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RESEARCH ARTICLE

Genome-wide Analysis and Functional Identification of *KCS* Gene Family under Drought and Salt Stresses in *Phaseolus vulgaris* L

Ceren Yılmaz[1] • Merve Yüce[2] • Ahmed Sidar Aygören[1] • Ayşe Gül Kasapoğlu[1] • Selman Muslu[1] • Murat Turan[1] • Emre İlhan[1] • Murat Aydın[3] • Ertan Yıldırım[2]✉

[1]Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum/Türkiye
[2]Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Türkiye
[3]Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum/Türkiye

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ABSTRACT

β-ketoacyl-CoA synthase (KCS) is an important enzyme that catalyzes the biosynthesis of very-long-chain fatty acids (VLCFAs). In this study, the genome-wide analysis and functional characterization of the *KCS* gene family members in common bean (*Phaseolus vulgaris* L.) plants were conducted, and the response of the identified gene family to abiotic stresses was evaluated. In this study, 19 *KCS* genes were identified and characterized in the *P. vulgaris* genome. The molecular weights of these KCS proteins ranged from 49.14 kDa to 60.57 kDa, their amino acid lengths varied from 437 to 534, and their pI values ranged from 8.81 to 9.47, indicating a basic nature. Segmental and tandem duplications were observed in the *Pvul-KCS* gene family. Phylogenetic analysis revealed that *Pvul-KCS* proteins clustered into three main groups with *Arabidopsis thaliana* and *Glycine max* species. Comparative mapping analysis was also conducted with *A. thaliana* and *G. max*. Expression profile comparisons indicated that these genes had different expression levels in common bean varieties and played a role in the plant's response to biotic and abiotic stresses. This study provides important insights into the biological functions of *KCS* genes in *Phaseolus vulgaris* and offers valuable information for improving drought and salt stress tolerance in common beans.

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1. Introduction

Recent environmental developments, such as unstable rainfall patterns, temperature extremes, and salinity, have been linked to variations in soil composition (Verslues et al., 2006). The need for crops to maintain or increase crop yields when faced with unfavorable environmental conditions, such as drought and high salinity, necessitates genetic improvement (Araus et al., 2008) or the use of precursors that interact with these crops and promote plant growth, such as bacteria, hormones, and vitamins (Glick, 2012). From the earliest phases of plant life, the two most common abiotic stressors-salt and drought-effect agricultural yield and production. Salt and drought stress have a negative impact on both the quality and yield of plants (Maggio et al., 2005). Water must be provided to plants in the best possible quantity and quality because it is essential to their effective growth. Extended periods of drought

✉Correspondence
E-mail address: ertanyil@atauni.edu.tr

and excessive salinity can have long-lasting harmful effects on plants, affecting their root and stem growth and resulting in a reduction in the quantity and width of their leaves. Additionally, a halt in plant development may result from cells' reduced water capacity. The effects of salinity and drought stress on photosynthesis, metabolism, and plant growth have been shown in several research (Liu et al., 2016; Ors et al., 2016; Sahin et al., 2018; Ekinci et al., 2020).

The cuticle wax layer, which is made up of long-chain hydrocarbon compounds such alkanes, aldehydes, primary and secondary alcohols, ketones, which are esters, and various other related substances, acts as a barrier to protection for plants when they are subjected to a variety of abiotic challenges (X. Wang et al., 2017). Plant cuticle wax has a very intricate chemical makeup. In the outer cells of the epidermal layer, plant epidermal waxes are synthesized, and transported. The production and release pathways for these waxes involve several distinct organelles and enzymes working in unity (Bernard & Joubès, 2013). All plant species that grow on land have cuticular wax covering their aerial portions, which helps to prevent bacterial and fungal invasion as well as non-stroma transpiration. It coats the fruit. In the growth of fruits and post-harvest storage, it defends against stress from both abiotic and biotic sources by lowering fruit water loss, changing fruit luster, and improving fruit storage quality.

The enzyme that restricts the rate of the synthesis of very long-chain fatty acids (VLCFAs), known as KCS (β-ketoacyl-CoA synthase), provides components for the biosynthesis of cuticular wax (H. Yang et al., 2021). Animals, microbial organisms, and plants all use fatty acids that contain greater than eighteen carbon molecules as biological building blocks for a variety of molecules. For instance, in plants, the epidermis is the primary location of VLCFA synthesis. Here, they play a role in the process of biosynthesis of cuticular waxes. In many seed oils, triacylglycerols are primarily composed of VLCFAs. The *KCS* genes regulate epidermal wax's volume and composition. At every stage of plant growth and development, they actively take part in biochemical and physiological processes that also help plants adapt to stress. VLCFAs are also the main component of triacylglycerols in various plant oils (Ghanevati & Jaworski, 2001). The deletion mutant of the fatty acid elongation gene (FAE1/KCS18) in *Arabidopsis* results in a significant decrease in VLCFA content (Tong et al., 2021). This gene is essential for erucic acid biosynthesis and is involved in the production of VLCFAs in seeds (Kunst et al., 1992). One of the most significant edible legumes in the world is arguably the common bean (*Phaseolus vulgaris* L.), with a global production estimated at 35.5 million hectares in 2020 (http://faostat.fao.org/). Common beans are an important source of the daily requirement for protein in many countries, particularly in Latin America, Africa, and parts of Asia. In North America and Europe, common beans constitute a significant vegetable and legume crop economically. African nations consume considerable amounts of common beans; for instance, per capita common bean consumption in Rwanda, Kenya, and Uganda varies from 50 to 60 kg per year (Broughton et al., 2003; Buruchara et al., 2011). In terms of amino acid content and carbohydrates, vitamins (including A, C, and folate), and biologically significant minerals like Mg, Cu, and Zn, common beans are very nutrient-dense (Broughton et al., 2003; Blair, 2013). Furthermore, through symbiotic nitrogen fixation (SNF), common beans contribute to better soil and environmental health.

One of the key legumes for direct consumption, common beans encounter numerous difficulties as a crop. In their many agroecological contexts, common beans, domesticated from wild ancestors occupying a relatively small ecological niche, are subject to several kinds of stress related restrictions. Abiotic challenges include drought, salinity, chilling, and nutrient deficits, or toxicity in the soil, while biotic stresses on common beans include multiple fungal, bacterial, viral, and insect and worm pests (Assefa et al., 2019). Given the importance of common beans in both agriculture and economics, it is crucial to comprehend the molecular processes through which *KCS* genes in *P. vulgaris* function, particularly in response to abiotic challenges. This study's objectives were to locate *KCS* genes in the common bean genome, describe their characteristics, and analyze how they react to abiotic stressors. This research may provide insights into the functional roles of *KCS* genes in *P. vulgaris* and contribute to the development of stress-tolerant common bean varieties.

