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Cold Shock Protein Gene Family in *Phaseolus vulgaris* L.; Genome-Wide Insights and Expression Profiles

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ABSTRACT

Cold shock proteins (*CSP*s) are evolutionarily conserved RNA-binding proteins that play critical roles in transcriptional and post-transcriptional regulation during plant development and stress adaptation. Despite their functional importance, the *CSP* gene family has not yet been systematically characterized in *Phaseolus vulgaris* (common bean), a major legume crop of global significance. In this study, a comprehensive genome-wide identification and characterization of *CSP* genes in *Phaseolus vulgaris* was conducted. Using *Arabidopsis thaliana CSP* protein sequences as queries, four *PvCSP* genes were identified in the *Phaseolus vulgaris* genome. These genes were distributed across three chromosomes and encoded proteins containing a conserved cold shock domain, a CCHC-type zinc finger motif, and glycine-rich regions. Phylogenetic analysis revealed that *PvCSP2* and *PvCSP4* clustered within the same clade, suggesting potential functional redundancy. Gene structure and motif analyses showed that all *PvCSP*s consisted of a single exon and shared two highly conserved core motifs, while exhibiting distinct motif arrangements indicative of subfunctionalization. *Cis*-acting regulatory element analysis identified a total of 488 *cis*-acting elements in the promoter regions of *PvCSP* genes, with hormone-responsive elements related to abscisic acid (ABA), methyl jasmonate (MeJA), and auxin being particularly enriched in *PvCSP4*. Protein–protein interaction predictions suggested that *PvCSP*s may interact with RNA metabolism–related proteins, including DEAD-box RNA helicases and mRNA decapping factors. Transcriptome-based expression profiling revealed that *PvCSP2* was ubiquitously expressed across tissues, whereas *PvCSP4* showed preferential expression in stems, roots, and developing pods. Overall, this study provides the first systematic insight into the *CSP* gene family in common bean, highlighting their structural features, regulatory potential, and tissue-specific expression patterns, and lays a foundation for future functional studies on *CSP*-mediated stress responses in legumes.

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Introduction

Cold stress is a particularly notable abiotic stress factor since it not only limits growth and development in many plant species but also influences planting time and geographical distribution (Yu et al., 2025). Glycine-rich proteins (GRPs) play a central role in plants' responses to stress conditions such as cold, UV radiation, salinity, and pathogen attack. Among these proteins, glycine-rich RNA-binding proteins (GR-RBPs) stand out, typically carrying a standard RNA recognition motif (RRM) or harboring a cold shock domain (CSD) in their N-terminal regions and a glycine-rich region at their C-terminal ends (Köster et al., 2020; Sachetto-Martins et al., 2000). GR-RBP proteins are important in cellular adaptation mechanisms because they guide mRNA transport between the nucleus and cytoplasm, as well as RNA processing and regulation and binding to specific transcripts (Mangeon et al., 2010). This binding mechanism is important for understanding the biological functions of GRPs in plants. Plant GR-RBPs are divided into four main classes based on their glycine content (20-70%) and additional motif structure. Within these classes, Class IV has two RRM domains in addition to the GR motif and is commonly found in plants (Krishnamurthy et al., 2015; Mangeon et al., 2010). Other classes (I-III) contain CCHC-type zinc finger motifs that contribute to RNA-binding activity. Subclass III, known as Cold Shock Protein (*CSP*), has a Cold Shock Domain (CSD) and is particularly important among cold-responsive proteins, as it is found in both prokaryotes and eukaryotes (Budkina et al., 2020). These proteins possess a compact β-barrel structure composed of five antiparallel beta (β) strands. The β2 and β3 sequences contain RNA-binding motifs RNP1 and RNP2, and the side chains of aromatic amino acids interact with nucleic acids, playing an important role in RNA melting activities (Horn et al.,

2007; Maris et al., 2005; Schröder et al., 1995). Plant *CSP*s contain a glycine-rich C-terminal domain along with a varying number of CCHC-type zinc finger motifs in addition to the cold shock domain. *CSP*s were first discovered in bacteria and are widely found in higher plants such as monocots and dicots, as well as in land plants (Karlson & Imai, 2003). *CSP*s not only respond to cold stress but also play a role in various biological processes such as cellular growth, embryo development, flowering, fruit formation, nutrient stress, and dormancy (Graumann & Marahiel, 1998; Karlson et al., 2002; Sasaki & Imai, 2012). The number of *CSP* genes exhibits variation across plant species. To date, the *CSP* gene family has been identified through genome-wide analyses in *Arabidopsis thaliana* (4 *CSP*s), *Brassica chinensis* (6 *CSP*s), *Camellia sinensis* (3 *CSP*s), *Triticum aestivum* (6 *CSPs*), *Oryza sativa* (4 *CSPs*), *Solanum lycopersicum* (5 *CSP*s), and *Prunus avium* (3 *CSP*s), as well as in four cotton species: *Gossypium arboreum* (8 *CSPs*), *Gossypium raimondii* (8 *CSPs*), *Gossypium barbadense* (14 *CSP*s), and *Gossypium hirsutum* (13 *CSPs*) (Guo et al., 2024; Huang et al., 2019; Khatun et al., 2018; Nakaminami et al., 2009; Radkova et al., 2014; Y. Yang et al., 2024).

AtCSP2 is localized to both the nucleus and cytoplasm, with its expression reaching the highest levels during the seedling stage, particularly in the shoot apical meristem and root tip (Fusaro et al., 2007; Sasaki et al., 2007). The *AtCSP3* gene is expressed in the meristematic tissues of both roots and shoots, and its expression is upregulated in response to cold stress, contributing to enhanced freezing tolerance (Kim et al., 2009). Unlike *AtCSP2* and *AtCSP3*, *AtCSP4* displays a more widespread expression profile across diverse plant tissues. During the vegetative phase, At*CSP*4 transcripts are detected in apical meristems, vascular tissues of developing leaves, and root tips (Y. Yang & Karlson, 2011). In addition, expression is notably higher in reproductive parts such as flowers and siliques (Y. Yang & Karlson, 2011). Analysis revealed that three *SlCSP* genes (*SlCSP2*, *SlCSP3*, and *SlCSP4*) in *Solanum lycopersicum* are significantly regulated at various time points in response to multiple stress conditions, including drought, cold, salt, heat, and abscisic acid (ABA). Notably, the *SlCSP3* gene showed higher expression levels specifically under cold stress (Khatun et al., 2018).

Phaseolus vulgaris L., commonly known as the common bean, is a diploid species (2n = 2x = 22) within the Fabaceae family, originally domesticated in Central and South America. As one of the world's most vital crops, it serves as a major dietary protein source for many populations (Broughton et al., 2003). The common bean thrives in warm climates and is highly sensitive to frost; exposure to temperatures below 10°C can severely impair its growth and development, classifying it as a cold-sensitive crop (C. Liu et al., 2019; Tang et al., 2025). Additionally, cold stress can adversely affect key yield components, ultimately reducing whole productivity (Gobatto et al., 2025). Despite their potential roles in stress adaptation and RNA metabolism, the *CSP* gene family has not been comprehensively identified or analyzed at the genome-wide level in common bean to date. Because common bean is a cold-sensitive legume, temperature fluctuations can directly compromise early vegetative growth, reproductive success, and yield stability (Gobatto et al., 2025; C. Liu et al., 2019). Moreover, legumes possess unique developmental and metabolic programs, including nodulation and symbiotic nitrogen fixation, which are highly sensitive to environmental cues and require robust post-transcriptional regulation (O'Rourke et al., 2014).

In this study, a genome-wide identification and characterization of *PvCSP* genes is presented, integrating phylogenetics, structural features, promoter architecture, protein interaction predictions, and tissue expression profiles. The resulting candidate-gene set and regulatory signatures provide a practical foundation for functional validation and for breeding-oriented applications, such as marker development targeting regulatory variation linked to cold adaptation.

