Journal of Agricultural Production (2025), *6*(1), 15-31 https://doi.org/10.56430/japro.1593604

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Genome-Wide Analysis of Vacuolar Iron Transporter (*VIT*) Gene Family in *Phaseolus vulgaris* L.: Functional Roles in Heavy Metal Stress

Esma Yiğider✉

Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum/Türkiye

ARTICLE INFO

Article History
Received: 29.11.2024
Accepted: 03.02.2025
First Published: 26.03.2025

Keywords
Ccc1 domain
Iron transport
RNAseq
Vacuole
VTL

ABSTRACT

Vacuolar Iron Transporter (*VIT*) genes have been characterized and indicated to play critical roles in iron homeostasis in various plants. Heavy metals pose a significant challenge to bean cultivation, necessitating the development of heavy metal-resistant cultivars as a key strategy to mitigate their impacts. Vacuolar detoxification is a crucial strategy for plants to survive and adapt to the adverse environment caused by heavy metal stress. The current study used various bioinformatic tools to characterize the *VIT* gene in the bean, a significant member of the legume family and an important agricultural product, for the first time. The study identified and characterized 11 *VIT* genes (PhvulVIT-1–PhvulVIT-11) in the bean's genome. These genes displayed molecular weights (MW) ranging from 16.48 to 28.92 kDa and comprised 155–269 amino acid residues. The distribution of the 11 *PhvulVIT* genes on the four chromosomes was not homogeneous, and eight genes were observed to be located on chromosome 2. Gene duplication events suggested purifying selection as the primary evolutionary force, ensuring functional stability of duplicated genes. Phylogenetic analysis classified *PhvulVIT* genes into three clades, reflecting evolutionary relationships with orthologs in *Arabidopsis thaliana* and *Glycine max*. Cis-regulatory element analysis of promoter regions revealed key stress-responsive motifs like MYB, MYC, and ABRE, which are essential for plant responses to environmental stresses and phytohormone signaling. Additionally, the expression patterns of PhvulVIT under heavy metal conditions were examined using RNAseq. This study enhances our understanding of the functional roles of *VIT* genes in nutrient homeostasis and environmental stress adaptation, offering valuable insights for crop improvement strategies, including biofortification and the development of stress-tolerant cultivars.

Please cite this paper as follows:

Yiğider, E. (2025). Genome-wide analysis of vacuolar iron transporter (*VIT*) gene family in *Phaseolus vulgaris* L.: Functional roles in heavy metal stress. *Journal of Agricultural Production*, *6*(1), 15-31. https://doi.org/10.56430/japro.1593604

1. Introduction

The common bean (*Phaseolus vulgaris* L.), a vital legume from the Fabaceae family, is widely grown worldwide, particularly in developing nations (Hammami et al., 2022). It is a rich source of protein, dietary fiber, starch, potassium, thiamine, B vitamins, and folic acid (Silva-Gigante et al., 2023; Wainaina et al., 2021). Legumes' nutritional and functional characteristics make them important for many demographic groups, not only in low-income nations but also in rich ones (Lisciani et al., 2024). With the world population expected to exceed 9.7 billion by 2050, achieving food security necessitates crops that are both nutrient-dense and environmentally sustainable (Gu et al., 2021; Hall et al., 2017). One of the most significant developmental issues in the coming years will be the ability of global food markets to fulfill the demands of a fast-rising population (Daszkiewicz, 2022). Furthermore, this substantial increase in population is accompanied by an increase in the pace of numerous anthropogenic activities, which degrade plant development and productivity by

---

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

introducing abiotic stressors such as heavy metals (Aizaz et al., 2023).

