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RESEARCH ARTICLE

Characterization of *Two-Component System* gene (*TCS*) in melatonin-treated common bean under salt and drought stress

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Abstract

The two-component system (*TCS*) generally consists of three elements, namely the histidine kinase (*HK*), response regulator (*RR*), and histidine phosphotransfer (*HP*) gene families. This study aimed to assess the expression of *TCS* genes in *P. vulgaris* leaf tissue under salt and drought stress and perform a genome-wide analysis of *TCS* gene family members using bioinformatics methods. This study identified 67 *PvTCS* genes, including 10 *PvHP*, 38 *PvRR*, and 19 *PvHK*, in the bean genome. PvHK2 had the maximum number of amino acids with 1261, whilst PvHP8 had the lowest number with 87. In addition, their theoretical isoelectric points were between 4.56 (PvHP8) and 9.15 (PvPRR10). The majority of *PvTCS* genes are unstable. Phylogenetic analysis of *TCS* genes in *A. thaliana*, *G. max,* and bean found that *PvTCS* genes had close phylogenetic relationships with the genes of other plants. Segmental and tandem duplicate gene pairs were detected among the *TCS* genes and *TCS* genes have been subjected to purifying selection pressure in the evolutionary process. Furthermore, the *TCS* gene family, which has an important role in abiotic stress and hormonal responses in plants, was characterized for the first time in beans, and its expression of *TCS* genes in bean leaves under salt and drought stress was established using RNAseq and qRT-PCR analyses. The findings of this study will aid future functional and genomic studies by providing essential information about the members of the *TCS* gene family in beans.

Keywords Expression profile · Histidine · Melatonin · *Phaseolus vulgaris* · Two component system (TCS)

Abbreviations

CHASE	Cyclases/histidine kinases associated sensing extracellular
HATPase	Histidine kinase-like ATPase
HisKA	His kinase A
HK	Histidine kinase
HP	Histidine phosphotransfer
RR	Response regulator
TCS	Two-component system

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Introduction

The bean, *Phaseolus vulgaris* L., is a new world legume grown as a monoculture crop everywhere from the low-lying tropics to the semi-arid parts of temperate climates (Thung and Rao 1999). Furthermore, it is recognized as the primary food source for millions of people in underdeveloped nations, as well as an essential source of energy, minerals, and total protein globally (Petry et al. 2015; Pereira et al. 2020). One hundred grams of beans contain approximately 20–25 g of protein, 60 g of carbohydrates, and 0.7–1.5 g of fat. It also includes various vitamins, minerals, and phytochemicals (Azarpazhooh and Boye 2012; Terzi et al. 2020).

Plants are subjected to multiple biotic and abiotic burdens throughout their lives as they remain stable in their environment. Drought and soil salinity are two main environmental factors that limit plant growth and development and result

in large output losses, especially in arid and semi-arid areas (Raza et al. 2022a, 2023a; Suleymanov et al. 2023). Salinity stress affects plants in three different ways. The first is that the roots cannot obtain enough water from the soil because salt reduces the osmotic potential of the soil. Second, an increase in Na^+ and Cl^- ions cause the breakdown of cellular organelles, followed by the reduction of protein synthesis and enzyme activity, and finally, decreased nutrition intake and/or transport to shoots (Yavaş and Hussain 2022; Karimi et al. 2023). Since salinity reduces the ability of plants to use water, it slows down the plant's growth rate (Hailu and Mehari 2021). Stomata close and the amount of CO_2 between leaf cells reduces under salt stress. Salt stress also decreases the use of NADPH by the Calvin cycle, inhibits the production of chlorophyll and Rubisco, and interferes with the photosynthetic electron transport system (Miao et al. 2023). Drought stress is an important limiting factor in bean cultivation worldwide, especially in marginal and unfavorable environments (Rodiño et al. 2020; Sánchez-Reinoso et al. 2020). More than 60% of bean-producing areas cannot provide the necessary water for growth, and drought-related yield losses of up to 80% have occurred in some regions (Losa et al. 2022). Many metabolic processes are disrupted by drought stress in plants, including photosynthesis, stomatal conductivity, leaf area, reduced biomass, and decreased pod and seed yield (Asfaw et al. 2017; Hussain et al. 2023; Raza et al. 2023b; Yang et al. 2023).

Cytokinin is a key plant hormone that plays a role in virtually all aspects of plant growth and development, such as root and leaf development, plant formation, flowering, and seed germination, as well as responses to environmental stimulants (Liu et al. 2020). A two-component system (TCS) converts the cytokinin signal in plants. This system was initially identified in bacteria, but slime molds, fungi, and plants also use it in other signal transduction pathways (Loomis et al. 1997; Schaller 2000; Stock et al. 2000; Hutchison and Kieber 2002). TCS is typically made up of two gene families: the response regulator (*RR*) gene family and the histidine kinase (*HK*) gene family (Stock et al. 2000; Liu et al. 2020). HK proteins can be phosphorylated, transferring a phosphoryl group to a conserved aspartate residue in the RR's response (Rec) domain. The phosphorylated RR protein directly or indirectly regulates downstream gene activity (Stock et al. 2000; Hwang et al. 2012). Plant TCSs are more complex than most bacterial TCSs, and a histidine phosphotransfer (*HP*) gene family has been discovered to be responsible for an extra phosphorylation step in plants (Urao et al. 1998; Thomason and Kay 2000). The HP protein links the phosphoryl group transfer between HK and RR (Schaller et al. 2008).

Plants have three *HK* subfamilies: phytochromes, cytokinin receptors, and ethylene receptors. In Arabidopsis, three more *HK*s (*ACKI1, AHK1,* and *AHK5/CKI2*) are not part of any group. The general structure of *HK*s includes several trans-membrane domains, an input domain located in the N-terminal region, a donor domain with an autophosphorylation site and a conserved histidine residue, and an acceptor domain (Rec) (Hwang et al. 2002). However, because they lack highly conserved residues and motifs, the three ethylene receptors (*ERS2, AETR2,* and *AEIN4*) and phytochrome donors are unlikely to display *HK* action. As a result, they are referred to as distinct *HK*s (Kiba et al. 2005; Mähönen et al. 2006). Furthermore, the cytokinin receptors *AHK2, AHK3*, and *AHK4* possess a cyclase/HK-associated sensory extracellular domain (CHASE) that cytokinin recognizes. *AER1, AETR2, AEIN4, ERS1,* and *ERS2*, are ethylene receptors with ethylene (C2H4) binding domains. Furthermore, phytochrome family members (*PHYA, PHYB, PHYC, PHYD,* and *PHYE*) have a chromophore (PHY) binding domain and two PAS folds (Per/Arndt/Sim) (Stock et al. 2000).

The phosphotransfer domain (Hpt) of the *HP* gene family contains a highly conserved motif (XHQXKGSSXS) required for the transfer of the phosphate group from the Rec domain of *HK*s to the Rec domain of *RR*s (Hwang et al. 2002). Another type of *HP*, known as pseudo-histidine-containing phosphotransfer protein, lacks the conserved histidine residue. This group is not a phosphor-transmitter protein, but it does participate in cytokinin signaling by inhibiting the phosphorus delay from phosphorylated *AHP1* to *ARR* (Mähönen et al. 2006).

The type-A, type-B, and type-C subgroups of the *RR* family are based on conserved amino acid sequences and domains. Cytokinin response proteins known as type-A RRs have Rec domains in C-terminal extensions. On the other hand, type-B *RR*s are transcription factors consisting of an N-terminal Rec domain and a C-terminal output domain (Lohrmann et al. 1999). Although type-C *RR*s have the same domain as type-A *RR*s, they are not induced by cytokinin (Mizuno 2004). In addition to these *RR*s, there is a different class of *RR*s, known as pseudo-*RR*s (*PRR*s). *PRR*s are missing a highly conserved D residue required for phosphorylation (Makino et al. 2000). *PRR*s contain a specific CCT (Co, Col, and Toc1) motif in their C-terminal extension, vital in regulating circadian rhythms (Más 2008; Tsai et al. 2012).

Melatonin (*N*-acetyl-5-methoxytryptamine) is found in many different plant specie's leaves, stems, fruits, and seeds (Raza et al. 2022b; Golding and Lee 2023). Seeds and leaves generally have the highest melatonin content than fruit (Xiang et al. 2023). It is an indolic substance that shares structural similarities with other vital substances such as indole-3-acetic acid (IAA), serotonin, and tryptophan (Raza et al. 2022c). Melatonin intermediates exhibit antioxidant properties and have synergistic effects with other antioxidant compounds such as ascorbic acid and glutathione (Ramasamy et al. 2023). Due to its solubility in lipids and water, melatonin functions as a universal hydrophilic and

hydrophobic antioxidant (Mir et al. 2022). This property allows it to quickly cross cell membranes and penetrate subcellular spaces (Marcantonini et al. 2022).

The purpose of this study was to analyze the genome-wide TCSs in bean genotypes and determine the effects of melatonin treatment against salt and drought on expressions of *TCS* genes. This study will contribute significantly to the literature on *PvTCS* genes and their functions in the context of gene evolution, genomic distribution, and expression patterns under varying environmental conditions. Moreover, these results may help future functional and genomic studies in other plants.

Materials and methods

Detection of *PvTCS* genes in the bean genome

Protein sequences of *HK, HP,* and *RR* genes encoding TCS elements in the bean genome were obtained from the Phytozome v13 database (Goodstein et al. 2012) using Pfam Accession Numbers His kinase A (HisKA) domain (PF00512), histidine kinase-like ATPase (HATPase) domain (PF02518), cyclases/histidine kinases associated sensing extracellular (CHASE) domain (PF03924), histidine phosphotransfer (Hpt) domain (PF01627) and response (Rec) domain (PF00072) in the Pfam database (Mistry et al. 2021). To identify proteins of all possible TCS elements in the bean (Schmutz et al. 2014), the annotated proteins from the *P. vulgaris* genome were scanned with default parameters, both blastp in the Phytozome v13 database and hidden Markov model (HMM) (http://www.ebi.ac.uk). The presence of HisKA, HATPase, CHASE, HPt, and Rec characteristic domains in the detected sequences was detected using the HMMER scanner. The molecular weight, amino acid numbers, and instability index, which if the index is less than 40, then protein is probably stable. If it is greater then it is probably unstable (Guruprasad et al. 1990) and the theoretical isoelectric point (pI) of the obtained TCS proteins was determined using the "ProtParam tool" (Gasteiger et al. 2005).