Materials and Methods

Characterization of the CSP Genes

Hypothetical *Cold Shock Protein* (*CSP*) genes in *Phaseolus vulgaris* were identified by searching the Phytozome database using the Pfam ID (PF00313). Putative sequences were verified for *CSP* domains using a Hidden Markov Model (HMM) and screened for CCHC-type zinc finger motifs with the CDD/SPARCLE tool. *CSP* protein sequences from *Arabidopsis thaliana* were obtained from the TAIR database and aligned with *P. vulgaris* candidates using the EMBL-EBI Pairwise Sequence Alignment tool. BLASTP searches were performed using an E-value threshold of 1×10^{-5}, the BLOSUM62 substitution matrix, and default gap penalties to ensure reliable homology detection. Genes exhibiting greater than 40% protein sequence identity with *A. thaliana CSP*s were identified as *CSP* genes and named according to their closest Arabidopsis homologs. Using the ExPASy program, the isoelectric point (pI) and molecular weight (MW) of the *PvCSP* proteins were determined (Gasteiger et al., 2003). The Plant-mPLoc web-based tool was used to estimate the subcellular localization of *PvCSP* genes (Chou & Shen, 2010).

Phylogenetic Analysis

The protein sequences of the *CSP* gene family from common bean and selected plant species (*Arabidopsis thaliana*, *Solanum lycopersicum*, *Brassica rapa* ssp. chinensis, and *Oryza sativa* ssp. japonica) were aligned using the T-Coffee algorithm under default settings. A phylogenetic tree was constructed using the Neighbor-joining method based on the Poisson substitution model with 1000 bootstrap replicates implemented in MacVector software (MacVector, Inc.). The resulting tree was visualized and annotated using iTOL (Letunic & Bork, 2021).

Chromosomal Localization, Calculation of Ka/Ks and Synteny Analysis

The chromosomal start and end positions of the *PvCSP* genes were retrieved from the Phytozome database, and their physical locations were visualized using the MapGene2Chrom (MG2C v2.0) mapping tool (Chao et al., 2021). To estimate evolutionary divergence, the *Ka* (nonsynonymous) and *Ks* (synonymous) substitution rates for each duplicated gene pair were calculated with

TBtools-II (Chen et al., 2023). In addition, TBtools-II was employed to investigate the collinearity relationships of the *CSP* gene family among *Phaseolus vulgaris*, *Arabidopsis thaliana*, *Glycine max*, and *Solanum lycopersicum*, providing insights into their potential evolutionary conservation (Chen et al., 2023).

Motif and Gene Structure Analysis of PvCSP Members

Conserved motif regions within the *PvCSP* amino acid sequences were identified using the MEME Suite, with the maximum number of motifs set to 10 and motif widths constrained between 10 and 80 amino acids, while all other parameters were maintained at their default settings (Bailey et al., 2015). The gene architecture of the *PvCSP* members was further assessed using TBtools-II, incorporating the GFF annotation file of the *Phaseolus vulgaris* genome to illustrate the distribution of exons and introns (Chen et al., 2023).

Identification of Cis-Regulatory Elements

Cis-regulatory elements located within the 2000 bp upstream region of the *PvCSP* genes were predicted using the PlantCARE database (Lescot et al., 2002). The resulting elements were then visualized with TBtools-II software (Chen et al., 2023).

3D Modeling and PPI of PvCSP Proteins

The I-TASSER tool was used to generate the 3D structures of the *PvCSP* proteins (J. Yang & Zhang, 2015). The Mol* Viewer tool was employed for the visualization of the results obtained from the I-TASSER (Sehnal et al., 2021). The protein-protein interaction network for *PvCSP* proteins was predicted using the STRING database (v 12.0) by applying a minimum interaction confidence score of 0.4, corresponding to medium confidence interactions. Interaction evidence was derived from curated databases, experimentally validated interactions, and gene co-expression data to improve the reliability of the predicted network (Szklarczyk et al., 2025).

RNA-seq Analysis

Transcriptome datasets corresponding to root, nodule, young trifoliate leaf, leaf, stem, flower bud, flower, young pod, and green mature pod tissues were retrieved from the Phytozome database (https://phytozome-next.jgi.doe.gov). Expression levels of *PvCSP* genes were obtained as RPKM (Reads Per Kilobase per Million mapped reads) values and used directly for subsequent analyses. RPKM normalization, accounting for sequencing depth and gene length, was performed at the data generation stage by the original data producers. As the aim of this analysis was to describe tissue-specific expression profiles rather than to conduct differential expression analysis, no additional logarithmic transformation was applied. The use of untransformed RPKM values allowed preservation of relative and tissue-specific expression patterns, particularly for genes exhibiting low to moderate expression levels across tissues. All RNA-seq datasets were derived from publicly available resources, in which biological replicates were provided as pooled samples in the original studies. Bar graphs illustrating tissue-specific expression patterns were generated and visualized using GraphPad Prism software (v 10.0.0).

Results

Identification of PvCSP Genes

To identify *CSP* genes in the common bean genome, amino acid sequences of *AtCSP* genes were employed in pairwise sequence alignments, with the result that *PvCSP* genes were identified based on identity scores. Moreover, the identification of *PvCSP* genes was supported by the presence of conserved CSD, CCHC-type zinc finger motifs and glycine-rich C-terminal domains (Figure 1). A total of four *PvCSP* genes were identified in the common bean genome: *PvCSP1* (Phvul.009G122300), *PvCSP2* (Phvul.011G033900), *PvCSP3* (Phvul.003G263300) and *PvCSP4* (Phvul.009G025100) (Table 1). The *PvCSP* members identified in the common bean genome exhibited an identity score that ranged from 50% to 70% with the *CSP* proteins of *Arabidopsis thaliana* (Table 1). The CDS sequence lengths of *PvCSP* genes ranged from 543 base pairs (bp) (*PvCSP1*) to 744 bp (*PvCSP3*), while the amino acid sequence lengths ranged from 180 amino acids (aa) (*PvCSP*1) to 247 aa (*PvCSP*3) (Table 1). The molecular weight of *PvCSP* proteins ranged from 18.63 kilodalton (kDa) (*PvCSP*1) to 24.38 kDa (*PvCSP*3), while their isoelectric points (pI) ranged from 5.92 (*PvCSP*4) to 9.32 (*PvCSP*1) (Table 1). A subcellular localization analysis indicated that *PvCSP* genes were predicted to localize to either the Golgi apparatus or the nucleus (Table 1). The positional information pertaining to the *CSP* domains and zinc finger motifs on the amino acid sequence of *PvCSP* members was presented in Table 1. The amino acid sequence analysis revealed that *PvCSP* members possess the same protein domains, including the conserved *CSP* domain, the CCHC-type zinc finger motif, and the glycine-rich region (Figure 1, 2). It was also observed that these domains are highly conserved (Figure 1).

Table 1. Sequence features of *PvCSP* genes in the common bean.

Gene	Locus	L	PS	Chr	SL	D	ZF	MW (kDa)	pI	IA
PvCSP1	Phvul.009G122300	543	180	9	Golgi apparatus Nucleus	(8-73)	118-135 159-175	18.63	9.32	51.3%
PvCSP2	Phvul.011G033900	603	200	11	Nucleus	(7-71)	124-137 180-193	18.9	6.29	70.6%
PvCSP3	Phvul.003G263300	744	247	3	Golgi apparatus	(7-72)	106-119, 133-146, 162-175, 192-205, 229-242	24.38	7.90	50.0%
PvCSP4	Phvul.009G025100	585	194	9	Nucleus	(10-75)	141-154, 176-189	18.7	5.92	67.8%

L: CDS length (bp); PS: Peptide sequence (aa); SL: Subcellular localization; D: *CSP* Domain (Amino acid range); ZF: Zinc-Finger (Amino acid range); IA: % Identity with *Arabidopsis CSP*s Protein; CDS: coding sequences; bp: base pairs; aa: amino acids; Chr: chromosomal location; MW: molecular weight; kDa: kilodalton; pI: isoelectric point.