Heavy metals have a dual role in plant metabolism: they function as essential micronutrients such as cobalt (Co), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and nickel (Ni) by acting as co-factors for critical metabolic enzymes, but they become toxic when their levels exceed permissible limits (Khalil et al., 2021; Salam et al., 2023). Non-essential heavy metals, including cadmium (Cd) and lead (Pb), exhibit phytotoxicity at minimal doses, negatively impacting plant growth and development (J. Li et al., 2020). When metal levels exceed an organelle's needs, it can upset the organelle's internal homeostasis, ultimately causing significant physiological and metabolic issues in plants (X. Chen et al., 2023). Excessive heavy metal exposure causes reduced biomass, leaf chlorosis, delayed root growth, and plant morphological anomalies, eventually culminating in plant death (Kumar et al., 2023). Bano et al. (2019) studied the impact of arsenic (As) on the growth of common bean plants, revealing reductions in shoot height, root length, and leaf surface area. Shahid et al. (2019) revealed that elevated Cd levels in beans lead to accumulation in roots and shoots, increased hydrogen peroxide (H_2O_2) production, and a significant reduction in chlorophyll content. Plants have developed mechanisms to avoid or tolerate heavy metal stress, such as antioxidant enzyme enhancement, ion homeostasis regulation, gene activation, and stress protein production (Kosakivska et al., 2021; Noor et al., 2022).

Vacuoles are exclusively found in eukaryotic species, vary in size, and can comprise up to 90% of the cell's volume (Cui et al., 2020). The vacuole has several functions, such as plant growth and development, turgor formation, storage of nutrients and metabolites, breakdown of proteins, and plant defense, the most important being maintaining ion and toxin balance (Mansour, 2023; Sharma et al., 2016). Metal transporters can remove metal ions from the cell or sequester them in vacuoles or other intracellular compartments to reduce HM-induced cytotoxicity (X. Chen et al., 2023). Tonoplast-localized vacuolar iron transporters (VIT) and VIT-likes (VTL) regulate plant Fe homeostasis and transport cytosolic ferrous ions into the vacuole via proton motive force (Kaur et al., 2021; Khoudi, 2021). This mechanism supports nutrient storage and redistribution while offering buffering capacity against heavy metal toxicity, which is crucial for plants in metal-contaminated soils. Previously, VITs were found to significantly function in keeping Fe within the appropriate physiological range and preventing cellular toxicity (Connorton et al., 2017). These *VIT* genes exhibit strong homology with a small family of nodulin-like proteins having a Ccc1 (Ca^{2+}-Sensitive Cross Complementer) like domain, with yeast Ccc1p1 (Gollhofer et al., 2011; Ram et al., 2021). Arabidopsis VIT1 was the first VIT protein discovered in plants, with 62% amino acid identity to Ccc1 and five projected transmembrane domains, which are compatible with the Ccc1 model. The AtVIT1 protein can transport iron into vacuoles, counteracting toxicity and promoting seedling development under high iron circumstances (Kim et al., 2006). In addition to their involvement in iron transport, VIT members have shown little selectivity for divalent metal ions (Zhu et al., 2016).

Using bioinformatics tools, previous studies have identified several VIT proteins across various plant species; however, a genome-wide investigation of this gene family in common bean has not yet been conducted. As an essential crop worldwide, characterizing vacuolar transporters in beans is crucial for preventing stress-induced crop losses and addressing global biofortification challenges. While biofortification remains a long-term objective, the primary goal of this study is to understand the molecular mechanisms underlying heavy metal tolerance in beans, particularly by enhancing metal homeostasis and stress adaptation through vacuolar sequestration. This study presents a genome-wide characterization of Phaseolus vulgaris VIT genes and includes in-silico expression analyses under different heavy metal treatments to explore the regulation of *VIT* genes under heavy metal stress.