Determination of gene structures, chromosomal locations, gene duplications, and conserved motifs of TCS elements in beans

Gene Structure Display Server v2.0 was used to obtain information about the exon–intron structures of genes of TCS elements in beans (Guo et al. 2007). Through a comparison of genome and coding DNA (CDS) sequences, the locations of the bean *TCS* element genes were established. The Phytozome v13 database was used to establish the chromosomal localizations and sizes of bean *TCS* genes. In addition, *PvTCS* genes were marked on the corresponding chromosomes and shaped with the Circos (Krzywinski et al. 2009); TBtools subsequently displayed a syntenic map (Chen et al. 2020). Non-synonymous values (Ka), synonymous values (Ks), and evolutionary strains' ratios (Ka/Ks) were calculated at the codeml program of the PAML package (Yang 2007) with PAL2NAL v14 online software which is the aligner for PAML (Suyama et al. 2006) using protein and transcript sequence of segmentally and tandemly-duplicated *PvTCS* gene pairs. In this online software, 'Remove mismatches' and 'selected positions' were selected as no, and Universal Code (NCBI: transl_table = 1) 'Removing Gaps' and 'Calculate dS and dN' were selected as yes.

Multiple EM for Motif Elimination (MEME) v5.1.0 tools were used to determine extra-conserved motifs of bean TCS element proteins (Bailey et al. 2006). The limits for the min (2)/max (50) width as well as the max number of motifs (10) were set. In addition, the motif regions were adjusted between 2 and 300. The determined motifs were investigated using the default settings of the InterPro scanner (Quevillon et al. 2005).

Phylogenetic analysis and multiple sequence alignment

Phylogenetic analyses were carried out using the Neighbor-joining (NJ) method with a bootstrapping value of 1000 replicates. The protein sequences of the bean TCS elements were aligned with the ClustalW (p-distance) program (online last version/https://www.genome.jp/tools-bin/clustalw) (Thompson et al. 1997) and a phylogenetic tree was drawn by the MEGA v7 tool (Letunic and Bork 2011) and visualized by Interactive tree of life (iTOL) (Letunic and Bork 2011).

Comparative mapping between beans and other species

The orthologs of the *TCS* genes of *A. thaliana* (Lamesch et al. 2012), *G. max* (Schmutz et al. 2010), and *P. vulgaris* were identified by using the Multiple Collinearity Scan Toolkit (MCScanX) (Wang et al. 2012). Protein sequences of orthologous genes were revealed using Phytozome v13. A Synteny map of *TCS* genes in these plants was generated using TBtools.

Determination of cis-acting elements of *PvTCS* genes and subcellular localization of PvTCS proteins

Cis-acting element analysis was made using the PlantCARE database (Lescot et al. 2002) by taking the 5′ upstream region including approximately 2 kb DNA fragments of the *PvTCS* genes. Subcellular localization was determined using PvTCS protein sequences with WoLF PSORT (last

version/https://wolfpsort.hgc.jp/) scanner (organism type: plants) (Horton et al. 2007).

Gene ontology analysis homology modeling, and protein–protein interactions of TCS proteins in bean

The STRING was used to describe protein–protein interactions' physical and functional linkages (Szklarczyk et al. 2021). The confidence score (0.4) and maximum additional interactions (5) were set in the STRING application in the Cytoscape program, and the interactions of PvTCS proteins with each other and with a few other proteins were determined. Furthermore, protein–protein interactions were reshaped with the Cytoscape tool (Shannon et al. 2003). Gene ontology data of *PvTCS* genes were accessed using the Blast2GO (Conesa et al. 2005) and Omics Box (Götz et al. 2008) tools to retrieve functional information on PvTCS proteins. Three-dimensional (3D) structures, created by comparing the reliability scores of the protein models, and protein homology patterns were determined using the Phyre2 database in the PvTCS protein sequences determined in this study (Kelley et al. 2015).

Identification of Pvu-miRNAs associated with *PvTCS* genes

All defined miRNA common bean sequences were obtained using PmiREN (Plant miRNA ENcyclopedia, http://www.pmiren.com). miRNA parameters were estimated using default settings in the psRNA Target Server (www.zhaolab.org/psRNATarget) (Zhang 2005). Targeted regions were predicted using PvTCS CDS sequences and Pvu-miRNA sequences. BLASTX online software (with a 1e-10 threshold) was used to compare in silico predicted miRNA targets to common bean-expressed sequence tags in the NCBI.

Tissue-specific in silico gene expression analysis of *PvTCS* genes

Illumina RNA-seq data to detect the expression of genes of TCS elements in bean leaves were obtained from the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) data library. Accession numbers of SRR957668 (leaf exposed to salt stress), SRR958469 (leaf salt control), SRR8284481 (leaf exposed to drought stress), and SRR8284480 (leaf drought control) determined as a result of detailed examinations were used (Hiz et al. 2014). RPKM (reads per kilobase of exon model per million mapped reads) algorithm was used to normalize gene expression profiles (Mortazavi et al. 2008). RPKM values were transformed to log2 and a heatmap map was obtained with the CIMMiner (https://discover.nci.nih.gov/cimminer/oneMatrix.do).

Plant material, stress, and melatonin applications

Serra and Elkoca-05 common bean cultivars determined in a previous study (Aygören et al. 2022) were used as plant material in this study. PEG 6000 (0 and 20%) and NaCl (0 and 150 mM) were used as drought and salt stress agents, respectively. Cultivation of plants, applications of Melatonin, and stress agents were carried out according to the instructions of Aygören et al. (2022). Melatonin application (0 and 200 mM) was made by spraying the leaves 24 h before applying stresses in the hydroponic system containing 1/10 Hoagland solution. Plants were exposed to salt and drought stress for 9 days and 24 h, respectively. At the end of the period, the leaf tissue of the bean cultivars was taken into liquid nitrogen and stored at − 80 °C. This research was carried out based on a completely randomized design with three replicates. Five plants from each replicate per treatment were selected and bulked. The bulked sample technique was used for molecular analysis in the research.

Isolation of total RNA, cDNA synthesis, and expression analysis

The Trizol® Reagent (Invitrogen Life Technologies, ABD) was used to isolate total RNA. The RNA quality was checked using a spectrophotometer before being photographed on a 1.5% agarose gel. The RevertAid RT Reverse Transcription Kit (Fermentas, ABD) was used to apply cDNA synthesis according to the manufacturer's protocol. Eleven *PvTCS* genes were preferred for qRT-PCR analysis based on RNAseq data. Table 1 contains information about primers designed for qRT-PCR. The qRT-PCR reactions were carried out on the RotorGene® Q Real-Time PCR System (Corbett Research, Qiagen GmbH, Germany) with the ABT SYBER Green mix (Cat No: Q03-02-01, Ankara, Turkey). The qRT-PCR reaction mixture was 20 µL, of which the cDNA was 200 ng, both forward and reverse primers were 0.4 µL, and the 2X ABT SYBER Green Mix was 10 µL, with the reaction volume being completed to 20 µL with double distilled water. The reaction was performed as follows: 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s, and 60 °C for 30 s. The *β-actin* gene from *P. vulgaris* was used as a housekeeping gene. qRT-PCR data were normalized using the $2^{-\Delta\Delta CT}$ method for relative quantification (Livak and Schmittgen 2001). GraphPad Prism 9 software (http://www.graphpad.com/) was used for statistical analyses based on the two-way ANOVA method and Dunnett's test at 0.05 significant

Table 1 The primer sequences used in qRT-PCR

Gene Name	Forward primer	Reverse primer
PvPHYA2	GTGAGTAAGAAGTTGGACGTAGAG	CAGATAGGCGCTGAATATGTAGT
PvHK2	CTTGACTTCCCAAGAGGGTTATT	CTCAGCTCCCTGTCGAATTATG
PvHK4	GTTCCATTTGATCTTCGTTCCATAC	CCCATGACAATATCCGGTACTTTA
PvHK6	CATGCTCTGGCTATCCTTGTATC	CAAATGCTGTACTCTCGGTATATTC
PvHK8	AGTAGAAGATGATTACGGTGAGATG	CTGATCTCGTGGGAAACAGTAG
PvHP5	AGAGTACTGCCTTGTGAAGAATAAA	CACTGAAACTCAGTTCCGTAGG
PvHP6	GAACAAGTGCCTGCTGATTTC	CTCACAGAAAGTCCTGAAGGTAG
PvRR12	CTGAAGAGCCTCCCACTATTTC	TACCCTGTCATTTCTGGCATAC
PvRR15	TGACAAGTGGTGGACAGAAC	GTAGCTCATAGCCTGTCATTCC
PvRR18	AAGTGACGGTGGTGGAAAG	GGCATGGAGTAGTCTGTCATTAT
PvRR28	GGAGGACGACCTGAATTTCTT	GCAACAAGCCTTGGATCATAAC
β-actin	TGAGCAAGGAGATTACAGCATTGG	CATACTCTGCCTTCGCAATCCAC

levels. The interaction effect for genotype x treatment was evaluated based on a simple main effect.

Results

Detection of *TCS* genes in bean genome

Current genome-wide analysis revealed the presence of *HK*, *HP*, and *RR* genes encoding TCS elements in the common bean genome. Except for Pv4, the *PvTCS* genes of the bean were found on Pv1, 2, 3, 5, 6, 7, 8, 9, 10, 11 chromosomes and scaffold_17. The start and stop positions of the *PvTCS* genes, as well as the molecular weights, amino acid lengths, instability indexes, and theoretical isoelectric points (pI) of the proteins, as calculated by the "ProtParam tool," have been listed in Table S1. PvHK2 had the most amino acids (1261), whereas PvHP8 had the fewest (87). Furthermore, the isoelectric points of *PvTCS* genes were determined to vary from acid to alkaline. PvRR11 had the lowest value (4.18) while PvPRR10 had the highest (9.15). A great majority of *PvTCS* genes are unstable.

Determination of gene structures, chromosomal locations, gene duplications, and conserved motifs of TCS elements in beans

Figure 1 presents chromosomal localizations and sizes of bean *TCS* gene family members. Figure 2A depicts information regarding the intron–exon numbers of *HK* gene family members. In *PvHK* genes, intron numbers vary between 1 and 12 and exon numbers vary between 2 and 13. *PvERS2, PvERS3, PvETR1,* and *PvETR2* genes had the lowest intron number, whereas *PvHK2*, *5*, and *6* genes had the greatest intron number. On the other hand, the *PvERS2, PvERS3, PvETR1,* and *PvETR2* genes had the fewest exons (2), whereas *PvHK2*, *5*, and *6* genes had the most exons (13). Figure 2B shows the intron–exon numbers of the bean *HP* gene family members as a result of structural analysis using Gene Structure Display Server v2.0. Among *PvHP* genes, intron numbers vary between 2 and 5 and exon numbers vary between 3 and 6. It was determined that the lowest intron number was in the *PvHP8* gene with 2, and the highest intron number was in the *PvHP1*, *2, 3, 5, 7,* and *9* genes. On the other hand, in terms of exon number, the lowest number of exons was detected in the *PvHP8* gene with 3, and the highest number of exons with 6 in *PvHP1*, *2, 3, 5, 7,* and *9* genes.