2. Materials and Methods

2.1. Identification and Characterization of KCS Genes in *P. vulgaris*

Using the Pfam Accession Number (PF08392) retrieved from the Pfam database, sequence information of the *KCS* gene family found in the *P. vulgaris* genome were obtained from the Phytozome v13 (https://phytozome-next.jgi.doe.gov/) database. To identify all possible KCS homologs in *P. vulgaris* (Schmutz et al., 2014), *G. max* (Valliyodan et al., 2019) and *A. thaliana* (Lamesch et al., 2012), the blastp in the Phytozome v13 database and the Hidden Markov Model (HMM) (http://www.ebi.ac.uk) search with default parameters were used. Additionally, the HMMER database was used to scan the presence of the KCS domain with the sequence information. KCS protein parameters were established by the earlier investigation by Aygören et al. (2023).

2.2. Phylogenetic Analysis

For the phylogenetic research, the Neighbor-joining (NJ) method was performed with a bootstrap value of 1000 replicates. Sequence alignment was performed using the ClustalW algorithm embedded in MEGA (Thompson et al., 1997). Following this, using MEGA v11 phylogenetic tree was

created (Tamura et al., 2011). The "Interactive Tree of Life" (iTOL) web interface was then utilized to model the evolutionary tree (Letunic & Bork, 2011).

2.3. Structure and Physical Location of *Pvul-KCS* Genes, Identification of Gene Duplication and Conserved Motifs, Comparative Mapping Between Other Species

"Gene Structure Display Server v2.0" (http://gsds.gao-lab.org/) (Hu et al., 2015) web interface was used for drawing exon and intron regions of *Pvul-KCS* genes to acquire information about gene structures.

KCS genes' chromosomal sites were found using the Phytozome v13 database. All *P. vulgaris* chromosomes had *Pvul-KCS* genes highlighted, which were then mapped using MapChart software (Voorrips, 2002). The "Multiple Collinearity Scan Toolkit" (MCScanX) database (Y. Wang et al., 2012) was used to search for gene duplication occurrences across *P. vulgaris*, *A. thaliana*, and *G. max*.

The exchange ratios between duplicated pairs of *Pvul-KCS* genes were calculated with values non-homologous (Ka) and homologous (Ks), and ratios non-homologous to homologous (Ka/Ks). The formulation $T=Ka/2\lambda$ was used to estimate the timing of duplication and divergence of each *KCS* gene ($\lambda = 6.56x10^{-9}$) (Z. Yang & Nielsen, 2000; Lynch & Conery, 2003; İlhan et al., 2023).

The "Multiple EM for Motif Elicition (MEME) Tool" (https://meme-suite.org/meme/index.html) was used to find further motifs that are conserved in Pvul-KCS proteins (Bailey et al., 2006). The MEME tool's settings were adjusted by earlier descriptions and discovered motifs were scanned with InterProScan database (Quevillon et al., 2005; Oner et al., 2022).

2.4. Promoter Analyses of the *Pvul-KCS* Gene Family, Intracellular Localization and Prediction of 3D Structures of Proteins

With PlantCARE (Lescot et al., 2002) database, *cis-acting* element analysis was performed in the 2 kb 5' upstream region DNA fragment of each gene of *KCS* (Rakhimzhanova et al., 2023). TBtools software was used to create phenograms (Chen et al., 2020) and WoLF PSORT tool was used to predict intracellular localization (Horton et al., 2007). The protein sequences obtained were visualized in 3D images using the Phyre2 database (Kelley et al., 2015). A single image was obtained by combining the protein images and intracellular localization data.

2.5. In Silico Gene Expression Analysis

The Sequence Read Archive (SRA) datasets in the NCBI database provided the Illumina RNA-seq data. SRR957668 and SRR958469 are for salt stress which are leaf tissue under salt treatment and salt control respectively (Hiz et al., 2014). SRR8284481 and SRR8284480 are for drought stress which are leaf tissue under drought stress treatment and drought stress control respectively (Gregorio Jorge et al., 2020). These accession numbers were used to access the expression level values and these expression values were normalized as demonstrated previously (Muslu et al., 2023). Heatmap graph was produced by using the CIMminer server (Weinstein et al., 1997).

3. Results and Discussion

3.1. P. vulgaris KCS Genes Features

The *P. vulgaris* genome in the Phytozome database v13 was searched for *KCS* gene family members using the PFAM accession number (PF08392). The investigation led to the discovery of 19 *KCS* genes in the common bean genome. The *Pvul-KCS* genes' locations on chromosomes, as well as their start and end positions, molecular weights, polypeptide lengths, isoelectric points, and stability/unstability index, are listed in Table 1.

It was observed that the identified *Pvul-KCS* genes were located on Chr1, Chr3, Chr4, Chr6, Chr7, Chr9, and Chr11 of the common bean genome and the unidentified scaffold_30 (Figure 1). As a result of the data obtained, the molecular weights of KCS genes were found to vary between 49.14 kDa and 60.57 kDa, and amino acid lengths between 437 and 534. The highest molecular weight of *Pvul-KCS-14* was 60.57 kDa, while *Pvul-KCS-16* was 49.14 kDa. *Pvul-KCS-14* contained the highest number of amino acids with 534, while *Pvul-KCS-16* contained the lowest number of amino acids with 437. It was also determined that the identified genes were mostly stable and the instability indices ranged between 29.59 and 49.80. It was observed that all genes were in the alkaline character and pI values ranged between 8.81 and 9.47.

In their study on the barley genome, Tong et al. (2021) found that the molecular weights and isoelectric points of the KCS proteins were 44.30-66.23 kDa and 6.95-10.2, respectively, with the length of these proteins in the range of 398-600 amino acid numbers.

Genome-wide characterization and identification of the *KCS* gene family in different species, Xiao et al. (2016) identified 58 *KCS* gene on *Gossypium hirsutum*, 31 *G. arboreum* and 33 *G. raimondii*, H. Yang et al. (2021) identified 13 *KCS* gene on *Atalantia buxifolia*, 16 *KCS* gene on *Citrus ichangensis*, 21 *KCS* gene on *Citrus medica*, 14 *KCS* gene on *Citrus grandis*, 16 *KCS* gene on *Citrus sinensis*, and 16 *KCS* gene on *Citrus clementina*, You et al. (2014) 38 *KCS* gene on *Linum usitatissimum* L., Lian et al. (2020) 28 *KCS* gene on *Malus domestica*, Xue et al. (2020) 58, 33 and 30 *KCS* genes on *Brassica napus*, *B. rapa* and *B. oleracea*, respectively, Tong

et al. (2021) *Hordeum vulgare* L. 33 *KCS* gene on, Dai et al. (2021) identified 18 *KCS* gene on *Malania oleifera*.

Table 1. Information about *Pvul-KCS* protein.