2. Materials and Methods

2.1. Identification and Analysis of *VIT* Gene Family

The genome, CDS (CoDing Sequence) and amino acid sequences corresponding to the *VIT* gene family in *Phaseolus vulgaris* (v2.1), *Arabidopsis thaliana* (TAIR10) and *Glycine max* (Wm82.a4.v1) were obtained from the Phytozome v. 13 database (https://phytozome-next.jgi.doe.gov/) using the PFAM ID: PF01988 (Sharma et al., 2020). To identify VIT proteins in the genomes of the three selected plants, the blast tool from the Phytozome database and the Hidden Markov Model (HMM) (http://www.ebi.ac.uk) were employed. The HMMER (http://hmmer.org/) database was utilized to assess the presence of the VIT domain in the retrieved sequences. The ProtParam (http://au.expasy.org/tools) tool was used to predict the protein's theoretical molecular weight (MW), amino acid numbers (aa), putative isoelectric point (pI), and instability index. The subcellular localization of *PhvulVIT* genes was predicted using the WoLF PSORT (https://wolfpsort.hgc.jp/) using peptide sequences (Horton et al., 2007).

2.2. Identification of Chromosomal Position, Structure and Conserved Motif Analysis of the *PhvulVIT* Genes

The sizes and locations of *PhvulVIT* genes on the bean chromosome were determined using the Phytozome database and this information was then used to create a genetic map using MapChart software (Voorrips, 2002). After determining the location of *PhvulVIT* genes on chromosomes, a synteny map was generated using TBtools (C. Chen et al., 2023). The *PhvulVIT* family gene structures were shown using Gene Structure Display Server (GSDS) v. 2.0 (Hu et al., 2015). CDS and genome sequences were used to obtain exon-intron information of PhvulVIT proteins.

The Multiple Em for Motif Elicitation (MEME) v. 5.5.7 tool was utilized to analyze conserved motifs of the 11 PhvulVIT sequences (Bailey et al., 2009). The parameters are as follows: the motif regions were revised between 2 and 300; the maximum motif number was set at 10; the site distribution was set at any number of repetitions (anr); the minimum width was set at 6; and the maximum width was set at 50. Furthermore, for the analysis of conserved regions in bean sequences, sequence logo analysis of motifs of PhvulVIT proteins was obtained using the MEME suit online tool.

2.3. Gene Duplication and Ka/Ks Analysis of *PhvulVIT* Genes

TBtools program was utilized to determine gene duplication events. The paralogous gene pairs were used to calculate the synonymous (Ks), non-synonymous (Ka), and non-synonymous to synonymous (Ka/Ks) ratio using the TBtools basic Ka/Ks calculator algorithm (Isıyel et al., 2024). The formula $T = Ks/2\lambda$ ($\lambda = 6.56E\text{-}9$) was used to calculate the divergence time (T) million years ago (Mya) (Aygören et al., 2023).

2.4. Sequence and Phylogenetic Analysis of VIT Family Genes in *P. vulgaris, A. thaliana*, and *G. max*

For the PhvulVIT proteins phylogenetic analysis, all the VIT proteins from *P. vulgaris*, *A. thaliana*, and *G. max* were aligned using ClustalW. The phylogenetic tree structure was generated using the Neighbor-Joining (NJ) method and 1000 bootstrap replicates in MEGA v. 11 (Tamura et al., 2021). The Interactive Tree of Life (iTOL) v. 6 online tool was used to draw the phylogenetic tree (Letunic & Bork, 2024).

2.5. Syntenic and Homology Modeling of VIT Proteins

Orthologous protein sequence information of *P. vulgaris, A. thaliana*, and *G. max*, was retrieved using Phytozome v13. The TBtools' One Step MCScanX tool was used to compare genome and transcript sequences of *P. vulgaris* with *A. thaliana* and *G. max*, analyzing homology and collinearity of *VIT* genes. The obtained files screened the *VIT* genes present in these three plants, and the interspecific collinearity analysis was visualized with the Multiple Synteny Plot function in the TBtools (Qi et al., 2023). The Phyre2 v. 2.0 (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) program was used to generate model three-dimensional (3D) structures using VIT amino acid sequences and the best 3D image was selected after evaluating the reliability rates (Kelley et al., 2015).