While the intron numbers of *PvRR* genes were between 1 and 10, their exon numbers varied between 2 and 11 (Fig. 2C). It was determined that the least number of introns was in *PvRR19, 24,* and *25* genes, and the highest number of introns was in *PvRR1* and *PvRR3* genes. The least number of exons were in *PvRR19*, *24,* and *25* genes with 2, and the highest number of exons were found in *PvRR1* and *PvRR3* genes with 11.

The rough time of duplication events was calculated using $T = Ks/2\lambda \times 10^{-6}$ Mya (million years ago) in terms of synonymous replacement rates (λ) in beans (Schmutz et al. 2014). The Ka/Ks ratio of *PvTCS* genes was found to be less than one in the calculated values. All duplicated gene pairs of beans *TCS* gene family members have undergone negative selection in the evolutionary process. *PvRR19–PvRR24 and PvETR1–PvETR2* gene pairs are tandemly duplicated genes. *PvHK1–PvHK7, PvHK2–PvHK5, PvHP1–PvHP5, PvHP1–PvHP6, PvHP3–PvHP8, PvHP3–PvHP9, PvHP4–PvHP10, PvHP5–PvHP6, PvHP8–PvHP9, PvPHYA1–PvPHYA2, PvPRR2–PvPRR6, PvPRR7–PvPRR9, PvPRR1–PvPRR16, PvPRR2–PvPRR4, PvPRR26–PvPRR28, PvPRR27–PvPRR8, PvPRR27–PvPRR18, PvPRR27–PvPRR5, PvPRR5–PvPRR8, PvPRR5–PvPRR18, PvPRR6–PvPRR21* and *PvPRR8–PvPRR18* gene pairs were found to be segmentally duplicated genes (Table S2).

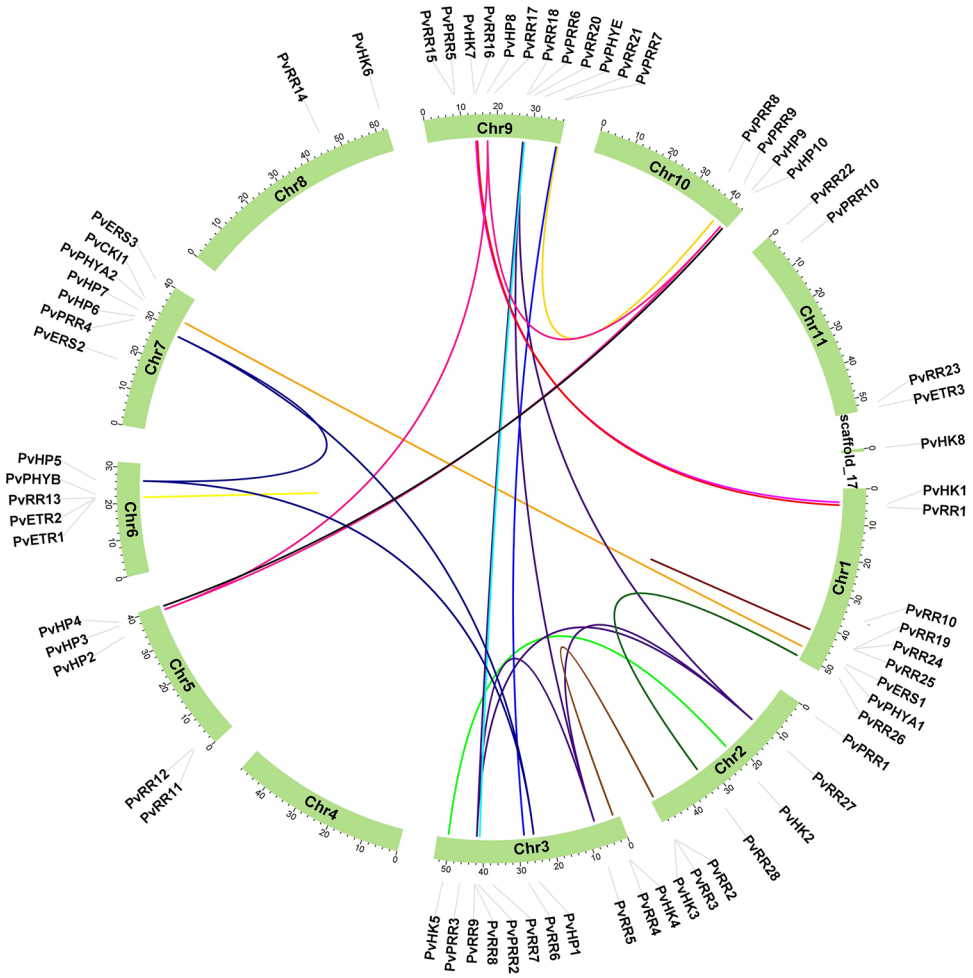


Fig. 1 Distribution of *PvTCS* genes in bean chromosomes. *The colored lines indicate segmentally and tandemly duplicated gene pairs. Solid lines indicate segmental duplication, while incomplete lines indicate tandem duplications

Ten conserved motifs were identified for each of the *PvTCS* gene family (*HK*, *HP*, and *RR*) members in the bean. Figure 3A–C, respectively, show possible conserved motif schemes for members of *PvHK*, *PvRR*, and *PvHP* gene families. According to the conserved motif analysis results,10 conserved motifs were determined per gene family and the amino acid numbers of the motifs ranged from 6 to 50. In the *PvHK* gene family members, PvPHYA1, PvPHYA2, and PvPHYE had the least motifs with 5 motifs while PvHK2 and PvCKI1 proteins had the highest motif with 8 motifs. HK-Motif-2, -4, and -7 were also found in all PvHK proteins. The HK-Motif-2 contains the HisKA domain. In the *PvHP* gene family members, the least motif (3 motifs) was found in the PvHP8 protein, and the highest number of motifs (8 motifs) was in the PvHP2 protein. In addition, HP-Motif-2 was found in the entire PvHP proteins and contained the HPt domain. HP-Motif-1 also contains the HPt domain and is located in all other PvHP proteins except PvHP2 and PvHP8. The best possible matches in the bean *TCS* genes and the domains in these sequences are given in Table S3. It was determined that the PvRR11 protein had the most minor motifs (2 motifs), while the PvRR6 and PvRR21 proteins had the most motifs (7 motifs). All PvRR proteins contain RR-Motif-1. Moreover, The RR-Motif-1, -3, and -4 motifs include the RESPONSE REGULATORY domain, that is, the RR domain. RR-Motif-3 was found in other PvRR proteins except for the PvRR11 protein, while the RR-Motif-4 was present in other PvRR proteins except for PvRR11 and PvRR23 proteins.

Phylogenetic analysis and sequence alignment

Phylogenetic analyses were performed according to the Neighbor-Joining (NJ) method with 1000 replicated bootstrap values. The protein sequences of the bean, soybean, and Arabidopsis TCS elements were aligned with the ClustalW program (Thompson et al. 1997). Phylogenetic trees were constructed by using the TCS protein sequences of bean, soybean, and Arabidopsis, and the phylogenetic trees of the *HK* (Fig. 4), *RR* (Fig. 5), and *HP* (Fig. 6) gene family members were divided into 6, 7, and 4 groups, respectively.

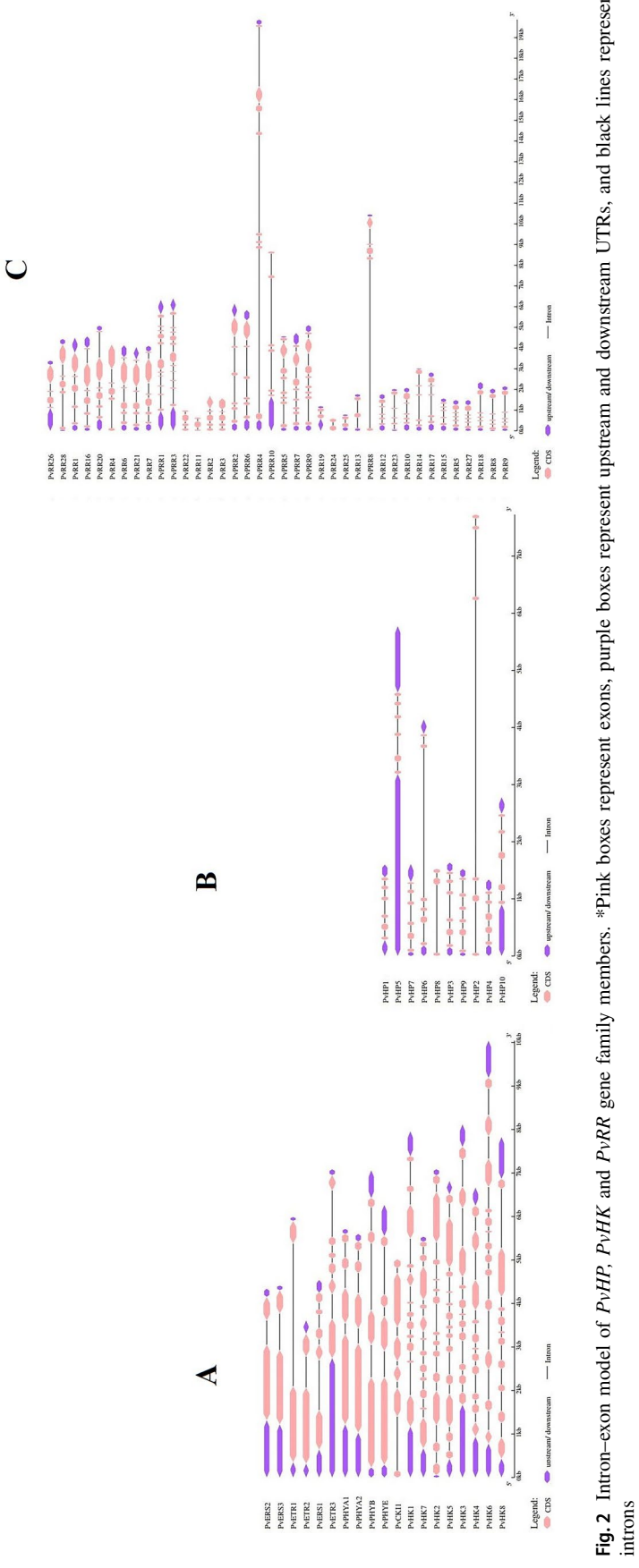


Fig. 2 Intron–exon model of *PvHP*, *PvHK* and *PvRR* gene family members. *Pink boxes represent exons, purple boxes represent upstream and downstream UTRs, and black lines represent introns

