it was evident that the alpha-helical structure predominated across all of them. While the alpha-helical structure was the predominant feature, there was also an observable presence of beta (β) pleated sheets within the protein structures in general. Specifically, 12 out of the 14 CHS proteins in *P. vulgaris* exhibited similar proportions of alpha-helical and beta-pleated structures. However, when examining the structural features of *Pvul*-CHS-6 and -14, it became apparent that the density of alpha-helical structures far exceeded that of beta pleated sheets especially in their active sites.

The intracellular localization of CHS proteins in common bean is also detailed in Fig. 7. According to the data from the WoLFPSORT database (Horton et al. 2007), *Pvul*-CHS-2 was found to have the highest diversity of intracellular localizations, with occurrences in eight different regions. In contrast, *Pvul*-CHS-4, -8, -10, -11, -12, and -14 exhibited the least diversity, each being localized in four different cellular compartments.

With the exception of the *Pvul*-CHS-6 and *Pvul*-CHS-14 proteins, the other CHS proteins appear to share almost identical homology. These proteins with similar homology are also predicted to be located in the same intracellular locations. CHSs are predominantly localized in the cytoplasm and chloroplast.

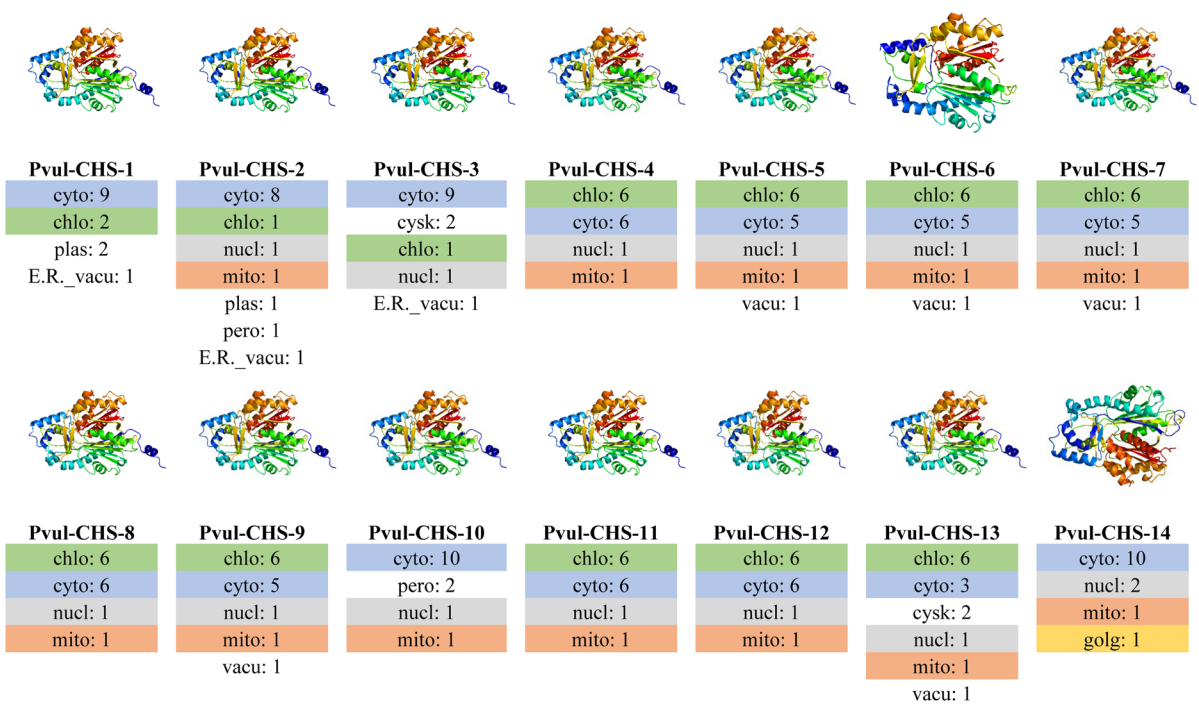

Fig. 7. 3D homology modelling and intracellular localization of *Pvul*-CHS proteins (cyto: cytoplasm, nucl: nucleus, chlo: chloroplast, mito: mitochondria, pero: peroxisome, vacu: vacuole, plas: plasma, cysk: skeleton, E.R:endoplasmic reticulum)