2.6. Cis-Regulatory Element Analysis and Protein-Protein Interactions of PhvulVIT Family

Cis-regulatory elements of *PhvulVIT* genes were investigated using 2 kb genomic sequences (excluding 5'UTR sequences) upstream of the start codon (ATG) with the PlantCARE database (Rombauts et al., 1999). The results obtained were visualized with TBtools. Protein-protein interaction (PPIs) was analyzed in the STRING database v. 12.0 (https://string-db.org/) using the amino acid sequences of each PhvulVIT family member (Szklarczyk et al., 2023).

2.7. In-silico Gene Expression Analysis

Illumina RNA-seq data were acquired from NCBI's Sequence Read Archive (SRA) database to analyze the *PhvulVIT* genes. The accession numbers for control [SRR21012358, SRR21012359, and SRR21012360 (root HM control with Na) and HM stress [SRR21012367, SRR21012369, and SRR21012370 (Zn stress treated root), SRR21012397, SRR21012398, and SRR21012399 (Mn stress treated root), SRR21012417, SRR21012418, and SRR21012419 (Cu stress treated root), and SRR21012427, SRR21012428, and SRR21012429 (Co stress treated root)] were used to find relevant RNA-seq data (Fang et al., 2022). Log2-transformed RNA-seq RPKM values were used for expression profiling (C. Chen et al., 2023). Heat maps were generated with CIMminer to illustrate high-dimensional data sets such as gene expression profiles (https://discover.nci.nih.gov/cimminer/oneMatrix.do).

3. Results and Discussion

3.1. Identification and Chromosomal Distributions of *VIT* Genes in Bean

The PFAM accession number (PF01988) was used to identify *VIT* gene family members in the *Phaseolus vulgaris* v2.1 genome, accessible in the Phytozome database VIT family genes in *P. vulgaris* were higher than in *A. thaliana* but lower than in *G. max*, with 6, 11, and 19 Arabidopsis, *P. vulgaris*, and *G. max* candidate *VIT* genes identified, respectively. Every *VIT* gene was given a specific name, i.e., from PhvulVIT-1 to PhvulVIT-11. Cao (2019) identified 114 *VIT* genes in 14 different plant species, including *A. thaliana, G. max*, and *Zea mays.* PhvulVIT-2 was the smallest protein (155 amino acids), while PhvulVIT-1 was the largest (269 amino acids). The molecular weights (MW) of *PhvulVIT* genes ranged from 16.48 (PhvulVIT-2) to 28.92 kDa (PhvulVIT-1), while their predicted isoelectric points (pI) varied from 4.69 (PhvulVIT-2) to 9.36 (PhvulVIT-6). Except *PhvulVIT-6* and *PhvulVIT-7, PhvulVIT* genes are generally characterized as acidic, with $pI < 7$. The instability index of a protein defines its structure and stability; values above 40 indicate instability and values below 40 indicate stability (Singh & Mukhopadhyay, 2021). The instability index of PhvulVIT proteins ranged between 29.41 to

43.54. All proteins are stable except PhvulVIT-3, 5, 7, and 11 (Table 1).

According to the subcellular localization prediction results by WoLF PSORT, all PhvulVIT proteins are localized in the plasma membrane, and all proteins except PhvulVIT-6 are localized in the vacuole. PhvulVIT proteins were also localized in organelles such as the golgi, endoplasmic reticulum, and cytoplasm (Table 1). Sharma et al. (2020) reported that TaVIT proteins in wheat are predominantly localized in the plasma membrane and chloroplast thylakoid membrane, while TaVTL proteins are found in the vacuolar membrane. Zhang et al. (2012) demonstrated that the transient expression of OsVIT1:EGFP and OsVIT2:EGFP protein fusions revealed the localization of OsVIT1 and OsVIT2 to the vacuolar membrane, suggesting they may also act as vacuolar membrane transporters. The chromosomal positions of VITs were determined using the TBtool program by uploading a mapchart file containing chr data.

Table 1. Detailed information of all *VIT* genes identified in the bean genome.