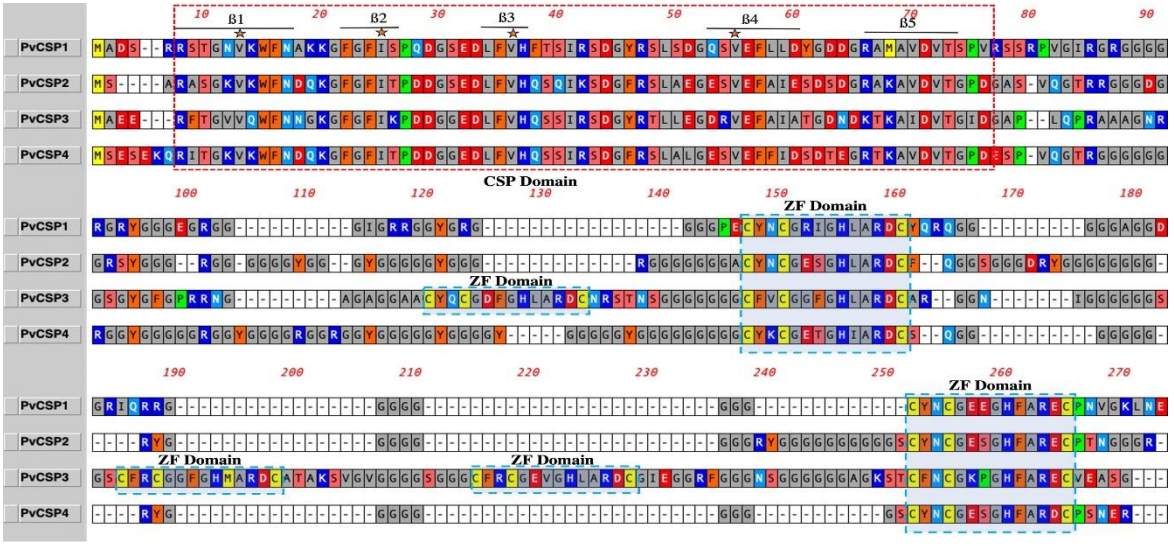


Figure 1. Multiple sequence alignment of *Phaseolus vulgaris* cold shock protein (*PvCSP*) family members. Conserved domains are highlighted, including the cold shock domain (CSD, red dashed box) and the CCHC-type zinc finger (ZF) domain (blue dashed box). The positions of the conserved β-strands within the CSD are indicated above the alignment. Glycine-rich regions are evident in the C-terminal portions of the sequences. Amino acid positions are shown above the alignment.

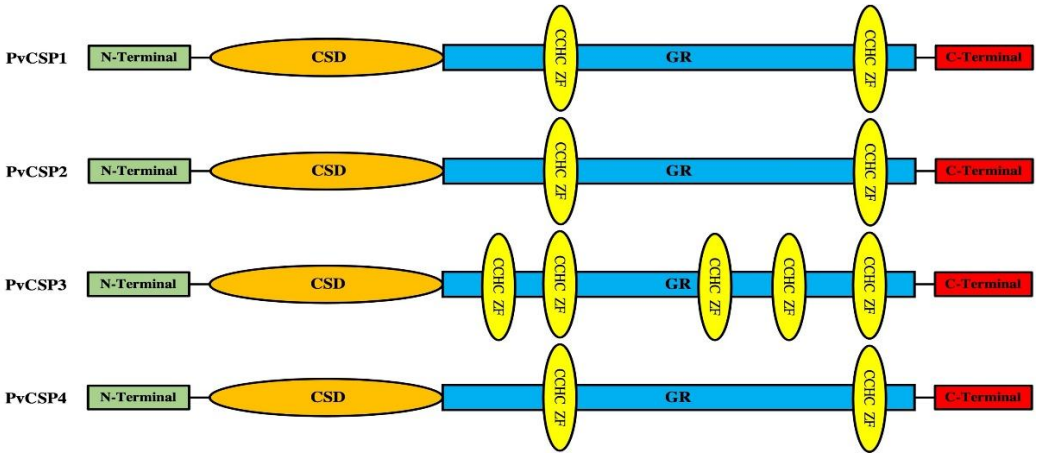


Figure 2. Schematic representation of the domain architecture of *Phaseolus vulgaris CSP* proteins. The diagrams illustrate the relative positions of the N-terminal region, the conserved cold shock domain (CSD), CCHC-type zinc finger (CCHC ZF) motifs, and the glycine-rich (GR) region within each *PvCSP* protein. Differences in the number and distribution of CCHC-type zinc finger motifs among *PvCSP* members are shown. The schematic representations are not drawn to scale.

Phylogenetic Analysis of CSP Genes

To investigate the evolutionary relationships of the *CSP* proteins of the common bean and other selected plant species, a phylogenetic tree was constructed using the Neighbor-joining (NJ) method and based on a total of 23 protein sequences from six species (Figure 3). Phylogenetic analysis grouped *CSP* genes from different plant species into three distinct clusters: Group I, II, and III (Figure 3). Group I and Group III comprised *CSP* members from monocot and dicot plant species. In contrast, Group II consisted exclusively of *CSP* members from monocot *O. sativa* and *S. bicolor* species (Figure 3). The Os*CSP1* and Sb*CSP2* genes were positioned in proximity within the same cluster. Similarly, *PvCSP*2 and *PvCSP4* were located within the same phylogenetic group and exhibited the closest evolutionary relationship among the *PvCSP* members (Figure 3).

Chromosomal Localization, Ka/Ks, and Synteny Analysis

The four *PvCSP* genes were identified in the common bean genome and were found to be distributed across three chromosomes. The *PvCSP1* and *PvCSP4* genes were located on chromosome 9, *PvCSP2* on chromosome 11, and *PvCSP3* on chromosome 3 (Figure 4).

To estimate the date of duplication events, the formula $T = (Ks/2\lambda) \times 10^{-6}$ million years ago was employed, assuming an average value of 8.46×10^{-9} for the synonym substitution rate (λ) (Hurst, 2002; Schmutz et al., 2014). The *Ka*/*Ks* ratio allows for the classification of selection types into three distinct groups: purifying (*Ka*/*Ks*<1), neutral (*Ka*/*Ks*=1), and positive selection (*Ka*/*Ks*>1) (Lynch & Conery, 2000). The *Ka/Ks* analysis showed that both segmentally duplicated *PvCSP* gene pairs, *PvCSP2/PvCSP4* and *PvCSP1/PvCSP3*, exhibited *Ka/Ks* ratios well below 1 (0.0746 and 0.3101, respectively), indicating that these duplicated genes have been subjected to purifying selection during their evolutionary history (Table 2).

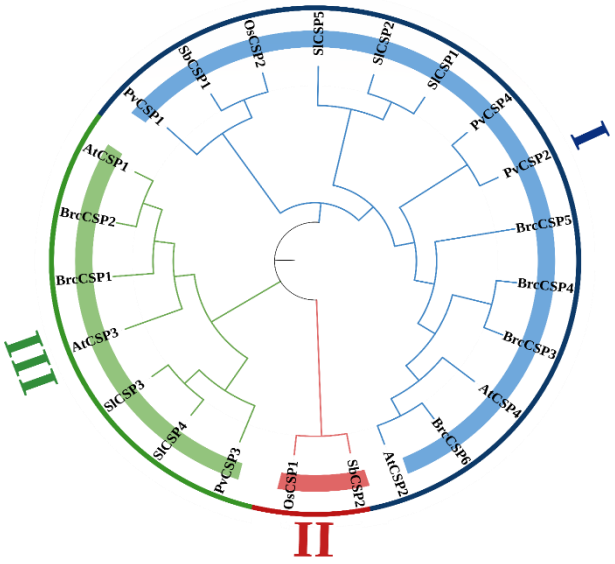


Figure 3. A phylogenetic analysis of *CSP* members of the common bean, along with five other selected plant species (*Arabidopsis thaliana CSP*/At*CSP*, *Solanum lycopersicum CSP*/Sl*CSP*, *Brassica rapa* ssp. *chinensis CSP*/Brc*CSP*, *Oryza sativa CSP*/Os*CSP*, *Sorghum bicolor CSP*/Sb*CSP*).