Pvul-CHS protein–protein interactions

The visualization of protein–protein interactions among *Pvul*-CHS proteins is presented in Fig. 8, and the STRING (Szklarczyk et al. 2023) database was used for this graphical representation. Analysis of protein–protein interactions and GO reveals that all *Pvul*-CHS proteins are involved in flavonoid biosynthesis. All protein sequences entered the database, and the STRING result was visualized using the export tab on the website. When considering protein–protein interactions, it is evident that all interacting proteins related to each other to an equal degree. Furthermore, in the image presented in Fig. 8, *Pvul*-CHS proteins are centered, while proteins that have not yet been characterized but are related are gathered on the right side of the image. On the other hand, the proteins gathered on the left side of the image are, from top to bottom, Glycosyltransferase (V7BUH3_PHAVU), 4-coumarate:coenzyme A (U3PHR7_PHAVU), Chalcone-flavonone isomerase family protein (V7BA56_PHAVU), Chalcone-flavonone isomerase family protein (V7AZH8_PHAVU) again and Aldo–keto reductase (V7AZH8_PHAVU) domain-containing protein. *Pvul*-CHSs were more intensely associated with uncharacterized proteins, suggesting that these uncharacterized proteins may be associated with plant growth and development and even may be related to flavonoid biosynthesis. In addition, the results of this study may be a resource for future research with the uncharacterized proteins shown in Fig. 8.

In silico expression profiles *Pvul-CHS genes under drought and salt stress and analysis of their expression during growth and development periods*

To assess the in silico expression patterns *CHS* genes in common bean under conditions of salt and drought stress, RNAseq data from the SRA (Sequence Read Archive) database were utilized. The heatmap illustrated in Fig. 9 utilizes the $\log_2$ transformed RPKM (Reads Per Kilobase Million) values derived from the RNAseq data analysis. This graph illustrates the fluctuations in *Pvul-CHS* gene expression levels that occur in response to treatments involving salt and drought stress in leaf tissues of *P. vulgaris*. As a result of the analysis, it was determined that the expression level of *Pvul-CHS-14* increased significantly, and *Pvul-CHS-3* increased slightly. On the contrary, the expression levels of all other *Pvul-CHS* genes decreased compared to the control conditions. Under drought stress, a significant decrease in the expression level of *Pvul-CHS-2* and *Pvul-CHS-14* was observed, while the expression level of *Pvul-CHS-12* increased significantly. In addition, there was a significant increase in the expression levels of *Pvul-CHS-8*, *Pvul-CHS-10* and *Pvul-CHS-13*, while no significant change was observed in the expression levels of other genes (Fig. 9a). The expression levels of genes during periods of growth and development provide as valuable information as under stress conditions. Changes in the expression levels of genes that naturally have significant effects on growth and development, also

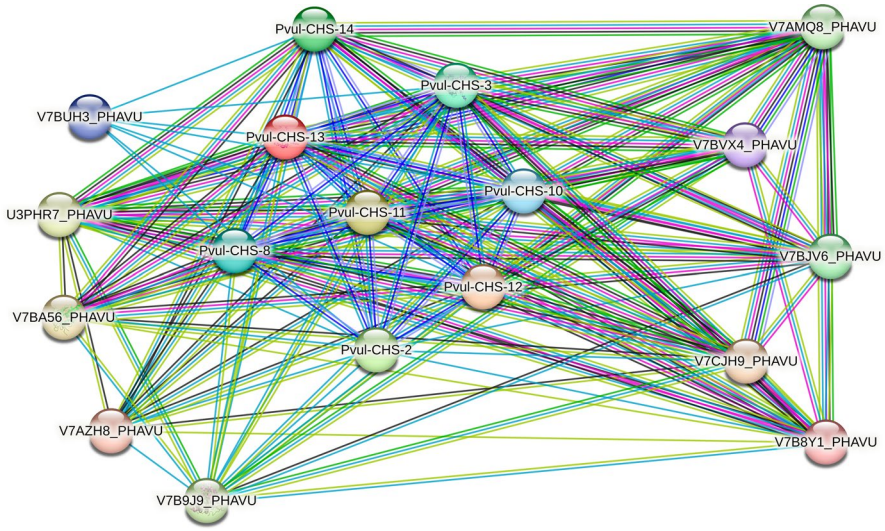

Fig. 8 Protein–protein interactions schematic representation found in *Pvul-CHS* gene products (The blue lines directly indicate relatively strong interactions; the green lines indicate weaker interactions, and the other colored lines represent values in between these two interaction strengths)