Gene Name	Pyhtozome ID	Chr No.	Start	End	Number of aa	MW (kDa)	pI	Instability Index
Pvul-KCS-1	Phvul.001G014100	Chr01	1058333	1061570	511	56.76	9.18	Stable
Pvul-KCS-2	Phvul.003G031300	Chr03	3165537	3167339	492	55.66	9.12	Unstable
Pvul-KCS-3	Phvul.003G138226	Chr03	34519665	34522309	516	57.79	9.03	Unstable
Pvul-KCS-4	Phvul.003G160400	Chr03	37852431	37855038	510	57.40	9.08	Stable
Pvul-KCS-5	Phvul.004G076300	Chr04	13184840	13190186	467	52.28	8.81	Stable
Pvul-KCS-6	Phvul.006G184900	Chr06	28607300	28609884	509	57.58	9.00	Stable
Pvul-KCS-7	Phvul.006G215000	Chr06	30773842	30775215	457	51.57	8.85	Unstable
Pvul-KCS-8	Phvul.007G009100	Chr07	657680	659317	483	54.81	9.06	Stable
Pvul-KCS-9	Phvul.007G009200	Chr07	661883	663334	483	54.98	8.92	Stable
Pvul-KCS-10	Phvul.007G027100	Chr07	2080156	2082926	496	56.00	9.15	Unstable
Pvul-KCS-11	Phvul.007G064000	Chr07	5712349	5713797	482	57.16	8.84	Unstable
Pvul-KCS-12	Phvul.007G279500	Chr07	39890399	39893153	519	58.07	9.33	Stable
Pvul-KCS-13	Phvul.009G023600	Chr09	5705803	5708064	512	57.58	9.47	Stable
Pvul-KCS-14	Phvul.009G084500	Chr09	13848281	13854143	534	60.57	9.14	Unstable
Pvul-KCS-15	Phvul.009G199000	Chr09	30198732	30201608	510	57.70	9.22	Stable
Pvul-KCS-16	Phvul.009G249200	Chr09	36980035	36983096	437	49.14	9.23	Unstable
Pvul-KCS-17	Phvul.009G257100	Chr09	37759369	37761230	525	58.57	8.82	Stable
Pvul-KCS-18	Phvul.011G068900	Chr11	6160872	6162607	469	53.31	8.61	Unstable
Pvul-KCS-19	Phvul.L003544	scaffold_30	265129	266550	473	53.59	8.94	Unstable

3.2. Chromosomal Location and Gene Duplication Analysis

KCS genes' chromosomal locations were specified, and the genes were found that unevenly distributed across several chromosomes. The evolution of the *Pvul-KCS* gene family was caused by both tandem and segmental duplications, which can be explained, in accordance with gene duplication studies. Tandem duplications were observed for several gene pairs, while segmental duplications involved genes located on different chromosomes.

As a result of gene duplication analysis, segmental duplication between *Pvul-KCS-2/Pvul-KCS-10*, *Pvul-KCS-8/Pvul-KCS-18* and *Pvul-KCS-18/Pvul-KCS-19* and tandem duplication between *Pvul-KCS-8/Pvul-KCS-9* and their Ka, Ks and Ka/Ks ratios are shown in Table 2. The Ka/Ks number implies positive selection in the evolutionary process when it is larger than 1, purifying selection when it is less than 1, and natural selection in duplication occurrences when it is equal to 1 (Juretic et al., 2005; İlhan, 2018; Kasapoglu et al., 2020).

3.3. Interspecific Phylogenetic Analysis of Pvul-KCS Proteins, Conserved Motif and Gene Structure

To explain the evolutionary relationships of Pvul-KCS proteins and to predict their potential functions, a phylogenetic tree was drawn using KCS-related proteins of *P. vulgaris*, *A. thaliana* and *G. max* species. The Neighbor-Joining (NJ) method was used with MEGA v11 (Molecular Evolutionary Genetic Analysis) software to perform phylogenetic tree analysis of a total of 70 KCS proteins from three plant species (Figure 2). At the phylogenetic tree classification of 19 *Pvul-KCS* gene were divided into 5 groups with *A. thaliana and G. max.*

Table 2. Ka/Ks ratios and segmental-tandem duplications for *P. vulgaris KCS* genes.

Gen 1	Gen 2	Ka	Ks	Ka/Ks	Duplication Type
***Pvul-KCS*-2**	***Pvul-KCS*-10**	1.300	0.0526	0.0404	**Segmental**
***Pvul-KCS*-8**	***Pvul-KCS*-18**	20.5922	0.3774	0.0183	**Segmental**
***Pvul-KCS*-18**	***Pvul-KCS*-19**	2.2121	0.1493	0.0675	**Segmental**
***Pvul-KCS*-8**	***Pvul-KCS*-9**	0.0369	0.0202	0.5478	**Tandem**

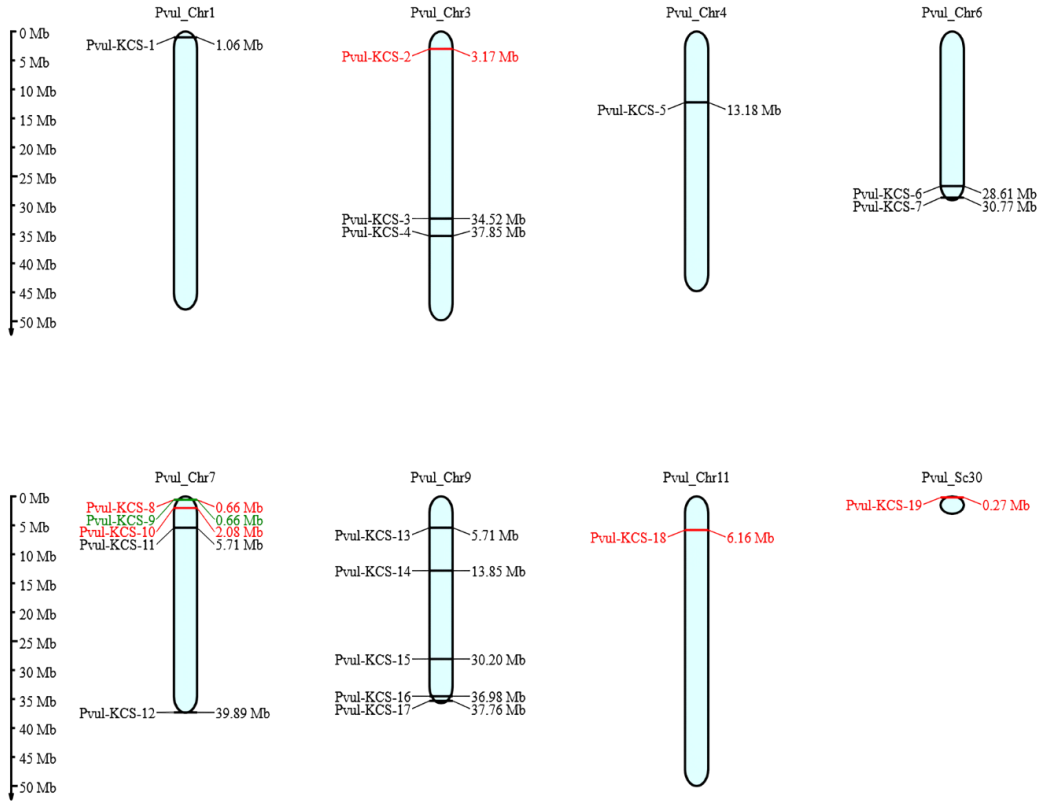

Figure 1. Chromosomal distribution of *Pvul-KCS* genes. Coloured parts indicate segmental duplications.