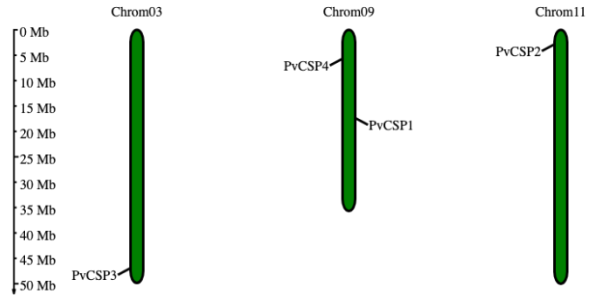


Figure 4. Chromosomal mapping of *PvCSP* genes across the common bean genome. The scale represents the length of chromosomes.

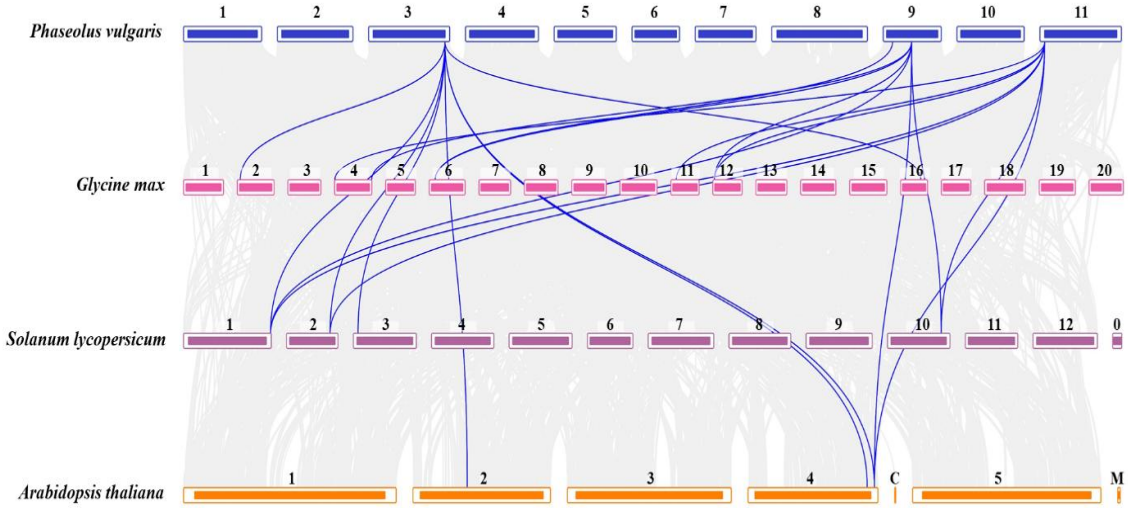


Figure 5. Schematic representation of the synteny relationships of the *CSP* genes in the genomes of *P. vulgaris*, *G. max*, *A. thaliana* and *S. lycopersicum*. Gray lines indicate genome-wide synteny relationships, while navy-blue lines highlight the syntenic *PvCSP* gene pairs and their chromosomal locations.

Table 2. The *Ka/Ks* ratio, duplication, selection types, and divergence time of the *PvCSP* genes.

Gene Pairs	*Ka*	*Ks*	*Ka/Ks*	Duplicated Type	Selection Type	Time (Million Year Ago)
PvCSP2/PvCSP4	0.137409	1.840944	0.074640	Segmental	Purify	61.36
PvCSP1/PvCSP3	1.210181	0.37528	0.310109	Segmental	Purify	40.33

Comparative synteny analysis revealed collinear relationships between *PvCSP* genes and their orthologs in *Glycine max*, *Arabidopsis thaliana*, and *Solanum lycopersicum* (Figure 5). Synteny maps were generated to visualize the chromosomal locations and conserved gene arrangements across these species (Figure 5). The analysis showed that *PvCSP* genes share colinear gene pairs with *G. max*, a legume species closely related to the common bean, as well as with *S. lycopersicum* and *A. thaliana*, which belong to different plant families (Figure 5).

Gene Structure and Conserved Motif Analysis

To explore the structural characteristics and variation among the *PvCSP* genes, their exon–intron arrangements were examined using TBtools-II. The analysis indicated that all members of the *PvCSP* family consist of a single exon (Figure 6). Among them, *PvCSP2* possesses the longest genomic sequence at 1260 base pairs, whereas *PvCSP1* has the shortest, measuring 1018 base pairs (Figure 6).

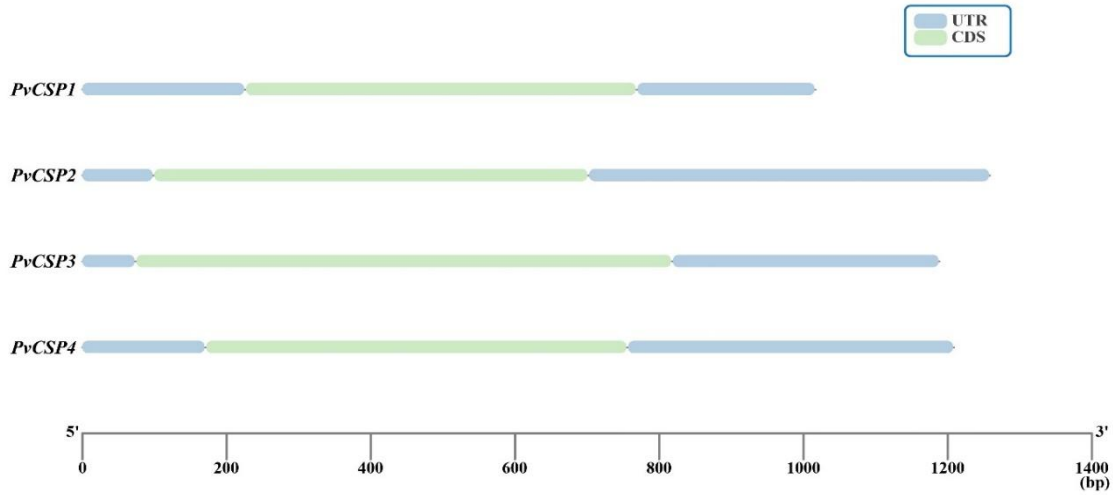


Figure 6. The gene structure of the *CSP* family member in the common bean genome. CDS: Coding sequence; UTR: Untranslated region

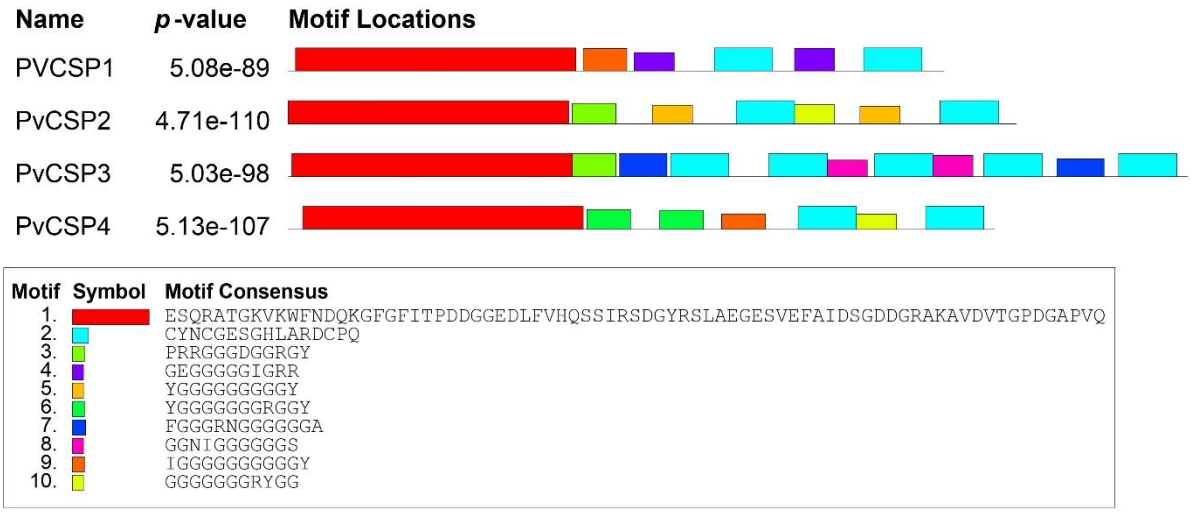


Figure 7. The conserved motif analysis of *PvCSP* proteins. Each of the ten preserved motifs was illustrated in a different color.