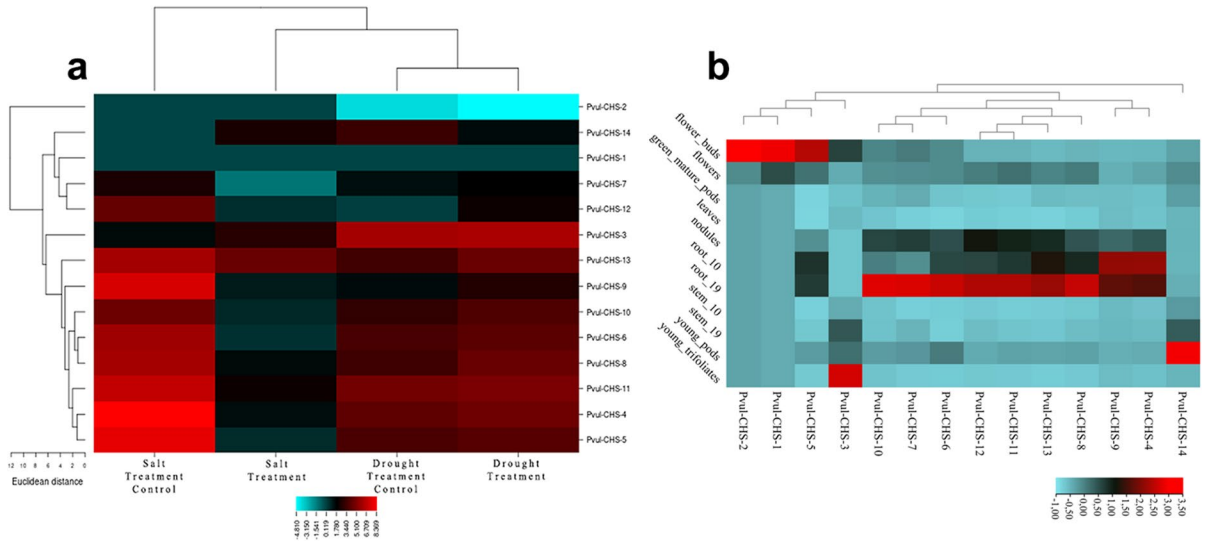

Fig. 9 **a**: In silico expression levels of *Pvul-CHS* genes in leaf tissue under salt and drought stress, **b**: indicating the expression of *Pvul-CHS* genes in some tissues (the graph illustrates the progression of expression level from cyan to red, the color scale gives a range of −4.81–8.37 for a, and 1–3.5 for b)

under stress conditions, and a correlation of these data, can be very valuable sources of information for researchers. In this study, FPKM values of *Pvul-CHS* genes for plant growth and development periods were accessed from the Phytozome v13 database and visualized using the TBtools program (Fig. 9b). While it was observed that *Pvul-CHS-3* gene was expressed more than other *CHS* genes in the early developmental period of the plant, it can be said that bean *CHS* genes in general may be associated with rooting based on expression analysis. Additionally, it can be seen from heatmap graph, bean *CHS* genes are generally expressed in tissues related to the late stages of the plant. In addition to these, *Pvul-CHS-1* and *-2* were found to be expressed in flower tissues and expression level could not be determined in other tissues.

Quantitative real-time PCR (qRT-PCR) analyses

To investigate the roles of *Pvul-CHS* gene family members under salt and drought stresses, they were exposed to 9 days of 150 mM NaCl and 24 h of 20% PEG6000 (polyethylene glycol) stress according to previous study (Aygören et al. 2023). For qRT-PCR analyses, primers specific to the identified genes were designed based on RNAseq data. The qRT-PCR results are presented in Figs. 10 and 11.

To assess changes in gene expression of *Pvul-CHS*, qRT-PCR analyses were performed using two cultivars, Elkoca-05 and Serra. In this study, the expression levels of *Pvul-CHS* genes under salt and drought stress were assessed using RNAseq data. Based on these data, an increase in the expression levels of *Pvul-CHS-2*, *-3*, *-6*, and *-13* genes was observed in the Serra cultivar following 150 mM NaCl treatment in both leaf and root tissues. However, no significant change was observed in the expression of the *Pvul-CHS-14* gene. Conversely, in leaf tissue under salt stress, the Elkoca-05 cultivar showed decreased expression levels of *Pvul-CHS-2*, *-3*, *-6*, and *-13* compared to the control, while *Pvul-CHS-14* exhibited increased expression. Likewise, expression differences were detected in *Pvul-CHS-3*, *-6*, *-13*, and *-14* genes in the Elkoca-05 cultivar under salt stress, with no significant change in *Pvul-CHS-2* expression (Fig. 10).