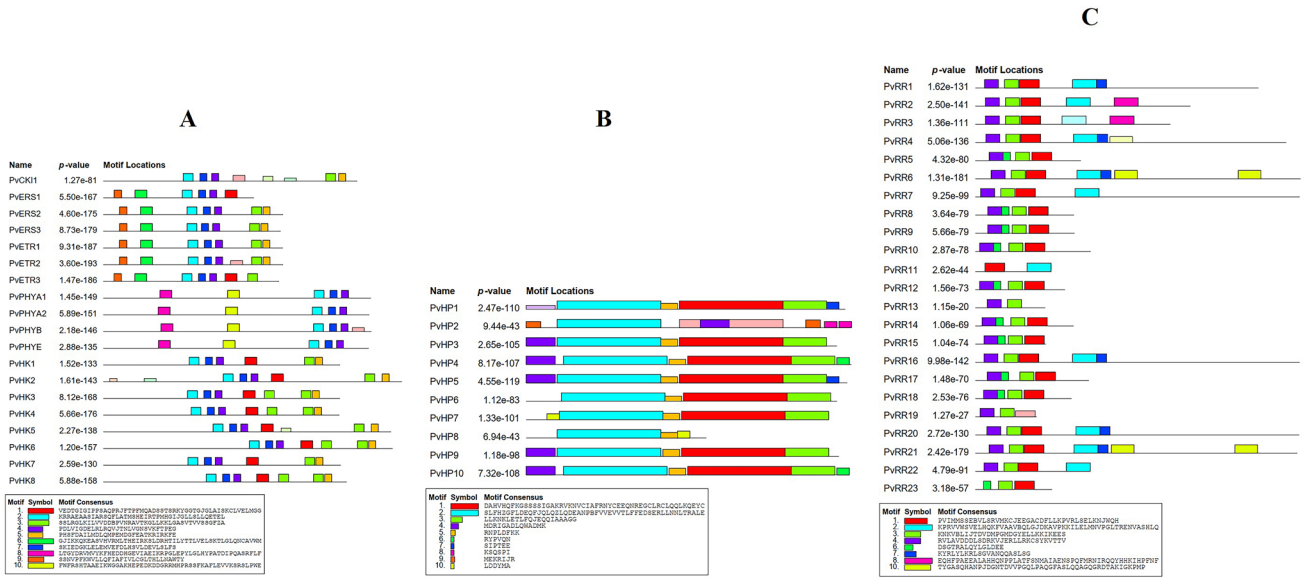


Fig. 3 Possible conserved motif scheme of *HK*, *RR*, and *HP* gene family members in bean and possible sequence data corresponding to colored box symbols

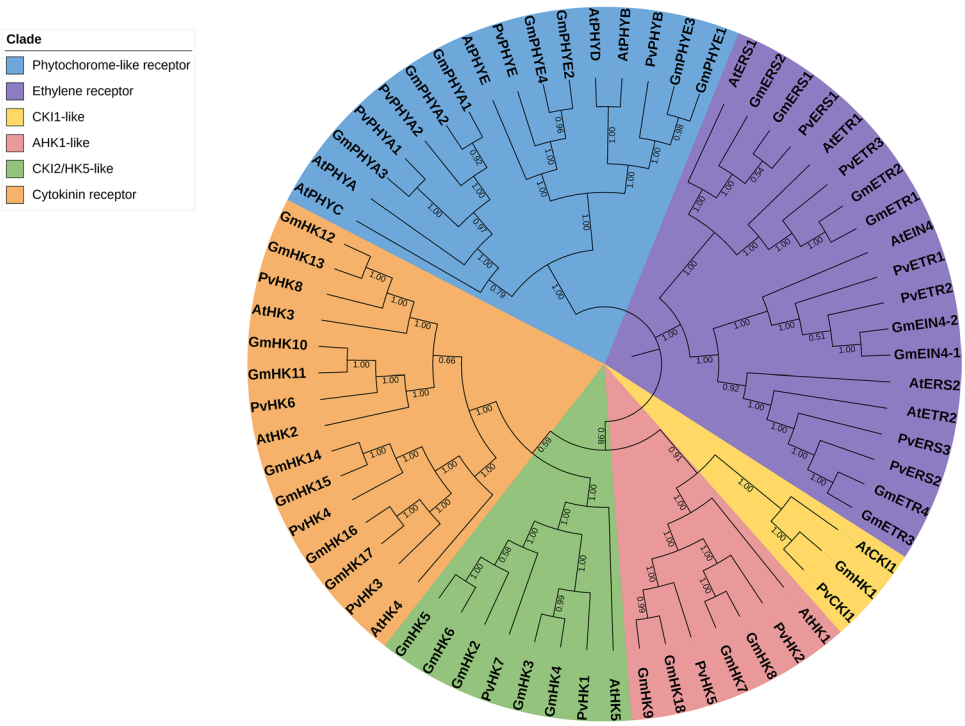


Fig. 4 Neighbor-joining phylogenetic tree of *P. vulgaris*, *G. max* and *A. thaliana HK* gene family members. The phylogenetic tree was created using the Mega v7 program and full-length amino acid sequences from soybean, Arabidopsis, and common bean. The tree was divided into 6 classes with a total of 68 proteins, including Phytochorome-like receptor group, Ethylene receptor group, CKI1-like group, AHK1-like group, CKI2/HK5-like group and Cytokinin receptor group. The numbers are bootstrap values based on 1000 replicates

Comparative mapping between beans and other species

Synteny analysis, one of the elements of comparative genomics, is essential for determining the evolutionary relationship between genomes. Figure 7 show synteny maps for bean with *G. max* and bean with *A. thaliana*, respectively. For *RR*, *HK*, and *HP*, 83, 31, and 26 syntenic relationships, respectively, were determined between the bean and *G. max*. In contrast, this number of synthetic associations between beans and Arabidopsis was determined as 36 for *RR* genes, 9 for *HK* genes, and 6 for *HP* genes.

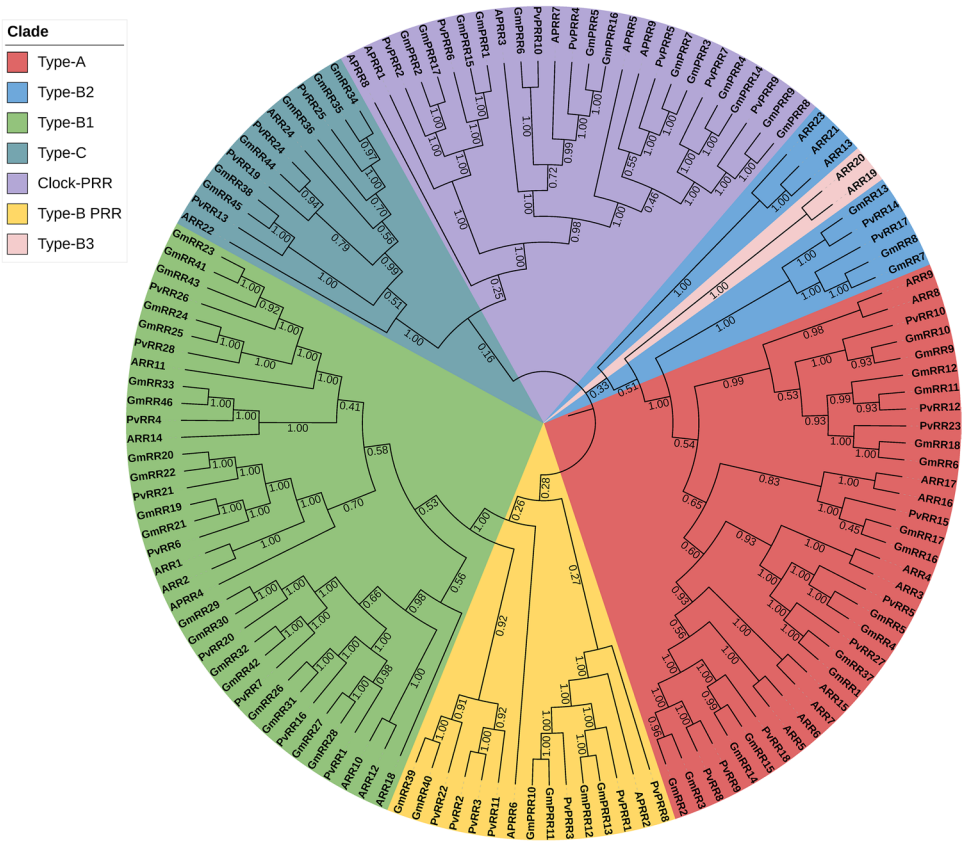


Fig. 5 Neighbor-joining phylogenetic tree of *P. vulgaris*, *G. max* and *A. thaliana RR* gene family members. The phylogenetic tree was created using the Mega v7 program and full-length amino acid sequences from soybean, Arabidopsis, and common bean. The tree was divided into 7 classes with a total of 135 proteins, including Type-A group, Type-B1 group, Type-B2 group, Type-B3, Type-C group, Clock-PRR group, and Type-B PRR group. The numbers are bootstrap values based on 1000 replicates

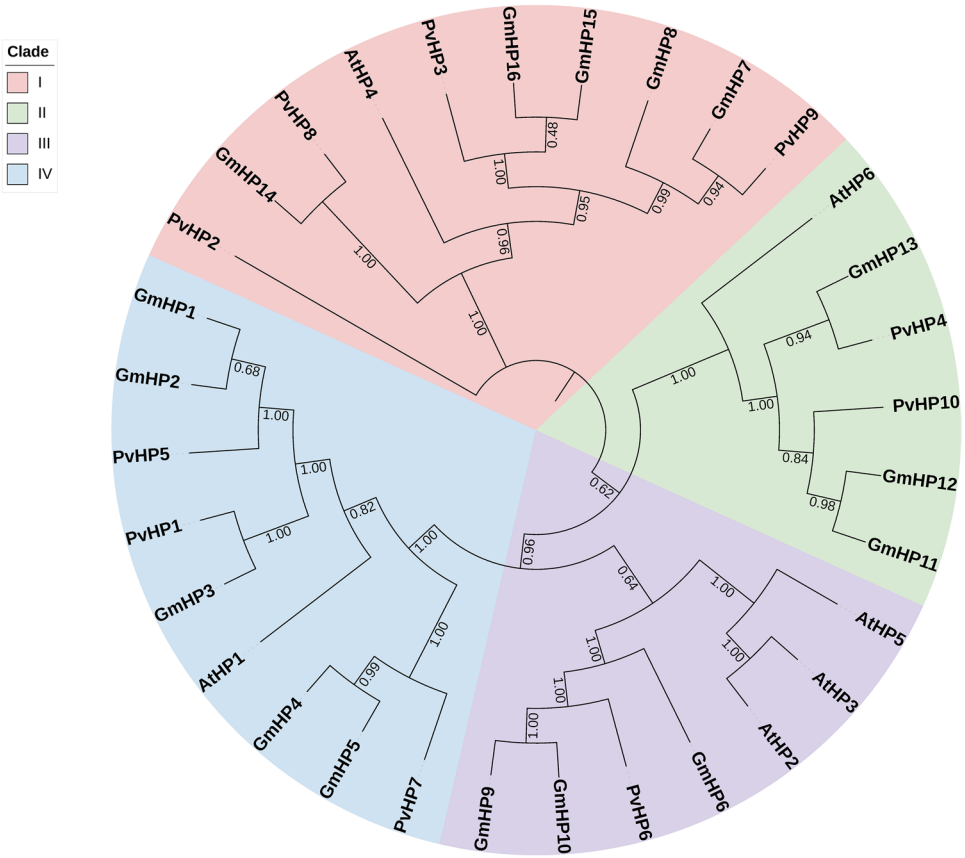


Fig. 6 Neighbor-joining phylogenetic tree of *P. vulgaris, G. max* and *A. thaliana HP* gene family members. The phylogenetic tree was created using the Mega v7 program and full-length amino acid sequences from soybean, Arabidopsis, and common bean. The tree was divided into 4 classes with 32 proteins, including Group I, Group II, Group III, and Group VI. The numbers are bootstrap values based on 1000 replicates