The MEME suite tool was employed to examine the conserved motifs of *PvCSP* members, with the conserved motifs expressed in different colors (Figure 7). The results of the analyses indicated the presence of 10 distinct conserved motifs in the *PvCSP* members, with the length of these motifs ranging from 11 (Motif 4, Motif 5, Motif 8, Motif 10) to 77 (Motif 1) amino acids (Figure 7). Motif 1 and Motif 2 were detected in all *PvCSP* members, whereas Motif 7 and Motif 8 were observed exclusively in *PvCSP*3 (Figure 7). Additionally, Motif 4 was observed to be present exclusively in *PvCSP*1, Motif 5 was present only in *PvCSP*2, and Motif 6 was found only in *PvCSP*4 (Figure 7).

Analysis of Cis-regulatory Elements of PvCSP Genes

The *cis*-regulatory element analysis identified a total of 488 cis-elements belonging to 36 different categories within the promoter regions of *PvCSP* genes (Figure 8). Hormone-responsive elements related to MeJA, auxin, salicylic acid, and abscisic acid were detected with varying frequencies among *PvCSP* members. MeJA- and ABA-responsive elements were more frequently observed in the promoter region of *PvCSP4* compared to other *PvCSP* genes (Figure 8). A total of 36 different types of *cis*-elements were categorized as follows: light-responsive elements (LRE), development-related elements (DRE), hormone-responsive elements (HRE), promoter-related elements (PRE), site-binding-related elements (SBRE), environmental stress-related elements (ESR) and other elements (OE) (Figure 8). Among the hormone response elements, the MeJA (Methyl jasmonate)-responsive elements (TGACG-motif and CGTCA-motif) were observed in the *PvCSP1*, *PvCSP3* and *PvCSP4* genes, while the auxin-responsive elements (TGA-box and TGA-element) were found in the *PvCSP1*, *PvCSP2* and *PvCSP4* genes (Figure 8). The salicylic acid responsiveness element (TCA-element) was observed exclusively in the *PvCSP3* gene, whereas the abscisic acid (ABA) responsiveness element (ABRE) was detected solely in the *PvCSP3* and *PvCSP4* genes (Figure 8). Furthermore, it was found that the MeJA and ABA responsiveness elements were found more intensely in the promoter region of the *PvCSP4* gene compared to other hormone response elements. These findings point to a potential regulatory role for MeJA and ABA hormones in the expression of stress related *PvCSP4* gene.

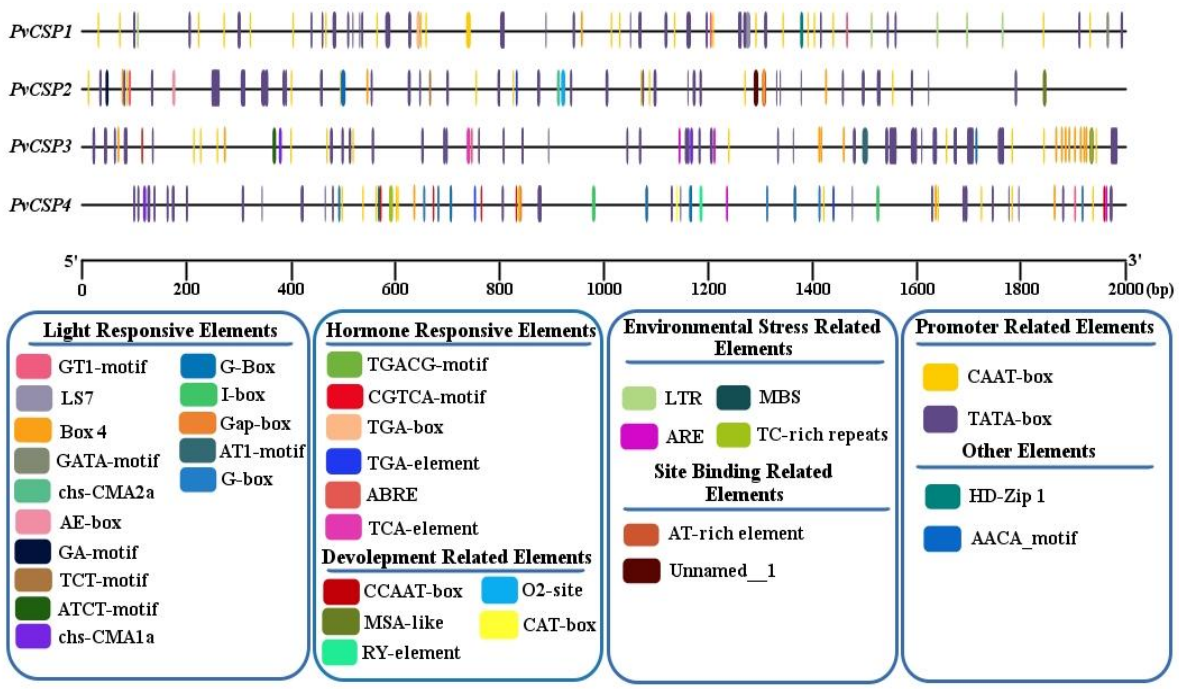


Figure 8. Distribution of *cis*-regulatory elements within the promoter regions of *Phaseolus vulgaris CSP* genes. Colored boxes indicate the positions of individual cis-elements along the promoter regions (5′–3′ orientation). *Cis*-acting elements were classified into functional categories, including light-responsive, hormone-responsive, development-related, environmental stress-related, site-binding-related, promoter-related, and other elements, as indicated in the legend.

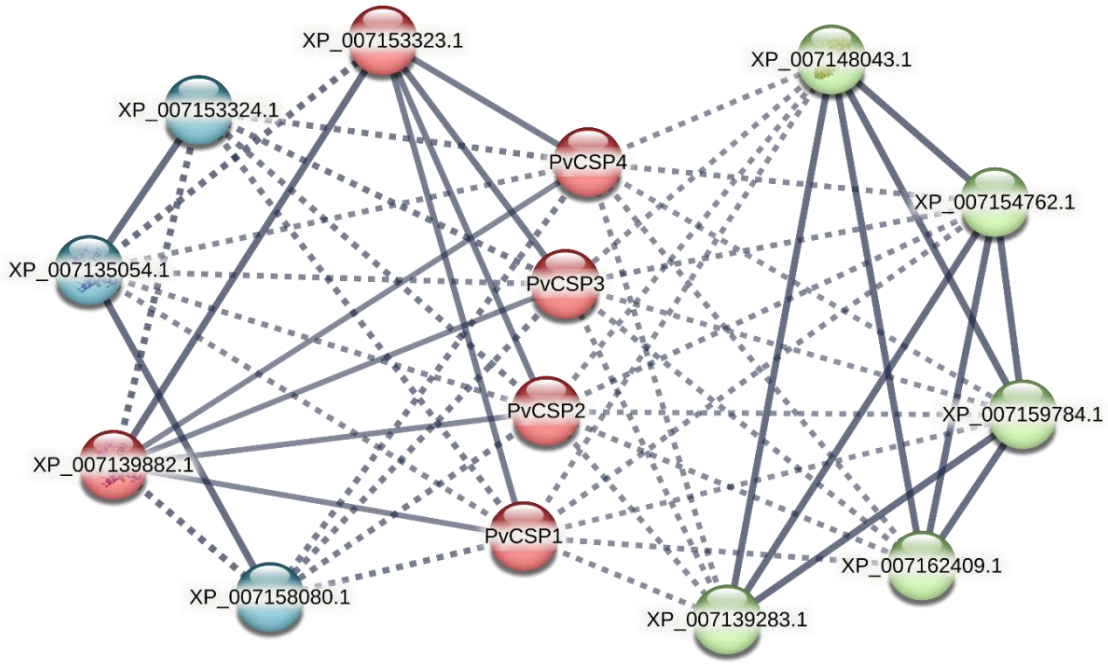


Figure 9. The protein–protein interaction network of *PvCSP* proteins

Prediction of Protein-Protein Interaction (PPI)

To examine the potential involvement of *PvCSP* proteins in cellular interaction networks, a protein–protein interaction (PPI) map was generated using the STRING database. Subsequent application of the K-means clustering algorithm organized the network into three distinct clusters. The first cluster (red bubble) comprised *PvCSP*1, *PvCSP*2, *PvCSP*3, *PvCSP*4, and XP_007139882.1 (Figure 9). The second cluster (green bubble) included XP_007148043.1, XP_007154762.1, XP_007159784.1, XP_007162409.1, and XP_007139283.1, while the third cluster (blue bubble) consisted of XP_007153324.1, XP_007135054.1, and XP_007158080.1 (Figure 9).