Following 20% PEG6000 application, expression differences were observed in *Pvul-CHS-2*, *-3*, *-6*, and *-13* in the Serra cultivar's leaf tissue due to drought stress, whereas no significant expression level changes were noted in *Pvul-CHS-14* compared to the control. Similarly, in root tissue under drought stress, expression differences were observed in *Pvul-CHS-2*, *-6*, and *-13*, while *Pvul-CHS-3* and

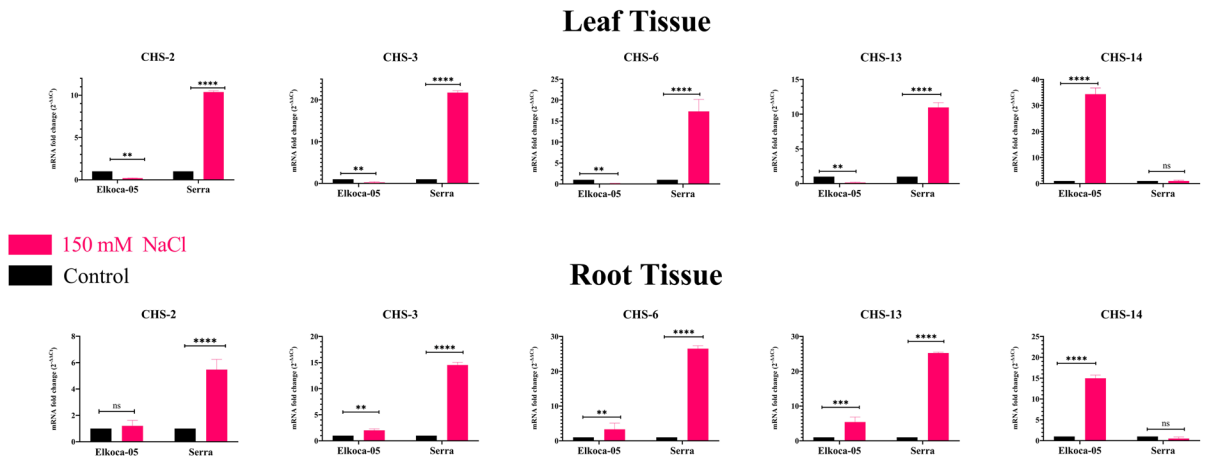

Fig. 10 qRT-PCR results of leaf and root tissues of Elkoca-05 and Serra cultivars under salt (150 mM NaCl) stress (expression levels were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001); bars: mean ± standard error (n = 3)). Significant differences between control and treatments were determined by Dunnett's test (*: importance rating, ns: non-significant)