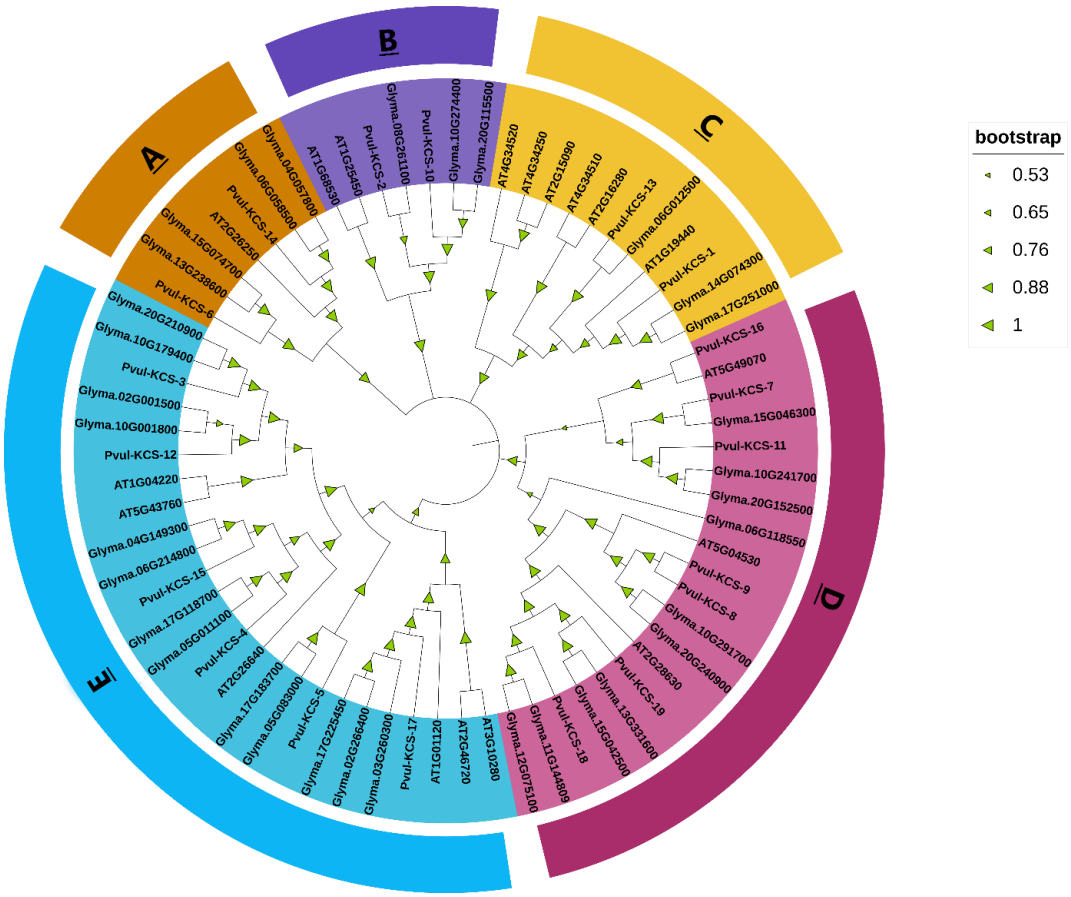

Figure 2. Phylogenetic tree constructed using three plant species' KCS proteins. *P. vulgaris* and two additional plant species' KCS full-length amino acid sequences were aligned with ClustalW, and a phylogenetic tree was created using the neighbor-joining (NJ) method with 1000 bootstraps using MEGA v11. Groups A, B, C, D, and E of the KCS subfamilies are denoted by the colours.

In their study, Zhang et al. (2022) observed that 25 *SbKCS* genes detected in the phylogenetic tree classification of *Sorghum bicolor* were divided into 5 groups with *A. thaliana*, *Oryza sativa*, *Zea mays* and *Brochypodium distachyon*.

Lian et al. (2020) observed that the 28 *MdKCS* genes identified in the phylogenetic tree classification of apple fruit (*Malus domestica*) were divided into 4 groups with *A. thaliana* and that there was a close relationship between different domains of KCS-related genes (KCS1-like, FAE1-like, FDH-like and CER6). In light of the information obtained as a result of the studies with KCS, it has been reached that *KCS* genes are divided into groups ranging from 3 to 8 in evolutionary terms and that these genes are mostly closely related to *A. thaliana* and *G. max* species.

In the conserved motif analysis of Pvul-KCS proteins using the MEME (v4.12.1) (Bailey et al., 2006) program, 10 conserved motifs were identified (Figure 3). It was discovered that the length of the identified motifs ranged from 21 to 50 amino acids. Pvul-KCS-5, -7, -8, -9, -16, -18 and -19 (9 motifs) had the least motifs, while the remaining Pvul-KCS's were equal and had the most motifs (10 motifs). Except for Motif 9, all motifs were detected in all KCS proteins. Also, the best matches corresponding to the motifs are given in Table 3.