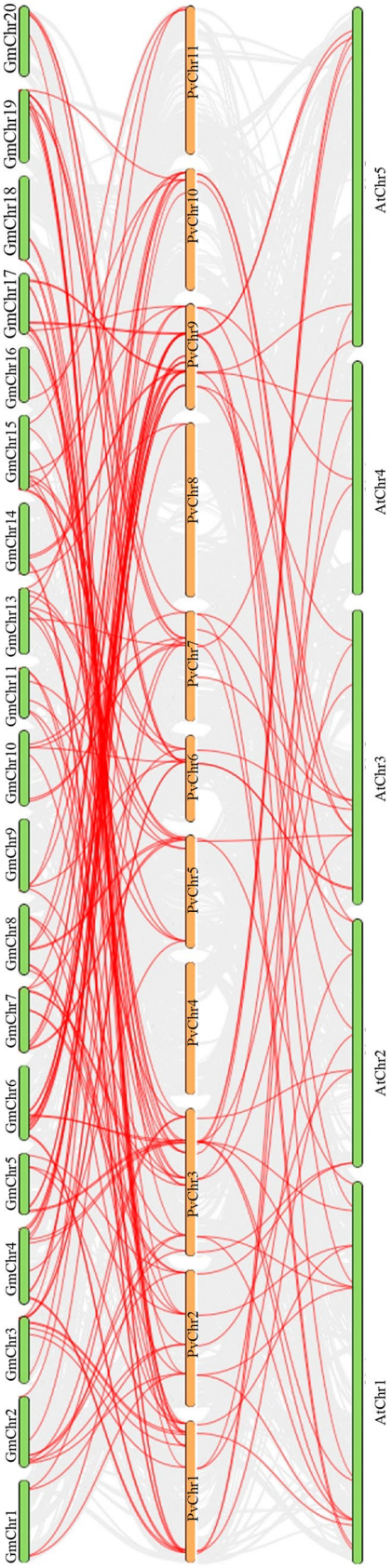


Fig. 7 TCS element synteny map for bean/*G. max* and bean/Arabidopsis. The upper green boxes represent the soybean chromosomes (GmChr), the middle orange boxes the bean chromosomes (PvChr), and the lower green boxes the Arabidopsis chromosomes (AtChr). The red lines show the orthology in the *PvTCS* and *GmTCS* genes and *PvTCS* and *AtTCS* genes

Intracellular localization and promoter analysis of the genes of the bean TCS elements

The intracellular localization of each gene of the bean TCS elements is given in Table S4. Moreover, 16 for *HK* genes, 15 for *HP* genes, and 14 for *RR* genes different cis-acting elements were detected in the promoter regions of the bean (Fig. 8).

Homology modeling of TCS proteins and protein–protein interactions in beans

Three-dimensional homology modeling of PvTCS proteins was visualized with the help of the Phyre2 database using TCS protein sequences (Kelley et al. 2015). The best 3D images were generated by analyzing the protein model reliability ratios, and 3D images of HP proteins were given in Table 2, RR proteins in Table 3, and HK proteins in Table 4. The confidence interval was taken as > 90% in the 3D homology modeling of proteins (Incili et al. 2023). Protein–protein interactions (PPI) represent many biological processes such as cell control, intercellular communication, and metabolic development (Braun and Gingras 2012). The STRING database was used to determine functional and physical interactions for ppi, and the data obtained were classified and integrated with the confidence score (Fig. S1).

Tissue-specific in silico gene expression analysis of *PvTCS* genes

The *PvTCS* gene family members' expression was analyzed using data from specific tissue libraries (RNAseq). When the expression of the *HK* genes in the bean leaves subjected to salt stress was analyzed, the expression of the *PvHK1, PvERS3,* and *PvHK2* genes decreased, while the expression of the *PvPHYA1, PvHK8,* and *PvHK6* genes increased as well as the expression of the *PvCKI1* gene did not change relative to the control. In response to drought stress, *PvHK2* gene expression decreased whereas *PvERS3* gene expression increased compared to the control. Other genes exhibited no significant variations (Fig. 9).

The expression levels of *PvHP7*, *PvHP4*, and *PvHP10* genes were reduced, while the expression of PvHP3 and *PvHP8* genes was raised in the digital gene expression of *HP* genes in salt stress. The expression levels of the other genes did not significantly change. In response to drought stress, the *PvHP3*, *PvHP8*, and *PvHP9* genes expression increased, while the *PvHP4*, *PvHP6*, and *PvHP7* genes expression decreased compared to the control. There were no important changes in how other genes were expressed (Fig. 9).

In salt stress, the expression levels of *PvPRR1*, *PvRR3*, *PvRR12*, *PvRR14*, *PvRR15*, *PvRR18*, *PvRR23*, *PvRR26*

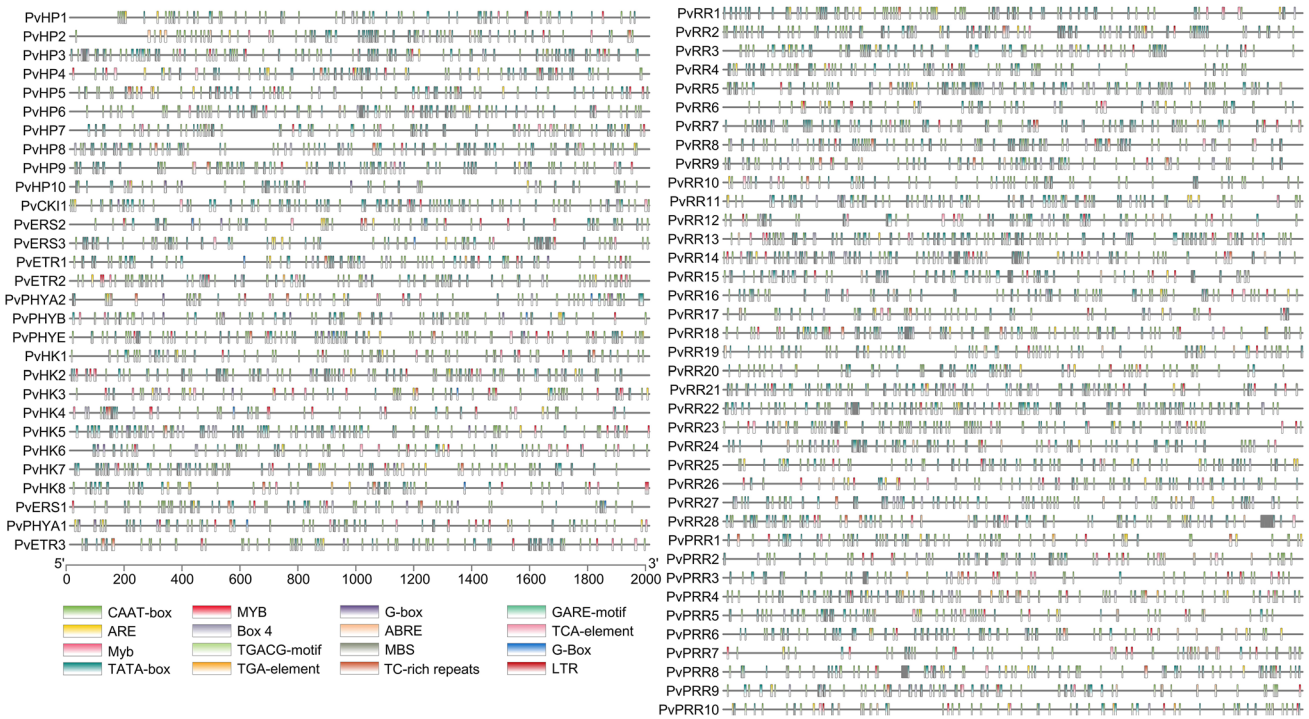
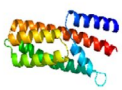
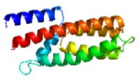
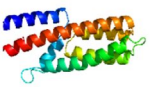
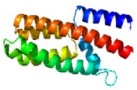
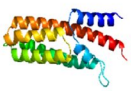
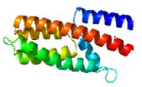
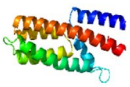
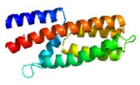
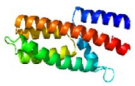


Fig. 8 Cis-acting elements in the promoter regions of *PvHK, PvHP,* and *PvRR* genes. This analysis was created in TBtools program by selecting cis-acting elements that function under various stress conditions

Table 2 3D images of bean HP proteins

				
PvHP1	PvHP2	PvHP3	PvHP4	PvHP5
				
PvHP6	PvHP7	PvHP8	PvHP9	PvHP10

and *PvRR28* genes decreased, the expressions of *PvRR7*, *PvPRR7,* and *PvPRR9* genes increased, and the expressions of the other genes were increased compared to the control. In drought stress, the expressions of *PvPRR1*, *PvPRR5*, *PvPRR8*, *PvRR3*, *PvRR10*, *PvRR12*, *PvRR15*, *PvRR16*, *PvRR18*, *PvRR22*, and *PvRR24* genes increased while decreasing the expressions of *PvPRR9*, *PvRR16*, *PvRR17*, and *PvRR28* genes (Fig. 9).

Expression profiling of *TCS* genes in bean

qRT-PCR primers for *PvHK2, PvHK4, PvHK6, PvHK8, PvPHYA, PvHP5, PvHP6, PvRR12, PvRR15*, *PvRR18*, and *PvRR28* genes were designed, and the expression levels of these genes in Elkoca-05 and Serra varieties treated with salt (0 and 150 mM NaCl), drought (0 and 20% PEG 6000), and melatonin (0 and 200 mM MEL) were determined by quantitative real-time PCR (Fig. 10).