Gene Name	Phytozome Gene ID	Chromosomal Location				aa	MW (kDa)	pI	Instability Index	Aliphatic Index	Subcellular Localization
		Chr Name	**Start**	**End**	**Strand**						
PhvulVIT-1	Phvul.008G070000	Chr08	6339673	6342165	R	269	28.92	5.44	37.43	0.171	vacu: 5, golg: 4, plas: 3, extr: 1, E.R.: 1
PhvulVIT-2	Phvul.002G323700	Chr02	48857306	48858670	R	155	16.48	4,69	33.80	98.32	cyto: 5, plas: 3, E.R.: 3, mito: 1, vacu: 1, golg: 1
PhvulVIT-3	Phvul.002G113500	Chr02	24370428	24371205	F	232	24.64	6.59	40.48	109.35	vacu: 8, plas: 6
PhvulVIT-4	Phvul.002G322800	Chr02	48774555	48776307	F	231	24.34	5.13	32.60	0.371	chlo: 6, plas: 3, mito: 2, vacu: 2, E.R.: 1
PhvulVIT-5	Phvul.007G079100	Chr07	7671484	7672393	F	223	23.7	5.44	41.69	101.93	vacu: 7, golg: 4, plas: 2, extr: 1
PhvulVIT-6	Phvul.002G205100	Chr02	37176245	37177230	R	221	22.94	9.36	29.73	99.37	plas: 8, E.R.: 5, chlo: 1
PhvulVIT-7	Phvul.004G096500	Chr04	15848520	15849195	R	224	23.71	6.15	43.23	99.38	vacu: 8, plas: 2, extr: 2, E.R.: 1, golg: 1
PhvulVIT-8	Phvul.002G205000	Chr02	37162537	37163665	R	229	24.24	8.63	29.41	104.37	vacu: 10, plas: 2, extr: 1, golg: 1
PhvulVIT-9	Phvul.002G322900	Chr02	48779469	48782786	R	247	26.17	5.48	31.68	105.22	plas: 10, vacu: 4
PhvulVIT-10	Phvul.002G205200	Chr02	37188536	37189462	R	221	23.25	6.74	30.14	105.57	plas: 4.5, vacu: 4, cyto_plas: 3, E.R.: 2, golg: 2, extr: 1
PhvulVIT-11	Phvul.002G205300	Chr02	37195862	37196667	F	209	22.7	4.71	43.54	108.76	vacu: 9, plas: 5

Chr: Chromosome, R: Reverse, F: Forward, aa: Amino acid, MW: Molecular weight, pI: Theoretical isoelectric point, vacu: Vacuole, golg: Golgi apparatus, plas: Plasma, extr: Extracellular, E.R: Endoplasmic reticulum, mito: Mitochondria, chlo: Chloroplast, cyto: Cytosol.

The unequal distribution of VITs on chromosomes is depicted in Figure 1. Chr2 (chromosomal 2) included the largest number of PhvulVIT (8), while Chr4, Chr7, and Chr8 each contained only one. Connorton et al. (2017) determined that the *TaVIT1* and *TaVIT2* genes of wheat are located on chromosomes 2 and 5, respectively. Thirty-one distinct VIT families of wheat have been identified in hexaploid wheat, with the majority of genes firmly positioned on chr2 (Sharma et al., 2020).

3.2. VIT Structure Analysis and Conserved Motif Composition

PhvulVIT genes exons and introns were discovered using GSDS structural analysis. The variety of exons and introns in gene families involves three processes: the gain or loss of exons and introns; exonization, which converts intronic or intergenic sequences into exonic states; and pseudoexonization, which reverses this transformation (Isıyel et al., 2024). The exon numbers of *PhvulVIT* genes range from 1 to 4, while the intron counts range from 2 to 3 (Figure 2). PhvulVIT-3, 5, 6, 7, 8, 10, and 11 are intronless genes with a single exon (Figure 2). PhvulVIT-2, PhvulVIT-4, and PhvulVIT-9 had the highest exon numbers, and of the 11 *PhvulVIT* genes, 2 had no UTRs (Figure 2). The *VIT* genes in wheat possess three to four intronic and exonic sections, whereas the *VTL* genes consist of a single exon apiece without introns. This structural distinction further categorises the VIT family into two sub-families (Sharma et al., 2020).