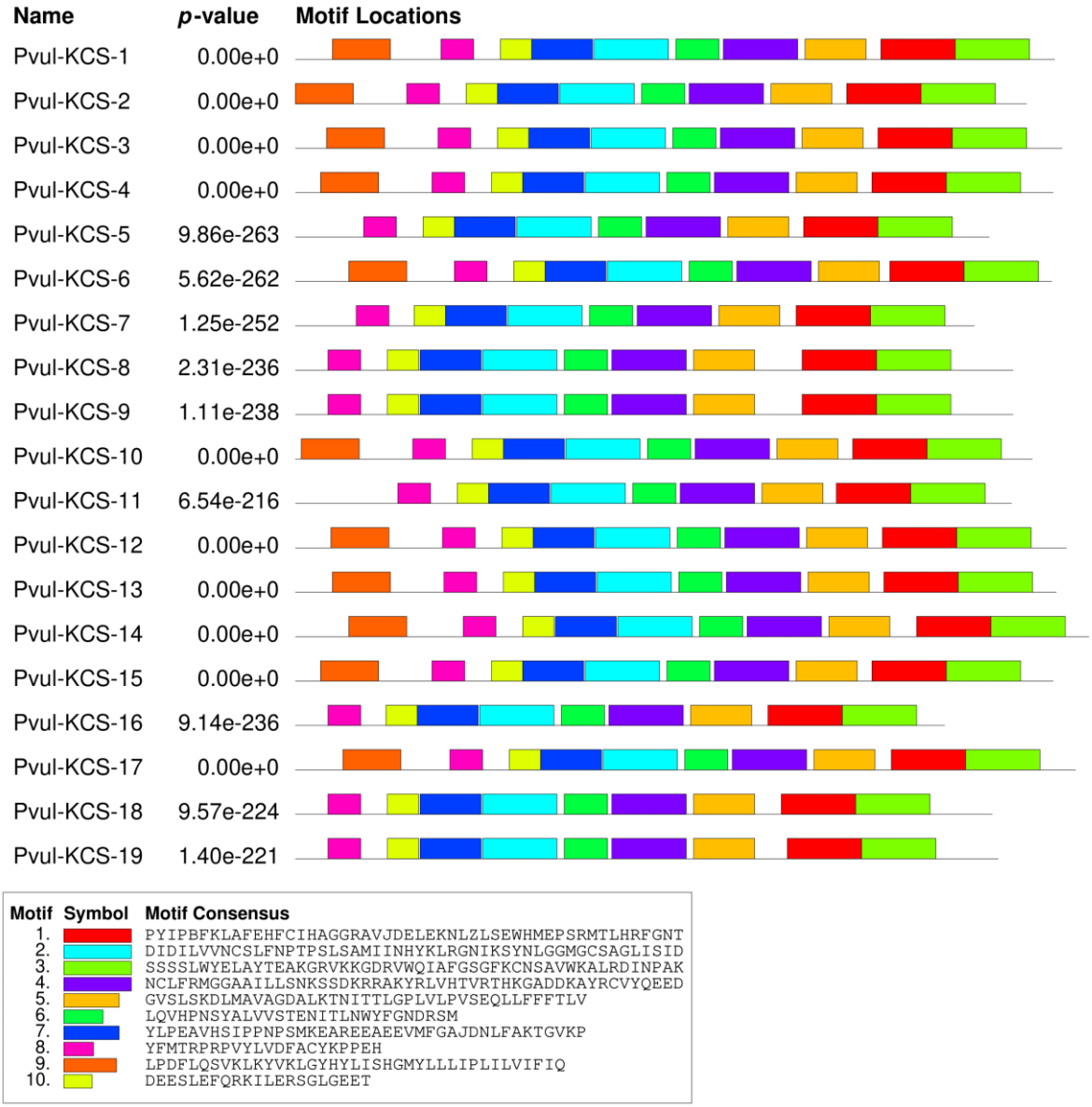

Figure 3. Predicted motif distribution in *Pvul-KCS* genes.

Zhang et al. (2022) predicted 10 motifs in *SbKCS* and identified the presence of five unique conserved motifs of these proteins; these five motifs were present throughout every single 25 SbKCS proteins, and they were placed in the same position throughout the protein sequences. This shows that practically all discovered *KCS* family genes exhibit a high degree of motif conservation.

Table 3. Predicted best possible matching information in *Pvul-KCS* genes.

MOTIF ID	WIDE	BEST POSSIBLE MATCH	Domain
MOTIF-1	50	PYIPDFKTAFEHFCIHAGGRAVIDELQKNLQLSEWHMEPSRMTLHRFGNT	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-2	50	DIDILVVNCSLFNPTPSLSAMIINHYKMRGNIKSYNLGGMGCSAGVISID	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-3	50	SSSSLWYELAYMEAKGRMKKGDRVWQIAFGSGFKCNSAVWKCMRDINPPK	NA
MOTIF-4	50	NCLFRMGGAAILLSNKPSDKRRAKYQLVHTVRTHKGADDKAYRCVYQEED	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-5	41	GVSLSKDLMAVAGDALKTNITTMGPLVLPMSEQLRFFFTLV	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-6	29	LQVHPNSYALVVSTENITPNWYQGNDRSM	NA
MOTIF-7	41	CPPEAVHYIPPNPTMKEAREEAEQVMFGAIDQLFAKTGVKP	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-8	22	YFMTRPRPVYLVDYACYKPPEH	NA
MOTIF-9	39	LPDFLQSVKLKYVKLGYHYLISHGMYLCLIPLIVVIFIQ	Very-long-chain 3-ketoacyl-CoA synthase
MOTIF-10	21	DEENLEFQRKILERSGLGEET	NA

NA: Not applicable.

Exon sizes (bp) and intron numbers were determined as a result of structural analyses performed on *Pvul-KCS* genes. As a result of the data obtained, *Pvul-KCS* was found to have 27 exons and 8 introns (Figure 4). The highest number of exons was 3 exons in *Pvul-KCS*-6 and *Pvul-KCS*-15 genes, while the other genes had 1 exon. However, region analysis data using the GSDS database showed that all *Pvul-KCS* members have symmetrical exons. Exons with symmetric splice sites at both ends are known as symmetric exons. Exon shuffling, recombination fusion, and protein domain exchange are probably facilitated by the abundance of phase 0 of symmetric exons (Gilbert, 1987; Patthy, 1987). The intron numbers in *Pvul-KCS* genes range from 0 to 2. It was observed that there were no intron regions in *Pvul-KCS* genes except *Pvul-KCS*-3, -5, -6, -7, -13, -15, and -17.

In their study, Zhang et al. (2022) demonstrated that the 25 *SbKCS* genes exhibited a range of intron counts, spanning from 0 to 1. Lian et al. (2020) showed that *KCS* genes within the same subgroup exhibited similar exon-intron distribution. In addition, it was observed that apple (*M. domestica*) *KCS* members have similar lengths and number of exon-introns as we determined in *P. vulgaris*.