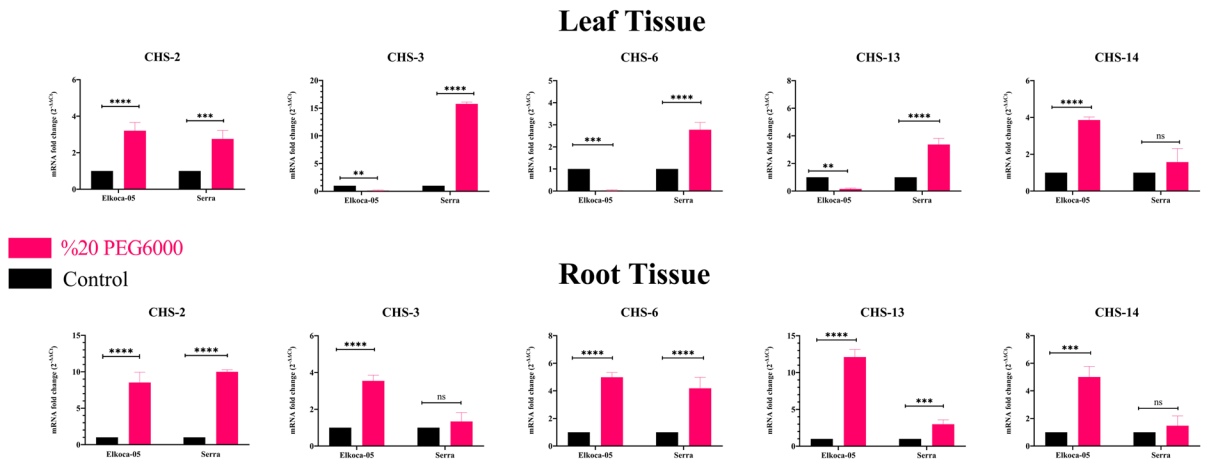

Fig. 11 qRT-PCR results of leaf and root tissues of Elkoca-05 and Serra cultivars under drought (20% PEG6000) stress (expression levels were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001); bars: mean ± standard error (n = 3)). Significant differences between control and treatments were determined by Dunnett's test (*: importance rating, ns: non-significant)

-14 showed no significant changes. Additionally, in the leaf tissue of the Elkoca-05 cultivar under drought stress, it can be said that *Pvul-CHS-2* and *-14* were increased, while *Pvul-CHS-3*, *-6*, and *-13* were decreased. When examining root tissue in the Elkoca-05 cultivar under drought stress treatment, it was observed that all five analyzed *Pvul-CHS* genes were up-regulated (Fig. 11). Upon reviewing the genes identified as significantly differentiated in qRT-PCR analyses, it was noted that the expression level of *Pvul-CHS-3* increased in the Serra cultivar's leaf tissue following 20% PEG6000 treatment compared to the control, with no significant change observed in the root. Based on these findings, it can be inferred that the *Pvul-CHS-3* gene is sensitive to drought stress and may contribute to plant tolerance.

Discussion

In this study, 14 *CHS* genes were found in the *P. vulgaris* genome. This gene, which is of great importance in flavonoid biosynthesis, has been previously identified in some other plant species. Specifically, 7 *CHS* gene family members were found in *Solanum melongena* L. (Wu et al. 2020), 5 in *Nicotiana tabacum* L. (Chen et al. 2017), 27 in *Oryza sativa* (Han et al. 2017), 16 in *Chrysanthemum nankingense* (Zhu et al. 2022), 11 in *Zostera marina* L.(Ma et al. 2021), 21 in *Mangifera indica* L. (Hu et al. 2022), 14 in *Zea mays* (Han et al. 2016), 14 in *Dendrobium catenatum* (Yang et al. 2023), and 17 in *Physcomitrella patens* (Koduri et al. 2010). The obtained CHS protein sequences were screened with the "ProtParam" tool to determine the characteristics of the proteins. After this screening, some data on the properties of the proteins were obtained. As shown in Table 1, the amino acid length of 3 proteins differs from the others. The highest pI value was 6.40 and all *Pvul*-CHS proteins exhibit acidic properties. The molecular weights of the proteins were generally determined as 42–43 kDa. Compared to the literature data, it can be said that CHS proteins do not only exhibit acidic properties and in general CHS proteins contain 300–500 amino acids. In *Mangifera indica* L., molecular weights from 33.47 to 46.06 kDa, protein lengths ranged from 304 to 411 amino acids, and isoelectric points were observed in both acidic and basic regions, ranging from 5.97 to 8.74 (Hu et al. 2022). For *Zostera marina*, molecular weights from 23.23 to 43.64 kDa, protein lengths ranged from 241 to 396 amino acids, and isoelectric points were found in both acidic and basic regions, ranging between 6.25 and 7.58 (Ma et al. 2021). In maize, protein lengths were determined to be between 326 and 472 amino acids, molecular weights between 35.27 and 49.69 kDa, and isoelectric points were observed in both acidic and basic regions, ranging from 5.35 to 7.58 (Han et al. 2016).