Table 3 3D images of bean RR proteins

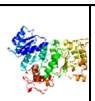
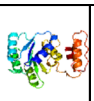
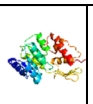
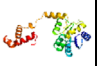
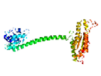
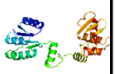
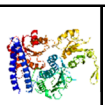
									
PvRR1	**PvRR2**	**PvRR3**	PvRR4	PvRR5	PvRR6	PvRR7	PvRR8	PvRR9	PvRR10
									
PvRR11	**PvRR12**	**PvRR13**	PvRR14	PvRR15	PvRR16	PvRR17	PvRR18	PvRR19	PvRR20
									
PvRR21	PvRR22	PvRR23	PvRR24	PvRR25	PvRR26	PvRR27	PvRR28		
									
PvPRR1	**PvPRR2**	**PvPRR3**	**PvPRR4**	**PvPRR5**	**PvPRR6**	**PvPRR7**	**PvPRR8**	**PvPRR9**	**PvPRR10**

Table 4 3D images of bean HK proteins

									
PvCKI1	**PvERS1**	**PvERS2**	**PvERS3**	**PvETR1**	**PvETR2**	**PvETR3**	**PvPHYA1**	**PvPHYA2**	**PvPHYB**
PvPHYE	PvHK1	PvHK2	PvHK3	PvHK4	PvHK5	PvHK6	PvHK7	PvHK8	

Drought stress and melatonin treatment altered the expression of some of the *PvTCS* genes in both cultivars (Fig. 10). When the effect of drought stress alone was evaluated, in Elkoca-05, a significant increase was observed in the expression of *PvHK2*, *PvHP5*, *PvHP6*, *PvRR12*, *PvRR18*, and *PvRR28* genes, while it happened a decrease in the expression of *PvHK4*, *PvHK6*, *PvHK8*, and *PvPYHA* genes. However, no change was observed in the expression of the *PvRR15* gene. In the Serra, on the other hand, A significant decrease was observed in the expression of *PvHK2*, *PvHK4*, *PvHK6*, *PvHK8*, *PvPYHA*, *PvRR15*, and *PvRR28* genes. No changes were observed in the expression of the other genes.

When the expression of genes studied in cultivars of melatonin application alone was evaluated, while a significant decrease was observed in the expression of *PvHK6*, *PvHK8,* and *PvRR15* genes in Elkoca-05, no change was observed in the expression of other genes. In Serra, there was a significant increase in the expression of *PvHK4*, *PvHK8*, *PvHP5*, *PvHP6*, *PvPYHA*, *PvRR15,* and *PvRR28* genes, while no change was detected in the expression of the other genes. When the combined effect of drought and Melatonin treatments was tested, a significant reduction in the expression of *PvHK2*, *PvPYHA*, and *PvRR15* genes was observed in the Elkoca-05. Still, no change in the expression of other genes was observed. On the other hand, while the expression

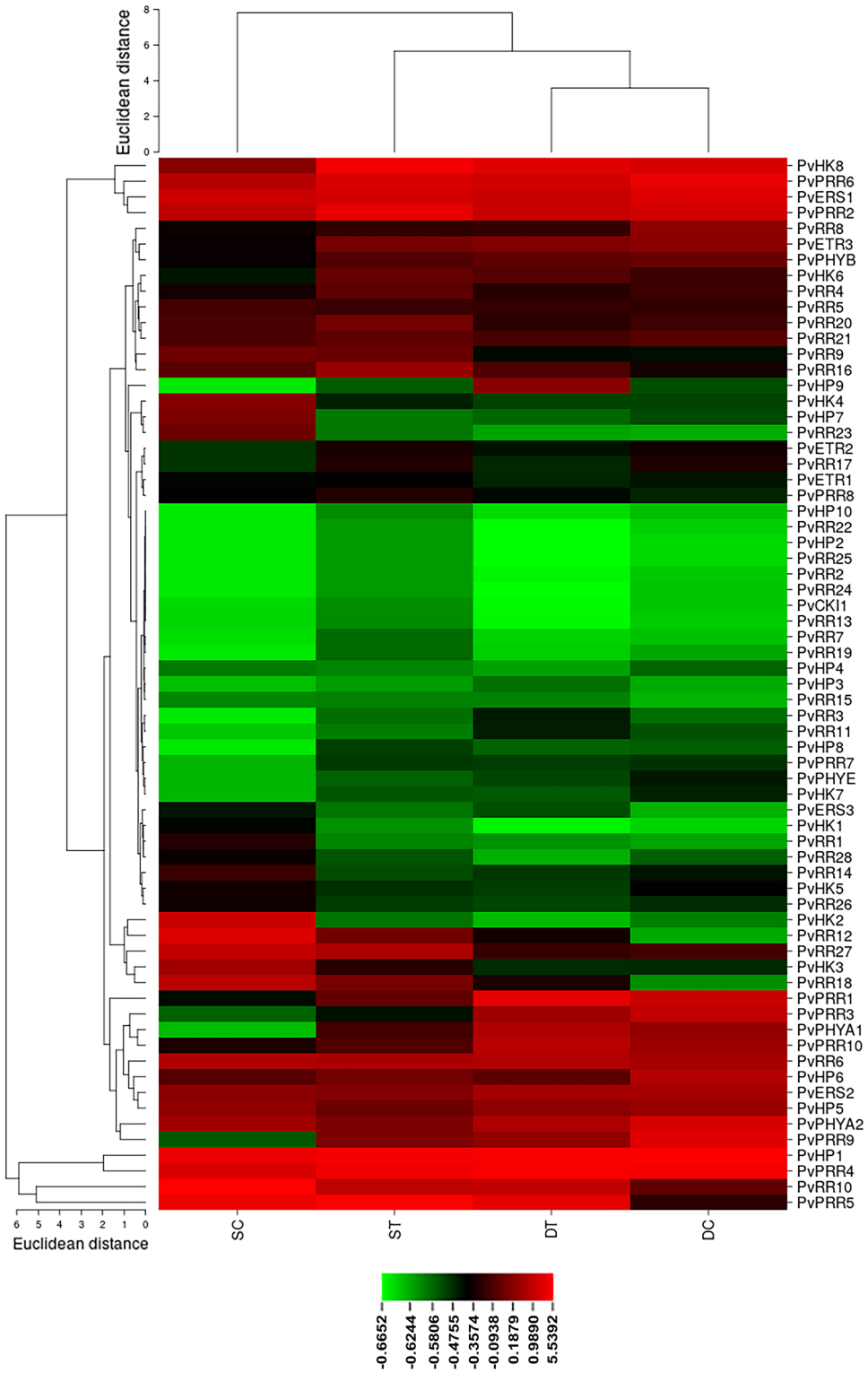


Fig. 9 In silico expression of *HK, HP,* and *RR* genes in bean leaves under drought and salt stress treatment. The expression values of each *PvTCS* gene identified in the study were obtained from RNA-Seq data, including salt-treatment (ST), salt-control (SC), drought-treatment (DT), and drought-control (DC)

of *PvHK2*, *PvHK8*, *PvPYHA*, and *PvRR28* genes decreased significantly in the Serra, the expression of other genes did not alter.

The salt stress and melatonin treatments altered the expression of some *PvTCS* genes in both cultivars (Fig. 11). When the effects of salt stress alone on the expression of some *PvTCS* genes in both varieties were evaluated, In Elkoca-05, there was a significant increase in the expression of *PvHK4*, *PvHK6*, *PvHK8*, and *PvHP5* genes, and a significant decrease in the expression of *PvPYHA*, and *PvRR12*

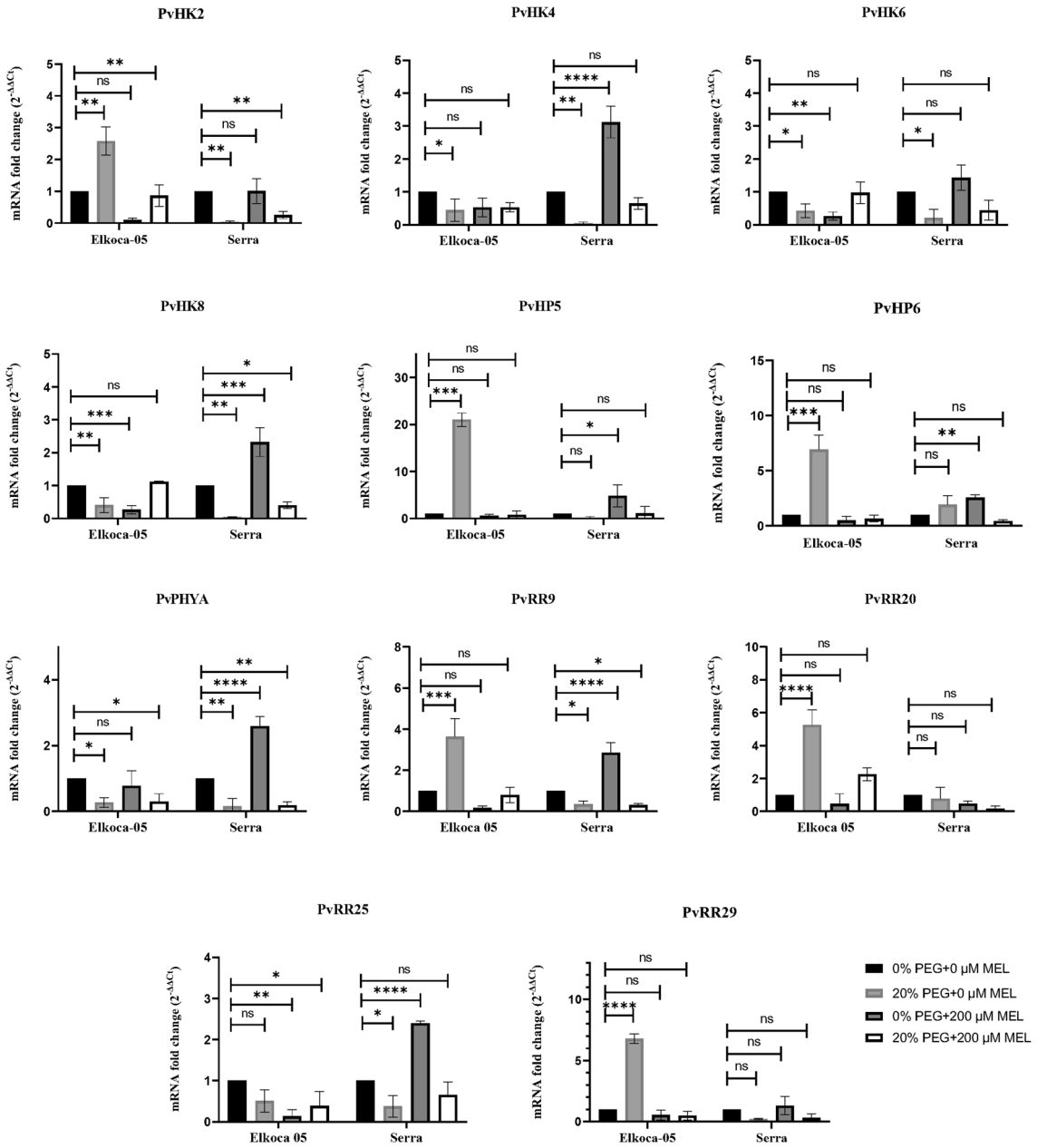


Fig. 10 Expression of *PvTCS* genes in Elkoca-05 and Serra cultivars under drought stress. qRT-PCR was used to analyze the expression patterns of eleven *PvTCS* genes under salt, drought, and melatonin treatments in Elkoca-05 and Serra. Real-time PCR was used to calculate relative transcript levels using β-Actin as an internal standard. This study was conducted using an all-random design with three replicates. The scale of the relative expression levels is shown by the Y-axes. Abiotic stress and melatonin treatments were displayed for each gene of Elkoca-05 and Serra on X-axes. The bars indicate means ± SD (n = 3). ****, ***, **, and * indicate significant at $p < 0.0001$, $p < 0.001$, $p < 0.01$ and $p < 0.05$ between means, respectively based Dunnet test whereas ns indicates non-significant $p > 0.05$

genes. Moreover, no changes were observed in the expression of other genes. In the Serra, a significant increase in the expression of *PvHK2*, *PvHK6*, *PvHK8*, *PvHP5*, *PvPYHA*, *PvRR15*, and *PvRR28* genes was observed while there were no changes in the expression of other genes.