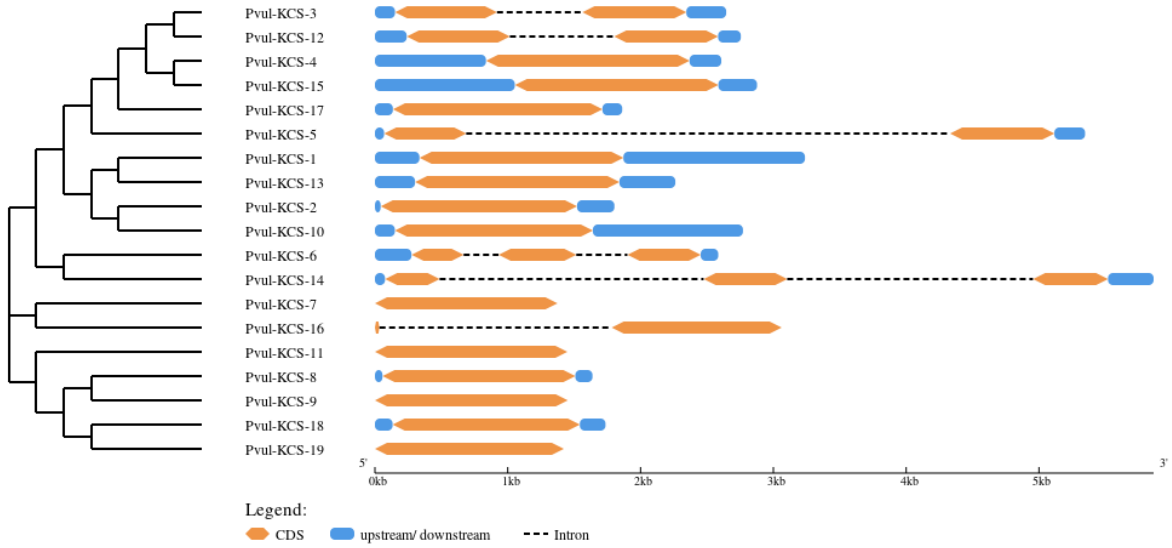

Figure 4. Exon and intron counts, lengths, and locations in *Pvul-KCS* genes.

3.4. Comparative Genomic Analysis

To better comprehend the *Pvul-KCS* gene family expansion and evolution in the *P. vulgaris* genome and the genomes of other species, synteny analysis was carried out. Tandem or segmental duplications of the *Pvul-KCS* gene were used to evaluate duplications. A comparative genomic analysis was conducted to identify homologous *KCS* genes in *Phaseolus vulgaris*, *Arabidopsis thaliana*, and *Glycine max*. The analysis revealed that some *KCS* genes were conserved among species, while others were unique to each species. Venn diagrams were used to visualize the shared and unique *KCS* genes among the three species (Figures 5 and 6).

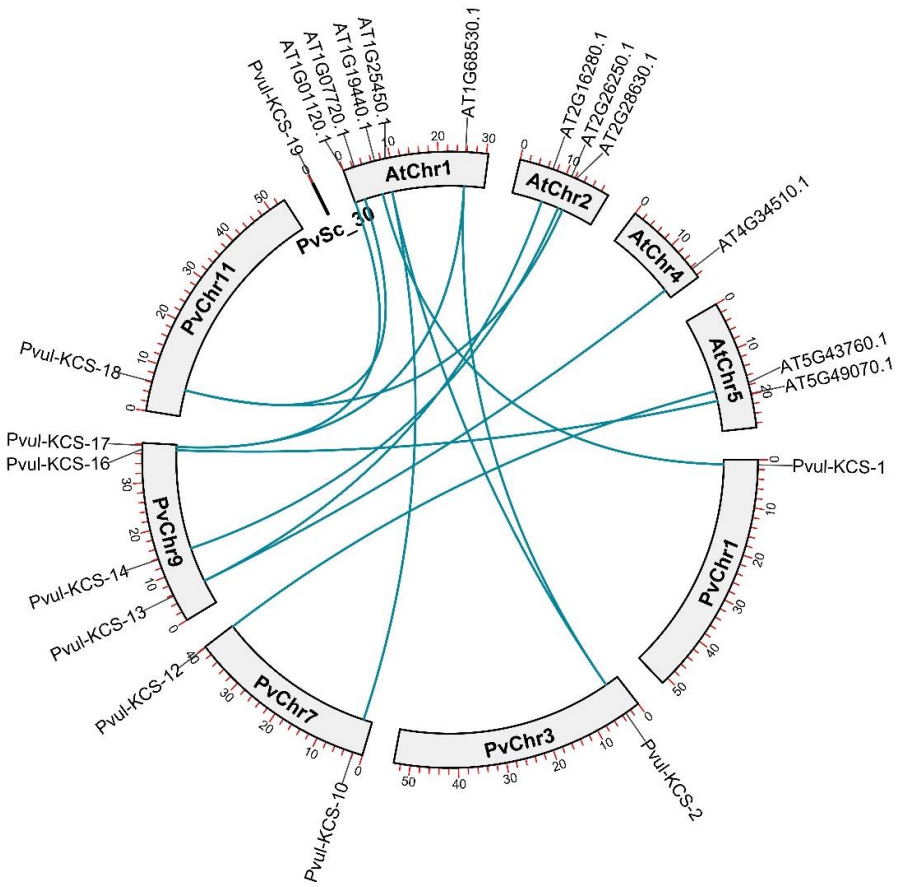

Figure 5. Synteny analysis of *P. vulgaris* and *A. thaliana* genes. *PvSc_30: scaffold_30 chromose.

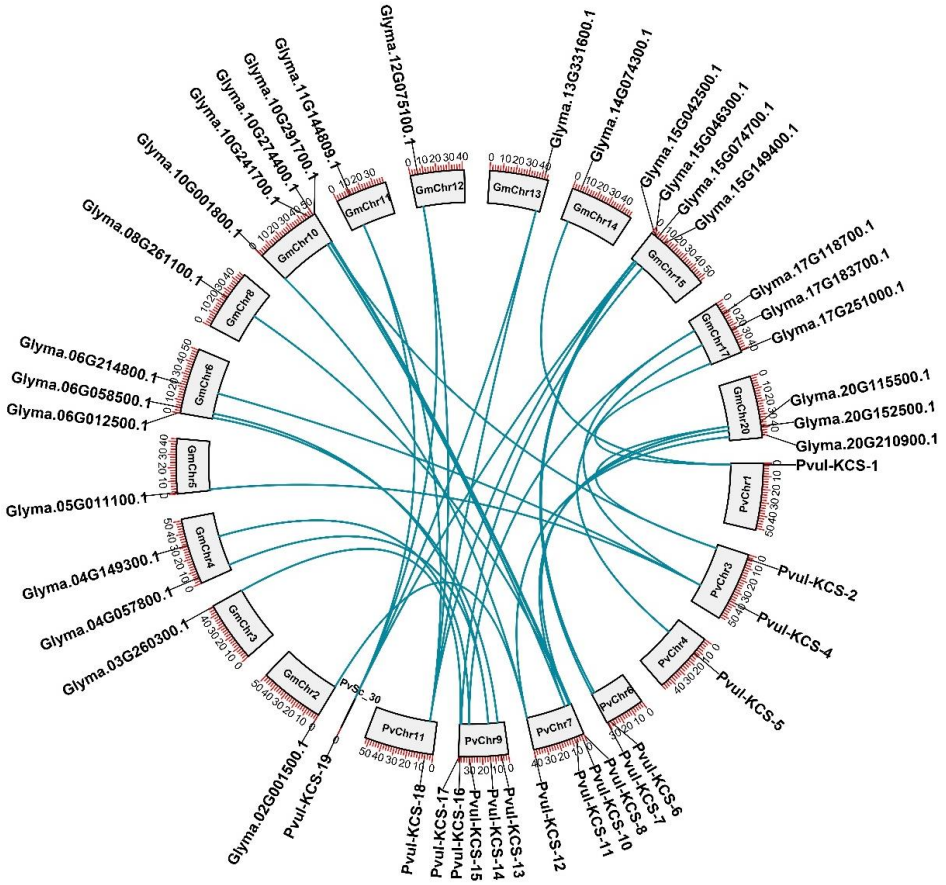

Figure 6. Synteny analysis of *P. vulgaris* and *G. max* genes. *PvSc_30: scaffold_30 chromose.