Three-Dimensional (3D) Structure Prediction of PvCSP Proteins

Three-dimensional models of the *PvCSP* proteins were generated using the I-TASSER platform and subsequently rendered through the PDB 3D Viewer

(https://www.rcsb.org/3d-view) (Figure 10). To assess the reliability of the predicted protein structures, the C-score was calculated (Roy et al., 2010). The reliability of the predicted *PvCSP* protein structures was assessed using the C-score, which ranges from -5 to 2, with higher values indicating greater confidence in the model (J. Yang et al., 2013). Additional evaluation metrics included the TM-score and root-mean-square deviation (RMSD). The TM-score varies from 0 to 1, where a value of 1 represents near-perfect alignment between structures (Zhang, 2008). RMSD reflects the average deviation between atomic positions and generally falls within 0 to 30 Å (Roy et al., 2010). Among the *PvCSP* proteins, *PvCSP*1 achieved the highest C-score at -1.04, suggesting the most reliable structural prediction, whereas *PvCSP*3 exhibited the lowest confidence with a C-score of -3.84 (Table 3).

Gene Expression Profiles of PvCSP Genes

RNA-seq data retrieved from the Phytozome v13 database were analyzed to explore the tissue-specific expression of four *Phaseolus vulgaris cold shock protein* (*PvCSP*) genes under non-stress conditions (Figure 11). The *PvCSP* family exhibited distinct yet overlapping expression patterns across vegetative and reproductive tissues, suggesting functional diversification. Expression profiling revealed that *PvCSP1* transcripts accumulated mainly in leaf tissues, where they may participate in RNA processing events linked to photosynthetic function. By contrast, *PvCSP2* showed consistently high transcript levels across nearly all organs, with a marked enrichment in flower buds, stems, and roots. Such a broad and intense expression pattern suggests that *PvCSP2* acts as the predominant and constitutively active member of this gene family (Figure 11). *PvCSP3* maintained moderate and relatively stable expression throughout the plant, typical of genes associated with fundamental cellular maintenance (Figure 11). In turn, *PvCSP4* expression was most evident in stems, developing pods, and roots-tissues characterized by rapid growth and strong metabolic demand-indicating a possible role in supporting dynamic developmental processes. Together, these results indicate that *PvCSP* genes are differentially regulated under normal physiological conditions (Figure 11). *PvCSP2* and *PvCSP4* appear to contribute broadly to RNA stability and general plant growth, while *PvCSP1* and *PvCSP3* likely perform simpler or more targeted functions that help maintain normal RNA activity in specific tissues (Figure 11).

Discussion

Previous studies on cold shock domain proteins (*CSP*s) have consistently demonstrated that plant *CSP*s play important roles in a broad range of biological processes associated with growth, development, and freezing tolerance (Chaikam & Karlson, 2008; Karlson & Imai, 2003; Park et al., 2009; Radkova et al., 2014; Sasaki & Imai, 2012). One of the earliest functional characterizations of plant *CSP*s was reported in wheat (*Triticum aestivum* L. cv. Chihoku), where the *CSP* gene *WCSP1* was shown to be strongly induced under cold stress, with protein accumulation increasing progressively during cold acclimation, thereby providing direct evidence for the involvement of *CSP*s in low-temperature adaptation (Karlson et al., 2002). Subsequent genome-wide studies have identified *CSP* gene families across diverse plant species, including five *CSP* genes in *Solanum lycopersicum* (Khatun et al., 2018), six in *Brassica rapa* ssp. chinensis and five in *B. rapa* ssp. pekinensis (Huang et al., 2019), three in *Prunus avium* (Guo et al., 2024), and an expanded *CSP* repertoire comprising sixty-two members across four cotton species (*Gossypium arboreum*, *G. raimondii*, *G. barbadense*, and *G. hirsutum*) (Y. Yang et al., 2024).

Table 3. The quantitative outputs generated by the I-TASSER modelling of *PvCSP* proteins.

Protein	C-score	TM-score	RMSD (Å)	Peptide sequence lengths (aa)
*PvCSP*1	-1.04	0.58±0.14	7.3±4.2	180
*PvCSP*2	-1.91	0.49±0.15	9.6±4.6	200
*PvCSP*3	-3.84	0.30±0.10	15.1±3.5	247
*PvCSP*4	-1.89	0.49±0.15	9.5±4.6	194

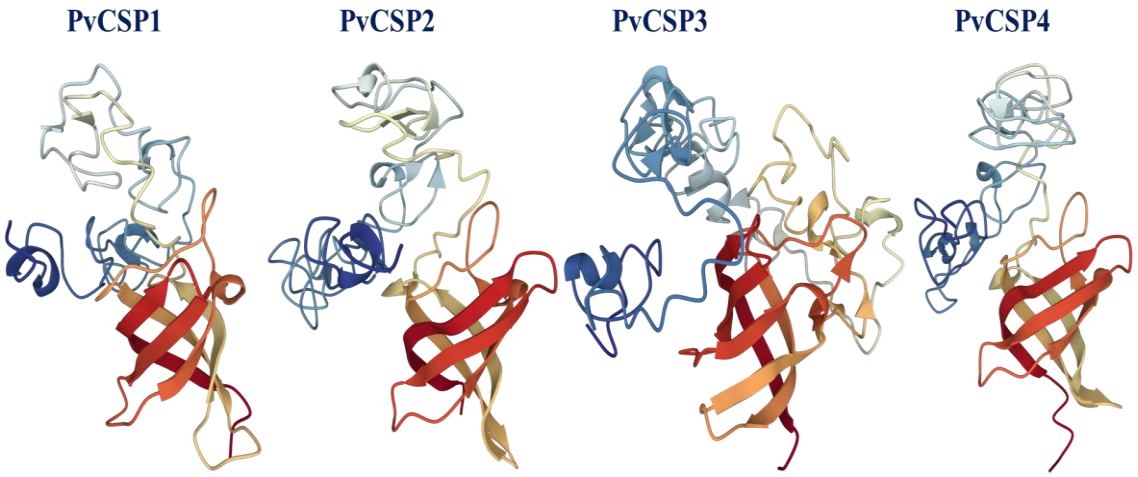


Figure 10. Three-dimensional structure prediction of four *PvCSP* proteins.