When the distribution of *CHS* genes on bean chromosomes was analyzed, a result far from similarity with other research was obtained. *Pvul*-CHS genes are distributed on only three chromosomes of beans. Upon reviewing relevant research, it was found that the distribution of *CHS* genes in *Zea mays* plants spanned chromosomes 1, 2, 3, 4, 5, and 7. Notably, two genes were situated on chromosomes 2, 5, and 7, while one gene was identified on chromosome 1, three genes on chromosome 3, and four genes on chromosome 4 (Han et al. 2016). In a similar study, the distribution of *CHS* genes on chromosomes in *G. max* plants was examined, revealing their presence on eight different chromosomes. Specifically, one *CHS* gene was located on chromosomes 2, 5, 6, 9, 11, and 19, with two genes on chromosome 1 and nine genes on chromosome 8 (Anguraj Vadivel et al. 2018). In the *Pvul-CHS* genes, only tandem duplication has occurred during the evolutionary process. Previous research has reported the presence of both segmental and tandem duplications within the *CHS* gene family (Han et al. 2016; Ma et al. 2021; Hu et al. 2022; Zhu et al. 2022). Due to the presence of segmental duplication in other species, it is thought that the distribution of *CHS* genes on chromosomes may be related to duplication events. In addition, *Pvul-CHS* paralog genes were subjected to purifying selection. Han et al. (2016) reported that the Ka/Ks ratio was <1 in all 2 *ZmCHS* paralog pairs in maize, indicating purifying selection. Similarly, Zhu et al. (2022) found that the Ka/Ks ratio was <1 in all 6 *CnCHS* paralog pairs in *Chrysanthemum nankingense*, also indicating purifying selection.

The structures of the *CHS* genes under research were visualized using GSDS. It was determined that all bean *CHS* genes contain one intron and two exons, for a total of 14 introns and 28 exons. On the other hand, two genes in *G. max*, one in *Z. mays*, one in *S. melongena* and two in *O. sativa* were found to contain multiple intron regions. For instance, there were 21 introns and 38 exons in *G. max* (Anguraj Vadivel et al. 2018), 14 introns and 28 exons in *Z. mays* (Han et al. 2016), 8 introns and 15 exons in *S. Melongena* (Wu et al. 2020), and 31 intron and 58 exons in *O. sativa* (Han et al. 2017). Based on the number of exons, it can be argued that *CHS* genes are conserved in the evolutionary process.

In this study in which the syntenic relationship between *CHS* genes of 3 different species was examined, it was determined that two of the bean *CHS* genes showed a total of four syntenic relationships. *Pvul*-CHS-14 showed syntenic relationships with *G. max* and *Pvul-CHS-3* showed syntenic relationships with *G. max* and *A. thaliana*. In terms of similarity with these data, a total of eight syntenic relationships were determined in *O. sativa*, four of which were with *Z. mays* and the other four with *S. bicolor* (Han et al.

2017). However, in addition to 2 *CHS* genes in bean, 3 genes from *OsCHS* were shown to be in syntenic relationship. No clear syntenic relationship visualization has been found in other research, and the first synteny map between *A. thaliana—G. max—P. vulgaris* is presented in this study.

In the 2 kb upstream region of *Pvul-CHS* genes, *cis*-regulatory elements ranging from 88 to 189 per gene were identified. In contrast, other research identified 57 *cis*-regulatory elements in *Mangifera indica* L. (Hu et al. 2022), 297 in *Zostera marina* (Ma et al. 2021), and 297 in *Solanum melongena* L. (Wu et al. 2020). Considering the functions of these elements listed in Fig. 6, it is seen that there are abundant elements in the upstream regions of *Pvul-CHS* genes that may play a role in the response to stresses. In similar research, 1 gene associated with the MBS element, 4 genes associated with the CGTCA-motif, 13 genes associated with MYB, and 5 genes associated with ABRE were identified in *S. melongena* L. (Wu et al. 2020).