In order to demonstrate the evolutionary process of the *VvKCS* gene family, Zheng et al. (2023) created two comparative syntenic maps of grapevine linked to four representative plant species (*A. thaliana*, *M. domestica*, *O. sativa*, and *Musa acuminata*). A syntenic link between 14 *VvKCS* genes and those in *A. thaliana*, *M. domestica*, *O. sativa*, and *M. acuminata* was identified. Additionally, they discovered 25, and 15 orthologous pairs between *M. domestica* and *A. thaliana*, respectively.

3.5. Promoter Analysis of *Pvul-KCS* Genes

The sequences obtained from 2000 bp upstream of the 5' upstream regions of all *KCS* genes were analysed and it was determined that the promoter motifs in *KCS* genes play important roles in plant growth and development, adaptation to environmental conditions, molecular responses to abiotic and biotic stresses. The cis-acting elements located in the promoter regions of the *KCS* genes identified as a result of the data obtained from the PlantCARE database in the *P. vulgaris* genome were analysed and the detected cis-elements were made visually understandable using TBtools software (Figure 7). As a result of the data obtained, 82 cis-acting elements were detected in *Pvul-KCS* genes. It was determined that cis-acting elements such as MBS, ARE, W box, LTR, TC-rich repeats, which are associated with abiotic and biotic stresses, were localized in all *Pvul-KCS* genes.

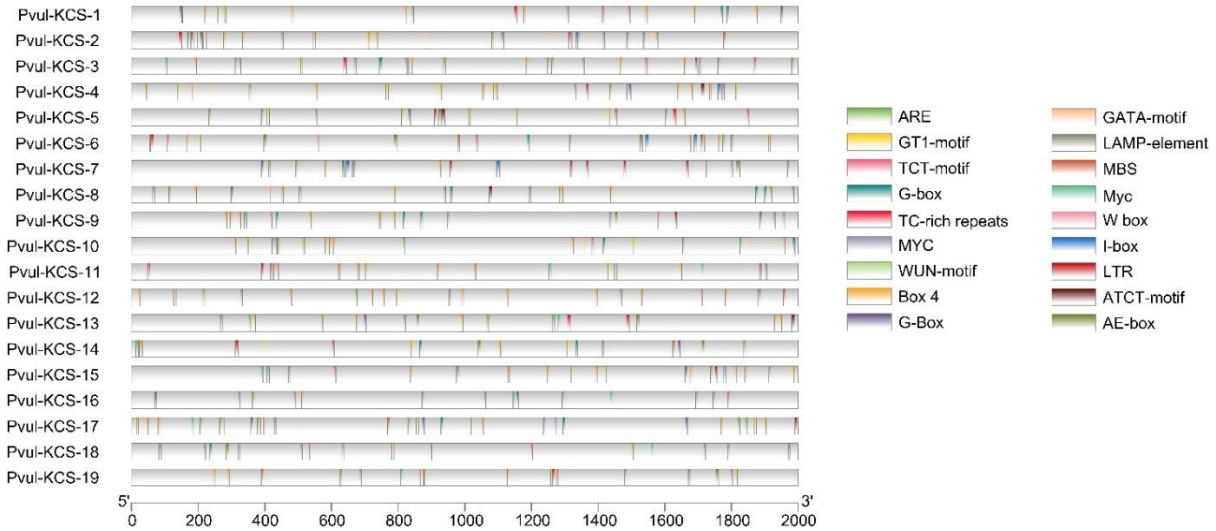

Figure 7. Promoter regions of *Pvul-KCS* genes. The promoter sequences (-2000 bp) of 19 *Pvul-KCS* genes were analysed with the help of the PlantCARE database. The scale indicates the upstream length along the translation codon. Different coloured boxes indicate different cis-acting elements.

In their study in *Passiflora edulis* fruit, Rizwan et al. (2022) found basically 4 different categories of cis-regulatory elements in PeKCS promoter regions. These are; 8 different cis-elements in plant growth and development, 10 different cis-elements in phytohormones, 15 different cis-elements in photosensitivity, and 7 different cis-elements in stress resistance.

3.6. Expression Profiling of *KCS* Genes in Response to Abiotic Stresses

To investigate the response of common bean *KCS* genes to abiotic stresses, RNA-Seq data from common bean leaf tissue subjected to drought and salt stress were analyzed. Differential gene expression analysis revealed that several *KCS* genes were differentially expressed under these stress conditions. This suggests that common bean *KCS* genes may play a role in the plant's response to abiotic stresses and could be potential targets for improving stress tolerance in common beans.

To determine the in-silico expression analysis of *Pvul-KCS* genes under salt and drought stress, the RNAseq data obtained from the SRA database were visualized (Figure 8). It was discovered that the expression levels of *Pvul-KCS* genes differed under salt and drought stress treatments based on the clustered heat map graph produced by CIMMiner with log2 transformation of RPKM values. While *Pvul-KCS*-9, *Pvul-KCS*-10, and *Pvul-KCS*-14 were the genes whose expression levels increased under salt stress, *Pvul-KCS*-13 and *Pvul-KCS*-15 were the genes whose expression levels increased under drought stress (Comparisons were made with the control group).