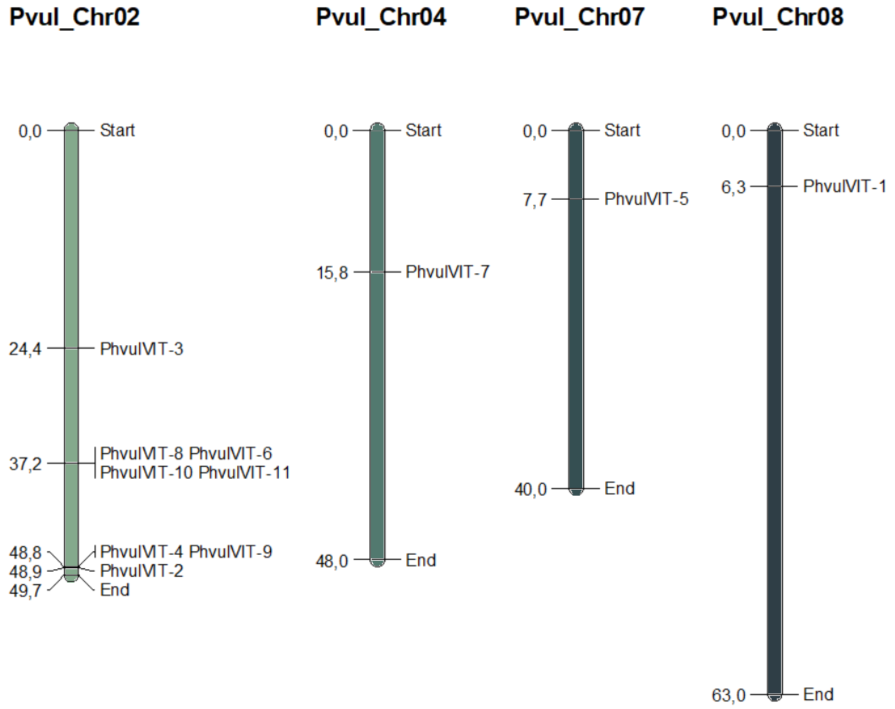


Figure 1. *PhvulVIT* genes distribution in bean chromosomes.

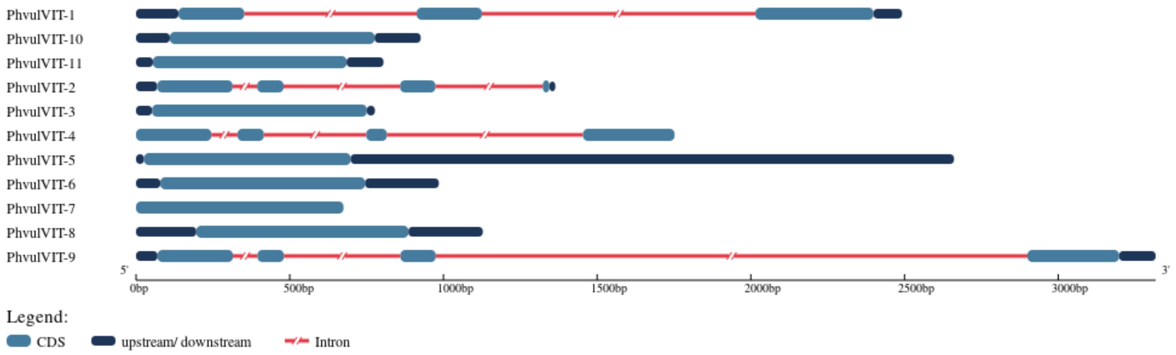
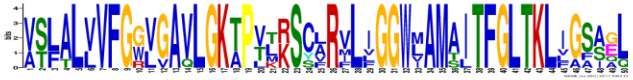


Figure 2. Gene structure of *VIT* genes in bean constructed by the GSDS 2.0. Exons, introns, and untranslated (UTR) regions are marked by blue boxes, red lines, and navy-blue boxes, respectively. The scale bar at the bottom shows the lengths of the exons, introns, and UTR sections.