Except for the *Pvul*-CHS-6 and *Pvul*-CHS-14 proteins, the other CHS proteins share almost identical homology. These proteins with similar homology were also predicted to be located in similar intracellular locations. CHS homologs are predominantly localized in the cytoplasm and chloroplast. In the light of the findings, the properties of CHS proteins were found to be similar among the species. When other genome-wide analyses with other plant species were taken into account, it was observed that the amino acid lengths of *P. vulgaris* were close to each other when compared with the CHS proteins of other species, and the molecular weights were very close except for *Zostera marina*. These findings further indicate that *CHS* genes are conserved in the evolutionary process. The data obtained from the motif analysis agreed with previous research. Only Motif 2 and Motif 3 were found to contain a CHS-related domain, while no specific domain was found in the other motifs. However, the other identified motifs different than Motif 2 and Motif 3 may be related to CHS proteins or flavonoid biosynthesis pathways. Motif analysis shows that all CHS proteins end with a C-terminal domain, while the N-terminal domain is spread throughout the proteins, but nevertheless all *Pvul*-CHS proteins were found to contain a free carboxyl group at their termini. A similar presence of these domains was observed in research involving CHS proteins from different plant genomes, including *C. nankingense* (Zhu et al. 2022), *Z. mays* (Han et al. 2016), and *S. melongena* (Wu et al. 2020), as reported in analogous research. Besides *Pvul*-CHS-14 was found to be different from other proteins in terms of the motifs they contain, homology analysis revealed that *Pvul*-CHS-14 has different homology compared to other proteins, indicating that motif analysis and homology modeling overlap. Similarly, the *Pvul*-CHS-6 is distinguished from other proteins in homology modeling due to the different folding of its amino acids. According to homology modeling, the active sites of the proteins shown in red and orange colors are almost the same in all proteins except *Pvul*-CHS-14, and it can be said that this protein may have a different function than the others based on the alpha helix and beta pleated structures. In this context, regarding the intracellular localization of the proteins, *Pvul*-CHS-10 and *Pvul*-CHS-14 were found more densely in the cytosol than the other proteins and *Pvul*-CHS-14 was predicted to be involved in the Golgi apparatus. This finding may indicate the homology models match with the intracellular location predictions. Similarly in the case of CHS proteins in *C. nankingense* (Zhu et al. 2022), five of them were reported to be located in the chloroplast, ten in the cytoplasm, and only one in the nucleus. In another study focusing on *S. melongena* L. (Wu et al. 2020), it was noted that among the seven CHSs identified, one was distributed in the nucleus, two in the chloroplast, and the remaining four in the cytoplasm.

Pvul-CHS genes and other *CHS* genes have been found to be associated with the flavonoid biosynthesis pathway. In similar research, gene ontology analysis of *CHS* genes in *Z. mays* revealed that it exhibited chalcone synthase activity dependent on the intracellular Chal_sti_synt_N domain, much like in beans (Han et al. 2016). In another investigation, gene ontology analysis in *Cucumis sativus* L. indicated that *CHS* genes play roles in plant growth and responses to abiotic stress factors (Ahmad et al. 2023).

Furthermore, qRT-PCR analyses were performed to profile the expression patterns of *Pvul-CHS* genes in two different *P. vulgaris* cultivars exposed to salt and drought stress conditions. Considering other research, it was determined that there was a change in the expression profiles of *Pvul-CHS* genes under drought and salt stress. In the expression levels of the 5 different genes examined, it can be said that while

an increase was observed in the leaf tissue generally for Serra cultivar, the same situation was observed in the root tissue for Ekoca-05 cultivar. *Pvul-CHS-14* gene may have an important role in the response to salt and drought stress, especially in Elkoca-05. However, *Pvul-CHS-2* gene has a significant role in response to drought stress in both cultivars. Considering that not only the increase in expression level but also the decrease in gene expression may be a response to stress, it is thought that *Pvul-CHS-3*, *-6* and *-13* genes may respond to stress by decreasing their expression in the leaf tissues of Elkoca-05 cultivar. In addition, since the expression of the same genes increased in the root tissues of the same cultivar, it is thought that these genes are trying to protect against the effects of drought and salt stress by increasing the expression level, especially in the roots of Elkoca-05 cultivar. On the contrary, the expression levels of the same gene did not show a significant change in both tissues under both stress conditions in Serra. In silico expression analyses revealed that the *Pvul-CHS-2* gene did not match the qRT-PCR results. In addition, it was determined that the data obtained for the *Pvul-CHS-3* gene were similar to the expression profiles of the Serra and the *Pvul-CHS-6* gene was similar to the expression profiles of the Elkoca-05. However, along with these data, *Pvul-CHS-13* gene in silico data were in agreement with salt stress data in Elkoca-05 and drought stress data in Serra. According to the qRT-PCR results of the *Pvul-CHS-14* gene, it was determined that the expression level did not change significantly in the leaf tissues of Serra, while this data did not match with the in silico expression profile. On the other hand, qRT-PCR experiments of the same gene in Elkoca-05 cultivar exhibited similarity in in-silico data with salt stress but contrasted with drought stress. Plants with a diverse range of genetic variations are better equipped to adapt to varying environmental conditions and thrive. Cultivars without genetic diversity, however, can grow more susceptible to various stressors (Kasapoğlu et al. 2024). The differences in quality attributes and stress tolerance among individuals belonging to the same species are referred to as genetic diversity. The variations in the expression levels of several genes are one of the reasons for this discrepancy (Salgotra and Chauhan 2023).