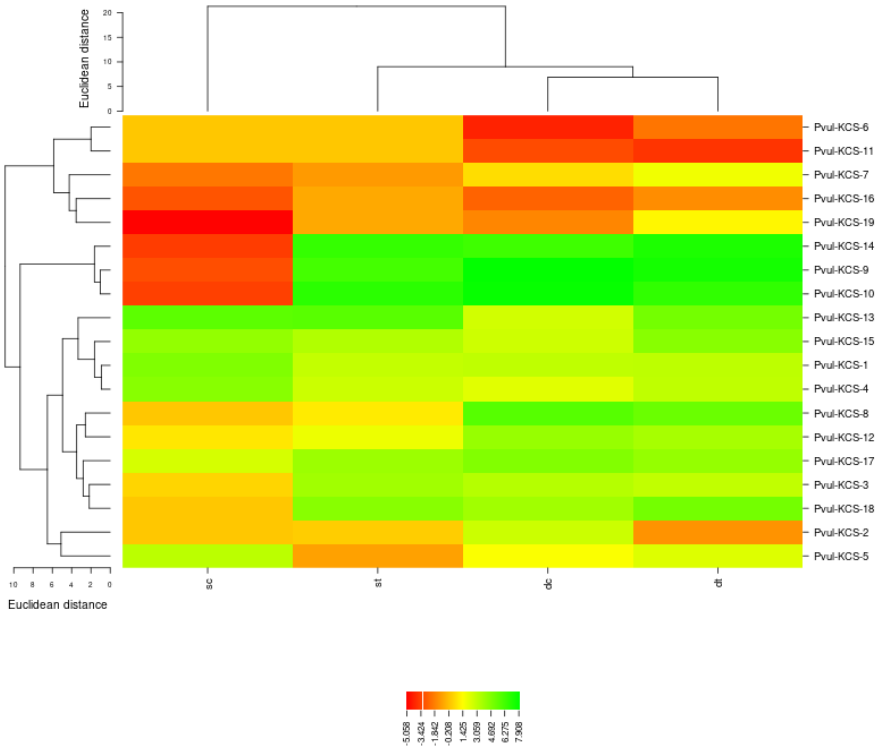

Figure 8. In silico expression analysis graph of *Pvul-KCS*.

Eight *MdKCS* genes in apple *MdKCS* genes showed an altered (down-up-down-regulation) trend under drought conditions in the study by Lian et al. (2020). Under drought-like conditions, *MdKCS12* and *MdKCS24* were observed to have up-regulated expression and *MdKCS6* expression was mostly down-regulated.

3.7. 3D Modelling of *Pvul-KCS* Genes and Their Intracellular Localization

With the help of Phyre2 database, blastp screening was performed with the data of KCS proteins obtained from the Protein Data Bank (PDB) and 3D homology modeling of KCS proteins was visualized with the help of these data (Figure 9).

In addition, the intracellular localization of Pvul-KCS proteins is shown in Figure 9. Using data from the WoLF PSORT database (Horton et al., 2007), all genes were predicted to be localized in regions such as plasma, vacuoles and endoplasmic reticulum.

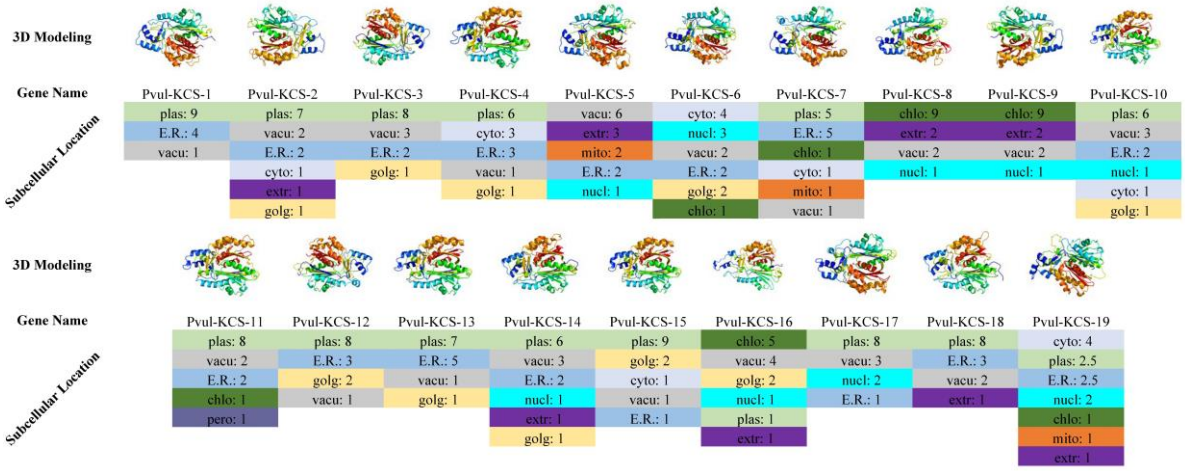

Figure 9. 3D structure modelling and intracellular localisation of Pvul-KCS proteins.

All 33 of the barley KCS proteins were found at the cell membrane in the study as a result of Tong et al. (2021), demonstrating that these proteins have highly conserved membrane protein catalytic activities.

Zhang et al. (2022) subcellular localization prediction analysis of Sorghum bicolor revealed that SbKCS proteins were primarily localized in the plasma membrane, followed by the mitochondria and chloroplast, indicating that SbKCS proteins may be expressed and function primarily in these organelles.

3.8. Protein-Protein Interactions of Pvul-KCS Proteins

Protein-protein interactions of Pvul-KCS proteins were visualized using Cytoscape software with the data obtained from the STRING database (Figure 10). Protein-protein interaction networks for particular gene families indicate the correlation between established members of the family (Piya et al., 2014). Rizwan et al. (2022) found that 31 PeKCS proteins demonstrated homology and interaction with established Arabidopsis KCS proteins in *P. edulis*. In their study, Rui et al. (2022) analysed the protein-protein interactions of *G. barbadense* KCS proteins, and found that over 90% of these proteins are involved in biosynthesis, elongation, and endoplasmic reticulum pathways related to fatty acids.

Figure 10. Protein-protein interactions (PPI) of identified KCS proteins.

4. Conclusion

In this study, a comprehensive analysis of the *KCS* gene family in common bean plants (*P. vulgaris* L.) was conducted. A total of 19 *KCS* genes were identified and characterized, providing insights into their molecular properties and subcellular localization. Phylogenetic analysis revealed the evolutionary relationships among KCS proteins in *P. vulgaris*, *A. thaliana*, and *G. max*. Additionally, the chromosomal location and gene duplication events of common bean *KCS* genes were investigated, shedding light on the expansion of this gene family in common beans.

The comparative genomic analysis showed that some KCS genes were conserved across common bean, Arabidopsis, and soycommon bean, while others were species-specific. This suggests that while certain *KCS* genes have essential functions shared among these plants, others may have evolved unique roles in each species. Understanding the conservation and divergence of *KCS* genes among different plant species can

provide valuable insights into their evolutionary history and functional significance.

Furthermore, the expression profiling of common bean *KCS* genes in response to abiotic stresses revealed differential gene expression patterns, implying potential roles in stress adaptation. These findings lay the foundation for future research aimed at elucidating the precise functions of specific *KCS* genes in stress tolerance mechanisms in common beans. Moreover, the information generated in this study can be leveraged for breeding programs focused on developing stress-tolerant common bean varieties, which are crucial for ensuring food security in regions susceptible to abiotic stress conditions.

Overall, this research contributes to our understanding of the *KCS* gene family in *P. vulgaris* and highlights their potential importance in stress responses, providing a basis for further functional studies and crop improvement efforts in common beans.

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Conflict of Interest

The authors declare that they have no known financial conflicts of interest or close relationships that might have appeared to have an impact on the research presented in this study.

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