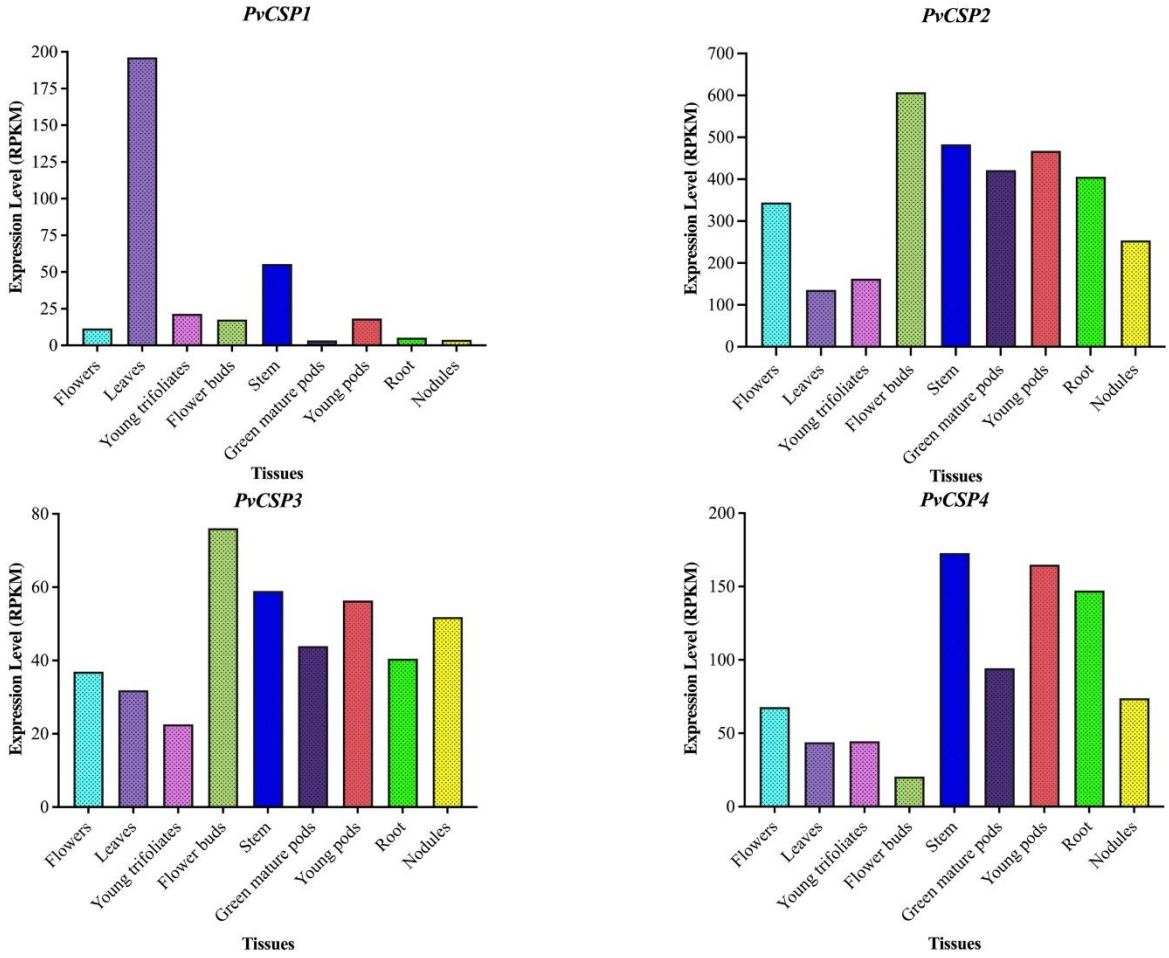


Figure 11. Tissue-specific expression profiles of *Phaseolus vulgaris CSP* genes under non-stress conditions based on RNA-seq analysis. Expression levels are presented as RPKM values across different tissues, including flowers, leaves, young trifoliate leaves, flower buds, stems, green mature pods, young pods, roots, and nodules. Bar plots illustrate the relative transcript abundance of each *PvCSP* gene across the analyzed tissues.

In addition, four *CSP* genes have been reported in the model plant *Arabidopsis thaliana* (Karlson & Imai, 2003; Sasaki & Imai, 2012), while *CSP* gene family size ranges from two in *Oryza sati*va to seven in the legume species *Glycine max* (Sasaki & Imai, 2012), collectively indicating that *CSP* genes are maintained in relatively low but conserved copy numbers across angiosperms. Common bean is a cold-sensitive crop in which chilling conditions can rapidly impair early seedling establishment, vegetative growth, reproductive development, and yield stability (Greaves, 1996; C. Liu et al., 2019). Under such conditions, *CSP*-mediated RNA regulatory mechanisms are thought to play critical roles in maintaining RNA stability and cellular homeostasis, thereby contributing to stress adaptation (Karlson et al., 2002; Nakaminami et al., 2009).

A comprehensive analysis of the *CSP* gene family in the common bean genome is currently lacking. In this study, four *CSP* genes were identified using a genome-wide *in silico* approach, a number comparable to that reported in *Arabidopsis thaliana* and consistent with the conserved *CSP* gene family size observed across plant species. The maintenance of a limited yet conserved number of *CSP* genes across phylogenetically diverse plant taxa highlights the strong evolutionary conservation of this gene family and underscores their fundamental roles throughout the plant life cycle (Karlson & Imai, 2003; Sasaki & Imai, 2012). Moreover, the promoter architectures and baseline tissue-specific expression profiles presented here provide a valuable framework for prioritizing *PvCSP* candidates for functional validation and for informing breeding strategies aimed at enhancing cold tolerance through regulatory variation rather than coding-sequence modification, an approach increasingly recognized as central to stress resilience and yield stability in crops (Mickelbart et al., 2015; Shinozaki & Yamaguchi-Shinozaki, 2007). In cold-sensitive legumes such as common bean, this regulatory perspective is particularly relevant, as stress-responsive RNA metabolism is closely linked not only to vegetative growth and yield stability but also to legume-specific processes including nodule development and symbiotic nitrogen fixation, both of which are highly sensitive to temperature fluctuations (O'Rourke et al., 2014; Oldroyd & Dixon, 2014). The phylogenetic evaluation identified twenty-three *CSP* proteins across six plant species, which clustered into three distinct evolutionary clades, designated as Group I, Group II, and Group III (Figure 3). A comparable diversification pattern has been reported in tomato (*Solanum lycopersicum*) (Khatun et al., 2018) and cotton species

(*Gossypium* spp.) (Y. Yang et al., 2024). Within the common bean, *CSP* protein members were distributed across two of these lineages: *PvCSP*1, *PvCSP*2, and *PvCSP*4 were assigned to Group I, whereas *PvCSP*3 clustered within Group III. Notably, *PvCSP*2 and *PvCSP*4 exhibited the closest evolutionary relationship among the *PvCSP* members, mirroring the relationship previously reported between At*CSP*2 (At4g38680) and At*CSP*4 (At2g21060) in *Arabidopsis thaliana* (Karlson & Imai, 2003). Earlier studies have demonstrated substantial sequence similarity between At*CSP*2 and At*CSP*4, accompanied by nearly identical expression patterns and comparable subcellular localization (Nakaminami et al., 2009; Sasaki et al., 2007, 2013; Y. Yang & Karlson, 2011). Interestingly, *atCSP2 atCSP4* double mutants display enhanced freezing tolerance, supporting the notion that these genes function as negative regulators of cold acclimation (Sasaki et al., 2013). Taken together, these findings suggest that *PvCSP*2 and *PvCSP*4 in common bean may serve analogous molecular functions, providing a promising basis for future functional characterization or breeding strategies aimed at improving frost resilience.

Examination of *PvCSP* protein architecture revealed the presence of three characteristic domains: an N-terminal cold shock domain (CSD), a glycine-rich region, and one or more CCHC-type zinc finger motifs (Figures 1 and 2). These structural features are well conserved among plant *CSP*s (Guo et al., 2024; Radkova et al., 2014; Sasaki & Imai, 2012). The number of CCHC zinc finger motifs in dicotyledonous species has been reported to vary widely, ranging from one to seven, which is consistent with the structural diversity observed among *PvCSP* proteins (Sasaki & Imai, 2012). Motif-level analysis further demonstrated that the N-terminal CSD is highly conserved across *PvCSP* members (Figure 7). Previous studies have proposed that conserved CSD and CCHC domains confer RNA chaperone activity, enabling *CSP* proteins to interact with both single- and double-stranded nucleic acids (Y. Yang et al., 2024). Gene structure analysis revealed that all *PvCSP* genes consist exclusively of coding sequences and lack introns (Figure 6). Intron-deficient *CSP* gene structures have similarly been reported in sweet cherry, tomato, Pak-choi, Chinese cabbage, and cotton (Guo et al., 2024; Huang et al., 2019; Khatun et al., 2018; Y. Yang et al., 2024). Such streamlined gene architectures may facilitate rapid transcriptional responses under stress conditions.