When evaluating the expression of some *PvTCS* genes in cultivars under melatonin treatment alone, there was a change in that it was reduced expression of *PvPYHA*, *PvRR12*, and *PvRR28* genes in the Elkoca 05. On the other hand, in the Serra, there was increased expression of *PvPYHA*, *PvRR12*, *PvRR15,* and *PvRR18* genes.

The combined effect of salt stress and melatonin treatment was evaluated, in the Elkoca-05, *PvHK2*, *PvHK8*,

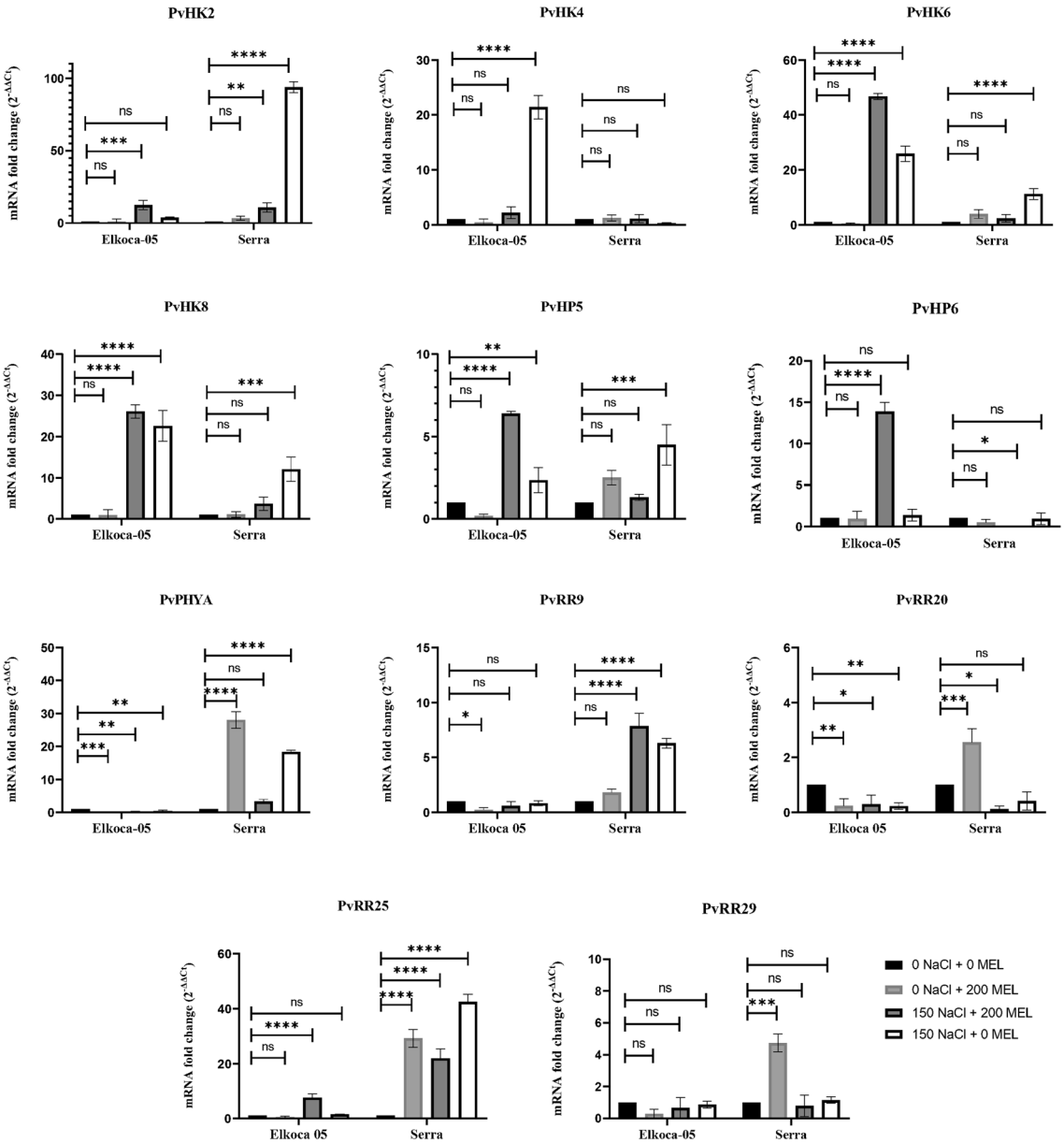


Fig. 11 Expression of *PvTCS* genes in Elkoca-05 and Serra cultivars under salt stress. qRT-PCR was used to analyze the expression patterns of eleven *PvTCS* genes under salt, drought, and melatonin treatments in Elkoca-05 and Serra. Real-time PCR was used to calculate relative transcript levels using β-Actin as an internal standard. This study was conducted using an all-random design with three replicates. The scale of the relative expression levels is shown by the Y-axes. Abiotic stress and melatonin treatments were displayed for each gene of Elkoca-05 and Serra on X-axes. The bars indicate means ± SD (n = 3). ****, ***, **, and * indicate significant at $p < 0.0001$, $p < 0.001$, $p < 0.01$, and $p < 0.05$ between means, respectively based Dunnet test whereas ns indicates non-significant $p > 0.05$

PvHP5, *PvHP6*, and *PvRR15* genes showed a significant increase in expression, while there was a significant decrease in the expression of *PvPHYA*, and *PvRR12* genes. On the other hand, in the Serra, a significant increase was observed in the expression of *PvHK2*, *PvRR15*, and *PvRR28* genes, while a significant decrease was observed in the expression of *PvHP6*, and *PvRR12* genes.

Discussion

The *TCS* gene family is one of the evolutionary highly conserved signal elements encoded by multiple gene families found in all organisms. Previous research has indicated that this signaling network plays a significant role in various plant processes, including plant growth

and development, as well as a variety of stress responses (Lohrmann and Harter 2002). In contrast, even though members of the *TCS* gene family have been examined in several plant species, no members of this signaling network have been identified in beans. As a result of a genome-wide examination of *TCS* genes in beans, 67 candidate *PvTCS* genes, including 10 *PvHP* genes, 38 *PvRR* genes, and 19 *PvHK* genes, were identified in this study. As a result of genome-wide analysis in numerous plants, numerous putative *TCS* genes were identified, including 55 in Arabidopsis (Hwang et al. 2002), 80 in banana (Dhar et al. 2019), 49 in poplar (Singh and Kumar 2012), 83 in soybean (Mochida et al. 2010), 51 in melon (Liu et al. 2020), 51 in chickpeas (Ahmad et al. 2020), 65 in tomato (He et al. 2016a), and 46 in cucumber (He et al. 2016b).

Segmental and tandem duplications are duplication events that contribute to the development of living things in the evolutionary process. The study for *TCS* genes determined that both segmental duplication and tandem duplication occurred in the evolutionary process in beans. Similarly, in a study on tomato, it was determined that both duplication types in *TCS* genes contributed to the evolution process of tomato (He et al. 2016a). In contrast to tomatoes and beans, only segmental duplication was the type of duplication that was effective in the evolutionary process of Arabidopsis, bock choy, and soybean (Schaller et al. 2008; Mochida et al. 2010; Liu et al. 2014). The Ka/Ks ratio determines the selection pressure. During their evolution, duplicated gene pairs may undergo gain-of-function, loss-of-function, and neofunctionalization (Lynch and Conery 2000). Nonsynonymous substitution value (Ka), synonymous substitution value (Ks), and Ka/Ks ratios are often used to help understand the direction of evolution and the selective power in the coding sequence (Li et al. 2009). These algorithms employ distinct models of replacement or mutation based on different assumptions that consider diverse sequence characteristics and permit us to create a variety of evolutionary process predictions (Muse 1996). Ka/Ks > 1 indicates accelerated evolution with positive selection, Ka/Ks = 1 indicates neutral selection, and Ka/Ks < 1 indicates functional restriction due to purifying (negative) selection (İlhan 2018; Kasapoğlu et al. 2020; Kizilkaya et al. 2020).

It has been determined that the Ka/Ks ratio of all *TCS* duplicate gene pairs in beans is less than 1 and exposed to negative, that is, purifying selection pressure. Moreover, in the duplicate gene pairs identified in melon and tomato, purifying selection pressure occurred as in beans. However, of the duplicate gene pairs detected in tomato, only the *SlRR18*/*SlRR26* duplicate gene pair had a Ka/Ks ratio greater than 1 and was subject to positive selection (He et al. 2016b; Liu et al. 2020).

As a result of the phylogenetic analysis, the phylogenetic tree of the *HK* gene family members for bean, *G. max*, and Arabidopsis was divided into 6 groups: Phytochrome-like receptor, Ethylene receptor, CKI1-like, AHK1-like, CKI1/HK5-like, and Cytokinin receptor. As a result of the study, *HK* gene family members contain HisKA, HATPase, Rec (receiver), CHASE, PAS (Per-ARNT-Sim), PHY (phytochrome), and GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA) domains in different combinations. All *HK* gene family members contain the HisKA domain. *HK* genes consist of three subfamilies cytokinin receptors, ethylene receptors (ETR and ERS), and phytochromes (PHY) (Hwang et al. 2002). Some *PvHK* genes (PvHK3, -4, -6, and -8) have additional CHASE domains. The CHASE domain is an important region for the recognition and binding of cytokinin, and proteins with this domain have been shown to be involved in cytokinin sensing and signaling (Dhar et al. 2019). There is an additional PAS domain to other domains in the *PvPHY* genes. The PAS domain is found in many signaling proteins in archaea, bacteria, and eukaryotes and functions as a signal sensor domain (Repik et al. 2000). While there is no CHASE domain in the structure of *PETR*, *PvERS,* and *PvPHY* genes, there is an additional GAF domain. The GAF domain binds molecules such as cAMP and cGMP (Chang and Shockey 1999; Karniol et al. 2005).