Ten conserved motifs were identified in the conserved motif analysis conducted on PhvulVIT proteins using the MEME suite v. 5.5.7 and named Motif 1–10. Furthermore, the MEME suite database was used to find conserved domain sequences and motif logos of *P. vulgaris VIT* gene families (Table 2). The number of amino acids in conserved motifs ranges from 6 to 50 (Table 2). Table 2 displays the sequences that correlate to the motifs that are identified as being the best possible matches. It was found that Motifs 1 was present in all the common bean *PhvulVIT* genes that belong to the same gene family. This suggests that these motifs could act as identifiers for distinguishing the *PhvulVIT* gene.

Table 2. Sequence details of possible motifs and motif logo in PhvulVIT proteins.

MOTIF ID	WIDTH	LOGO	POSSIBLE BEST MATCH	DOMAIN
1	41		LMMGVGAVKQDIKAMLTAGFAGLVAGACSMAIGEFVSVYTQ	Ccc1
2	50		VSLALVVFGWVGAVLGKTPVTKSCLRVLIGGWMAMAITFGLTKLIGSAQL	Ccc1
3	41		EPEKEKLPNPFQAALASALAFSIGALVPLLPYAFIRSYKIR	Ccc1
4	29		HHNIDYSQRAQWLRDAVLGANDGLVSPFS	N/A
5	21		YDIYMRQMKREQERNNGGPRD	N/A
6	26		GHRTNEYSINQHEYPPHYNGLEPNPN	N/A
7	50		VVVTMFALLVFGYAKGHFTGNKPFRSALETALIGAIASAAAFGMAKAFHP	Ccc1
8	15		EHGISLDQEKTNLPN	N/A
9	6		WLDFMM	N/A
10	7		QDNKHWK	N/A

Motif 1, Motif 2, Motif 3, and Motif 8 contain the Ccc1 domain. The Ccc1 family comprises a group of putative vacuolar ion transporters. This protein family includes yeast Ccc1, which is involved in Ca and Mn homeostasis (Lapinskas et al., 1996). Arabidopsis VIT1, the first plant VIT protein to function as a vacuolar Fe^{2+} uptake transporter, is 62% amino acid similar to Ccc1 and has five predicted transmembrane domains consistent with the Ccc1 mode (Kim et al., 2006). The proteins with the fewest motifs are PhvulVIT-1, PhvulVIT-8, and PhvulVIT-11 (5 motifs), while the gene with the most motifs is PhvulVIT-9 (8 motifs) (Figure 3). All *VIT* and *VTL* genes exhibited the characteristic Ccc1-like superfamily domains observed in yeast (Sharma et al., 2020).