In a similar study conducted on *Nicotiana tabacum*, examining the heatmap results generated from analyses performed on different tissues of *N. tabacum* under high salt and drought stress conditions, it was reported that while four out of the five *CHS* genes exhibited high expression levels, significant expression differences were observed in the *NtCHS-5* gene (Chen et al. 2017). Chen et al. (2017) reported that, similar to the expression pattern of the *Pvul-CHS-3* gene, an increase in the *NtCHS-2* gene occurred in the root under 20% PEG6000 treatment compared to the control, while no change was detected in leaf tissue. Based on this study's results, it suggested that the *NtCHS-2* gene is sensitive to drought stress and could be used to develop drought tolerance in plants. In a different study, Han et al. (2016) investigated the expression levels of *CHS* genes in *Zea mays* under salicylic acid treatment for biotic and abiotic stress responses. The data indicated that *ZmCHS-1*, *-2*, *-3*, *-5*, *-7*, *-11*, and *-12* genes were down-regulated, while *ZmCHS-13* and *-14* were up-regulated. In parallel with these findings, *Pvul-CHS-3*, *-6*, and *-13* genes exhibited down-regulation under both salt and drought treatments, whereas *Pvul-CHS-14* displayed up-regulation in response to both salt and drought treatments in Elkoca-05. Yang et al. (2023), it was determined that the expression of *CHS* genes in *Dendrobium catenatum* generally increased in root tissues under drought stress. On the other hand, except for *DcCHS-1*, *DcCHS-8* and *DcCHS-12* genes, no significant changes they found in the expression of other genes in leaf tissues. Koduri et al. (2010) mentioned the similarity of *PpCHS10* with *AthCHSL1* and also reported that this gene is associated with fertilization in plants. It was mentioned that this gene, which is associated with pollen development and formation, may be involved in exine biosynthesis. In addition, it has been reported that *AthCHSL1* and *AthCHSL2* genes are also responsible for alkylpyrone production (Mizuuchi et al. 2008; Koduri et al. 2010). Similarly, overexpression of the *CpCHS1* gene was reported to protect sweet strawberry (Chinese strawberry) plants against drought stress (Hou et al. 2022). In the same research, *PavCHS1* and *PavCHS2* genes were also reported to be associated with leaf development and size and fruit development. These results suggest that *CHS* genes produce similar results in tolerance applications under abiotic stress conditions in different plant species. In the light of all these

findings, it is thought that *CHS* genes are associated with plant development and that these genes potentially develop a response against stress factors.

In this study, genome-wide analysis, and evaluation of *CHS* genes in common bean plants were performed. 14 candidate *CHS* genes were identified. Among these genes, 5 were selected based on their in silico expression levels and qRT-PCR was performed. The expression levels of these 5 genes were analyzed in the root and leaf tissues of two common bean cultivars subjected to salt and drought stress. These genes, which are thought to be a guide for further molecular biology, genetics, and breeding research, were detailed in this study.

Conclusion

In this study, 14 *CHS* genes in the genome of *P. vulgaris* were identified, a plant of considerable commercial and nutritional significance. We conducted a comprehensive characterization of the *Pvul-CHS* genes, encompassing their chromosomal positions, gene structures, evolutionary insights, intracellular localizations, conserved motifs, and functional attributes, utilizing a range of bioinformatics tools. Additionally, to gain deeper insights into the evolutionary dynamics of *Pvul-CHS* genes, comparative phylogenetic mapping and synteny analysis involving CHS proteins from *G. max* and *A. thaliana*, were conducted.

Notably, this study represents the first comprehensive characterization of *CHS* genes in *P. vulgaris*. The elucidation of *CHS* gene functionality under drought and salt stress conditions adds a distinctive dimension to our research. The insights attained from this research may serve as a valuable reference for future investigations in the subject areas of molecular biology, physiology, and breeding within the common bean researches globally. It may also serve as an essential resource for cloning research and functional investigations related to *P. vulgaris*.

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Author contributions All authors contributed to the study conception and design. MI, BMÖ and Eİ made bioinformatic analyses. The cultivation of the plants and initial stress treatments were carried out by EY and EY. MI, AGK and SM made laboratory experiments. This paper edited by MA and supervised by Eİ and MA.

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Data availability The datasets generated for this study are available online.

Declarations

Competing interest None of the authors declare a conflict of interest.

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