The phylogenetic tree of the *RR* gene family members of beans, *G. max,* and Arabidopsis is divided into 7 groups as Type-A, Type-B2, Type-B1, Type-C, Clock-*PRR*, Type-B *PRR,* and Type-B3. The *RR* gene family is separated into three subfamilies based on conserved domains. Type A *RR*s have a conserved D residue and an acceptor domain with a long C-terminal extension. Type B *RR*s contain a conserved Rec domain and a DNA binding domain (Myb). C-type *RR*s have the same domain structure as A-type *RR*s, except for the C-terminal extension. Another class of *RR*s, known as pseudo-regulators (*PRR*), contain a CCT motif and a conserved Rec domain at the C-terminus (Ahmad et al. 2020). The CCT motif at the C-terminus of *PRR*s is critical in regulating circadian rhythms (Más 2008; Tsai et al. 2012). Plant-specific type-B *RR*s are produced by combining *RR* and Myb domains (Riaño-Pachón et al. 2008).

The phylogenetic tree of the *HP* gene family members of the bean, *G. max*, and Arabidopsis were divided into 4 groups I, II, III, and VI. *TCS* gene family members also contain a His-containing phosphotransfer (HPt) domain that functions as a signaling module that binds to *RR*s (Schaller et al. 2008). *HP* gene family members transfer a phosphate group from the Rec domain of *HK* genes to the Rec domain of *RR* genes (Hwang et al. 2002). It contains a highly conserved xHQxKGSSxS motif. However, some *HP* genes lack the conserved histidine residue and are in a group known as His-containing phosphotransfer proteins. This group cannot function as phosphor transmitter proteins and phosphorylated *HP* genes regulate Cytokinin signaling by inhibiting phosphorylation in *RR* genes (Dhar et al. 2019). The

phylogenetic analysis performed in this study was based on the classifications made in Arabidopsis (Hwang et al. 2002). The same grouping types are also seen in the phylogenetic analyses performed on *Cucumis melo* and *Cicer arietinum*. However, in the study by Mochida et al. (2010), it was concluded that there is no phytochrome-like receptor group in the *HK* gene family in the *G. max* phylogenetic tree. There is also no Type C group in the *RR* gene family. It was determined by new examinations of the development and regeneration of the *G. max* genome that these two families contain individuals from these groupings (Le et al. 2011).

Due to the bean's limited genetic and genomic resources, it is crucial to compare it to other plant species. Moreover, the knowledge gained from genes from closely related species is also crucial for establishing the biological functions of their counterparts in beans (Melotto et al. 2011). In the comparative mapping performed between beans and other species, 140 syntenic relationships were detected in the syntenic analysis performed with *G. max*, while a total of 51 syntenic relationships were found in Arabidopsis. Almost all the *TCS* genes from *G. max* and bean were orthologous, indicating that they may have a closer evolutionary link and may have evolved from a common ancestor, consistent with prior findings (Schmutz et al. 2014).

Bean *TCS* genes were found at 17 locations in the cell, mainly in the nucleus. *TCS* gene family members are engaged in various biological functions, from stress response to circadian rhythm regulation. *TCS* genes, distributed almost everywhere in the cell, can affect the total cellular machinery either alone or in combination (Mochida et al. 2010). This control is achieved through cis-regulators in the promoter region. Cis elements activated by various stimuli trigger the expression of many genes. Cis-acting elements trigger other genes related to the maturation or stress (biotic and abiotic) of *TCS* genes. Many cis-acting elements have been discovered, including the Myb element, which is related to drought and salinity, the MBS element, which is connected to Myb, the gibberellin-sensitive element (GARE-motif), and the abscisic acid-sensitive element (ABRE) (Yamaguchi-Shinozaki and Shinozaki 2005). Type B *RR* genes containing Myb binding sites regulate the expression of some type A *RR* genes (Taniguchi et al. 2007). The cis-acting element analysis discovered regulatory elements, including hormone-sensitive and stress-responsive elements. Of the 67 *TCS* genes, 53 have the ABRE element, 59 have the ARE element, and 18 have the GARE-motif element. In addition, the light-sensitive element Box 4 is found in the 63 *TCS* gene.

Three-dimensional prediction algorithms of proteins are of great practical value, especially as they enable efficient exploration of sequence space in design applications (Huang et al. 2020). The 3D structure of PvTCS proteins was predicted using homology modeling with a modeling confidence level > 90% (Table 2, 3, and 4). It was determined that all the examined PvHP proteins have alpha helix structures. Most PvRR and PvHK proteins, on the other hand, have several beta-sheet structures in addition to the dominating alpha helix form.

Protein–protein interactions (PPI) are essential mediators in biological processes (Liddington 2004). The three-dimensional arrangement and dynamics of interacting proteins govern most interactions. These interactions are addressed under two sections: permanent and transitory (Nooren and Thornton 2003; Keskin et al. 2008). Some protein–protein interactions are specific to a pair of proteins, whereas others are promiscuous and interact with a wide range of proteins (Bryant et al. 2022). As a result of the PPI analysis of PvHK proteins, it was determined that PvHK proteins interacted mostly with PvHP proteins and cGMP-dependent protein kinase proteins. It was determined that PvHP proteins were mostly in interaction with PvHK and PvRR proteins while PvRR proteins interacted mostly with homeobox proteins, actin-dependent regulatory proteins, and histone-acyl transferase proteins.

MicroRNAs (miRNAs) are a class of small non-coding ribonucleic acids (RNAs) that are present in both animal and plant organisms that negatively regulate gene expression (Hwang et al. 2011; Petrov et al. 2019). The mature miRNAs that are conserved across plants typically consist of 20–22 nucleotides (nt) and originate from stem-loop regions of roughly 70 nt RNA precursors through the action of Dicer-like enzymes, which belong to the RNase III (Jing et al. 2022; Zhao et al. 2023). As a result of microRNA analysis, mir172 and mir390 target HK gene family members, mir164 and mir156 target HP gene family members, and mir166, mir171, and mir395 target RR gene family members (Table S5). The role of miR156, miR164, miR166, miR171, miR172, miR390, and miR395 under stress conditions has been reported in different plant species (Akdoğan et al. 2016; Singroha et al. 2021; Bakhski and Fard 2023; Ghorbanzadeh et al. 2023).

The effects of salt, drought, and melatonin on the expression of *TCS* genes were examined using in silico and in vitro gene expression analysis. As a result of melatonin application alone, the expression of some genes in the Elkoca-05 cultivar was down-regulated, while these genes were up-regulated in the Serra cultivar. Endogenous melatonin either suppresses or induces the expression of genes encoding important enzymes and transcription factors involved in defense (Weeda et al. 2014). Drought stress caused a decrease in the expression of almost all genes in the Serra cultivar. In the Elkoca-05 cultivar, on the other hand, differences were determined in terms of increase and decrease in the expression of genes. The most significant rise occurred in *PvHP5* (about 21-fold), and *PvHP6* (about sevenfold) genes in Elkoca-05 cultivar.

Under salt stress, there was a rise in the expression of all genes in the Serra genotype while a decrease in the expression of the *PvPHYA* (about tenfold), and *PvRR20* (about fivefold) genes was observed in the Elkoca-05 cultivar. In the application of melatonin against drought stress, an important decrease in expression of *PvHK2* (about fivefold) and *PvPYHA* (about fivefold) genes was detected in both cultivars. On the other hand, a significant rise in the expression of genes in both cultivars was detected in the application of melatonin against salt stress. It was shown that phytochrome-defective tobacco mutants, especially for phyA and phyAB were more tolerant to salt stress (Yang et al. 2018). Differences in *PHYA* expression in beans may be due to genotypic differences. In *A. thaliana*, the ortholog of *PvHK2*, *AHK1* functions in abscisic acid (ABA) signaling and as an osmosensor (Kiba et al. 2004; Pils and Heyl 2009). Accordingly, increased expression of *PvHK2* is highly correlated with its potential role in drought stress and osmosis sensing. Kumar and Verslues (2015) found that the mutants in Arabidopsis exhibit specific *AHK2* and *AHK3*-related traits that are also associated with cytokinin signaling for proline buildup and stimulation of *NCED3* and *P5CS1* gene expression under low water potential circumstances.

The expression of cytokinin receptor genes (*PvHK4*, *PvHK6*, and *PvHK8*) in Serra and Elkoca-05 cultivars was reduced under both stress conditions. In contrast to beans, in rice, drought stress activated *HK* genes (*HK5* and *HK3*) homologous to Arabidopsis CK receptor genes (*AHK2*, *AHK3,* and *CRE1*) while *HK6* homologous to *CKI2* was suppressed when exposed to drought stress (Pan et al. 2009). Similarly, soybean *HK* genes (*GmHK10-17*) homologous to Arabidopsis CK receptor genes (*AHK2*, *AHK3,* and *CRE1*), such as *GmHK12*, were induced under dehydration stress while *GmHK11* was suppressed (Mochida et al. 2010). Accordingly, it was understood that the genes in the same groups of the phylogenetic tree did not show similar response patterns to abiotic stresses.

Drought stress generally caused a rise in the expression of *PvRR* genes. *PvRR12*, *PvRR15*, and *PvRR18* are Type-A *RR*s and are among the genes whose expression was evaluated in this study while *PvRR28* is a Type-B *RR*. In Arabidopsis, drought enhanced the expression of a subset of Type-A *RR* genes, *ARR5*, *ARR7*, and *ARR15*, whereas in rice, drought stress inhibited the expression of virtually all genes. Type B *RR*s do not directly but indirectly participate in cytokinin signaling during the plant life cycle (Ishida et al. 2008). Moreover, type-B *RR*s regulate the transcription of type-A *RR*s (Cannon et al. 2004; Garg et al. 2015). It has also been demonstrated that Arabidopsis B-type *RR*s are involved in drought stress response (Wohlbach et al. 2008). However, comprehensive functional characterization is required to clarify the overall significance of *TCS* genes in the cell.

Conclusions

As a result of in silico analyses of the bean genome, 67 *PvTCS* genes including 10 *PvHP*, 38 *PvRR,* and 19 *PvHK*, located on 9 different chromosomes and scaffold_17 of the bean, were determined. Moreover, this gene family member classification was contributed by exon and intron structure, conserved motifs and domains, and phylogeny.

Phylogenetic analysis results show the closest relationships between beans, Arabidopsis, and soybeans. Gene duplication events contributed to the evolution of *PvTCS* gene family members in the evolutionary process, and it was determined by Ka/Ks analysis that all of these genes were subjected to negative selection in the evolutionary process. The expression changes of *TCS* genes in bean leaves under salt and drought stress and melatonin treatment of the bean plant were shown by gene expression analysis. Exogenous treatment of melatonin can improve the resistance against a series of stressors drought and salinity which is an answer to the question of how gene expression changes are regulated under stress. This study has revealed that the expression of *TCS* genes, which are effective in many reactions such as plant growth and development, response to stress, and signal transduction, differ under stress conditions. The results of this study will shed light on functional and genomic studies as well as on metabolic and physiological events such as plant growth and development and signal transduction in salt and drought stress in beans.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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