The *Ka/Ks* analysis indicates that segmentally duplicated *PvCSP* gene pairs have been predominantly shaped by purifying selection during evolution (Table 2), highlighting strong evolutionary constraints acting on this gene family. In particular, the markedly low *Ka/Ks* ratio observed for the *PvCSP2/PvCSP4* pair reflects intense selective pressure against amino acid-altering substitutions, consistent with long-term functional conservation of these paralogs. This pattern suggests that while synonymous substitutions accumulated over extended evolutionary time scales, non-synonymous changes were selectively constrained, thereby preserving essential protein structure and function. Such evolutionary behavior is characteristic of genes involved in core regulatory processes, including RNA binding and post-transcriptional control. By contrast, the *PvCSP1/PvCSP3* gene pair displayed a relatively higher, yet still sub-unitary, *Ka/Ks* ratio, indicating weaker but nevertheless effective purifying selection. This difference in selective pressure between duplicated *PvCSP* gene pairs suggests heterogeneous evolutionary lineages following duplication and may reflect early stages of functional partitioning or regulatory divergence rather than complete neofunctionalization. Similar patterns of purifying selection combined with differential constraint intensity among *CSP* paralogs have been documented in sweet cherry and cotton, underscoring a conserved evolutionary regime for *CSP* genes across diverse plant species (Guo et al., 2024; Y. Yang et al., 2024).

Comparative synteny analysis further revealed that *PvCSP* genes maintain conserved collinear relationships with their orthologs in *Arabidopsis thaliana*, *Solanum lycopersicum*, and *Glycine max*, indicating that *CSP* loci have been preserved within stable chromosomal contexts across angiosperm lineages (Sasaki & Imai, 2012). In legumes, the stronger and more continuous synteny observed between *Phaseolus vulgaris* and *Glycine max* is consistent with their shared evolutionary history and ancient whole-genome duplication events characteristic of legume genomes (Schmutz et al., 2014). Importantly, the retention of *PvCSP* genes within conserved syntenic blocks argues against rapid genomic turnover or lineage-specific expansion and instead supports the presence of strong functional constraints acting on these loci. Together, the concordance between conserved synteny patterns and low *Ka/Ks* ratios provides compelling molecular evidence that *PvCSP* genes have retained essential biological roles throughout angiosperm evolution.

A detailed analysis of the promoter regions of *PvCSP* genes revealed the presence of *cis*-regulatory elements associated with developmental processes, hormonal responsiveness, and environmental stress responses (Figure 8). Genes harboring diverse *cis*-elements within their promoter regions are often regulated by multiple transcription factors, enabling integration and cross-talk among abiotic stress signaling pathways (J. H. Liu et al., 2014). Comparable promoter architectures have been reported for *CSP* genes in sweet cherry and cotton, suggesting a conserved regulatory framework across plant species (Guo et al., 2024; Y. Yang et al., 2024).

Beyond transcriptional regulation, protein–protein interaction analysis provided further evidence supporting a post-transcriptional role of *PvCSP* proteins. Network analysis revealed that all *PvCSP* members were consistently connected to the DEAD-box RNA helicase XP_007139882.1, a core component of RNA metabolism involved in RNA unwinding and remodeling (Figure 9) (Linder & Jankowsky, 2011). In addition, each *PvCSP* protein was predicted to associate with the decapping factor DCP5 (XP_007153323.1), which plays a critical role in translational repression and mRNA turnover through decapping-dependent pathways (Figure 9) (Xu & Chua, 2009). Collectively, these interaction patterns indicate that *PvCSP* proteins are closely linked to post-transcriptional regulatory machinery and may function as integral components of RNA stability and translation control networks.

The maintenance of a limited yet conserved number of *CSP* genes across phylogenetically diverse plant taxa

highlights the strong evolutionary conservation of this gene family and supports their fundamental roles throughout the plant life cycle (Karlson & Imai, 2003; Nakaminami et al., 2009; Sasaki & Imai, 2012). Consistent with this evolutionary constraint, comparative transcriptome profiling across *Gossypium hirsutum*, *Solanum lycopersicum*, and *Phaseolus vulgaris* revealed both conserved and species-specific *CSP* expression patterns in vegetative and reproductive organs (Khatun et al., 2018; Y. Yang et al., 2024). In all three taxa, *CSP* transcripts preferentially accumulated in tissues characterized by high metabolic activity or rapid developmental transitions, in line with the established roles of plant *CSP*s as RNA-binding proteins involved in RNA stabilization, RNA chaperone activity, and post-transcriptional regulation (Khatun et al., 2018; Y. Yang et al., 2024).

In model and crop species, *CSP* genes display clear functional partitioning between broadly expressed and tissue-biased members. In *Arabidopsis thaliana*, *AtCSP2* and *AtCSP4* exhibit constitutive expression across multiple organs, consistent with roles in general RNA homeostasis, whereas other *AtCSP*s show more spatially restricted expression patterns associated with specific developmental contexts (Nakaminami et al., 2009; Sasaki et al., 2013). Similarly, in tomato, *SlCSP* transcripts preferentially accumulate during flowering and early fruit development and decline during later stages of fruit maturation, reflecting their involvement in early reproductive development and intensive transcriptional reprogramming (Khatun et al., 2018). In cotton, *GhCSPs* paralogs exhibit pronounced tissue specialization, a pattern likely shaped by sub-functionalization following allopolyploid origin, underscoring the plasticity of *CSP*-mediated RNA regulation in complex plant genomes (Yang et al., 2024). Within this comparative framework, common bean exhibits both conserved and legume-specific *CSP* expression features. Notably, the elevated expression of *PvCSP2* in nodules is of particular interest, as nodules are characterized by sustained transcriptional activity, extensive cellular differentiation, and high metabolic demand during symbiotic nitrogen fixation (O'Rourke et al., 2014). This expression feature, which is absent from non-legume reference systems, highlights a legume-specific dimension of *CSP* function and gains further relevance given that low-temperature stress can disrupt nodulation and nitrogen fixation processes (Zahran, 1999). In contrast, *PvCSP4* exhibits a more selective expression profile, with pronounced enrichment in stems, roots, and developing pods (Figure 11). Developing pods are rapidly expanding sink tissues that require tightly coordinated transcriptional and translational regulation to support seed formation, nutrient allocation, and storage program establishment. The preferential accumulation of *PvCSP4* transcripts in these tissues, together with the enrichment of hormone-responsive cis-regulatory elements in its promoter region, supports the view that *PvCSP4* integrates RNA metabolic control with developmental and hormonal signaling pathways during reproductive growth. Importantly, this pod-associated enrichment accompanied by strong expression in stems and roots distinguishes *PvCSP4* from *CSP* homologs characterized in *Arabidopsis* and tomato (Khatun et al., 2018; Nakaminami et al., 2009), suggesting functional specialization within the *PvCSP* family that may reflect legume-specific reproductive and developmental requirements.

Conclusion

In this study, four *PvCSP* genes that are encoded in the common bean genome were identified using bioinformatic analyses with a genome-wide approach. The results of the phylogenetic analysis indicated that members of the *PvCSP* are divided into two subgroups according to their evolutionary affinities. The presence of an N-terminal CSD domain, a glycine-rich region and a CCHC zinc finger domain was exposed in the *PvCSP* members. Analysis of the gene structure indicated that members of the *PvCSP* are devoid of introns. The analysis of the promoter revealed the presence of elements associated with developmental processes, hormonal sensitivity, and environmental stress response. The results of protein-protein interaction predictions indicated the potential involvement of *PvCSP* proteins in the regulation of RNA metabolism. Comparative analysis across cotton, tomato, and common bean highlights how genome complexity and organ specialization shape *CSP* gene expression and function in higher plants. The present study proposes four *PvCSP* genes for further investigation into their functionality, in relation to the common bean *CSP* genes.

Declarations

Ethical Approval Certificate
Ethical approval is not applicable

Author Contribution Statement
Ugur Sari: Data collection, conceptualization, methodology, investigation, formal analysis, and writing the original draft

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The author declares no conflict of interest

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