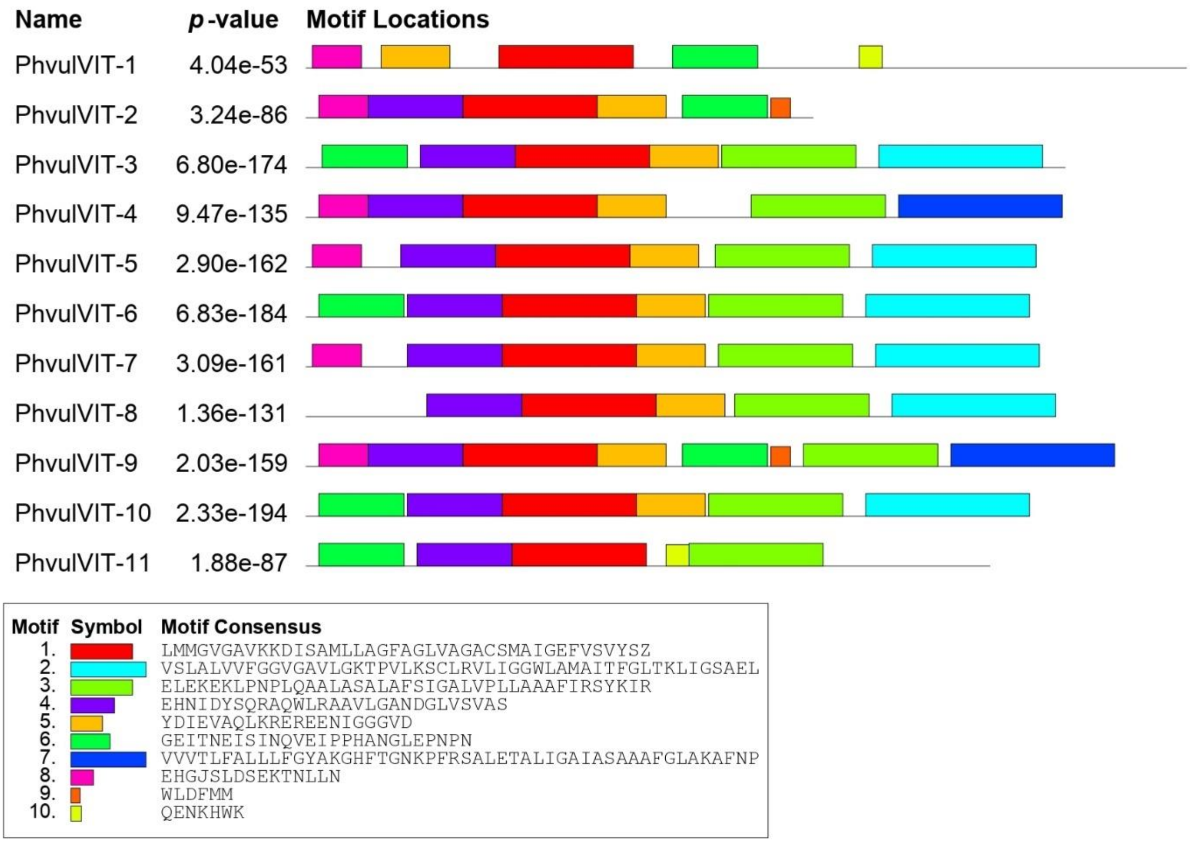


Figure 3. Motif composition of VIT proteins in bean. Boxes of various colors represent numerous motifs. Motif’s location in each sequence is marked.

3.3. Gene Duplication Events and Ka/Ks Analysis of *PhvulVIT* Genes

In this research, it was determined four tandem duplicated genes (*PhvulVIT-4*/*PhvulVIT-9*, *PhvulVIT-6*/*PhvulVIT-10*, *PhvulVIT-8*/*PhvulVIT-6*, and *PhvulVIT-10*/*PhvulVIT-11*) pair among 11 *PhvulVIT* genes. The Ka/Ks ratio is a useful tool for describing the degree of selection; values above 1 suggest positive selection, values below 1 indicate purifying selection, and a value of 1 indicates neutral selection (Inal et al., 2024). The Ka/Ks ratio of genes exhibiting tandem duplication was smaller than 1. This hypothesis shows that the duplicated *PhvulVIT* genes underwent purifying selection pressure. Table 3 shows that the *PhvulVIT-8* and *PhvulVIT-6* genes were determined to be the first to diverge approximately 216 billion years ago. Cao (2019) determined that tandem/segmental duplication and transposition events contributed to the evolution of the *VIT* gene family.

Table 3. Divergence time of the VIT paralogues in bean.

Gene 1	Gene 2	Ka	Ks	Ka/Ks	Selection Pressure	Duplication Types	Divergence Time (Mya)
PhvulVIT-4	*PhvulVIT-9*	0,076324	0,266241	0,286673	Purifying Selection	Tandem Duplication	43,65
PhvulVIT-6	*PhvulVIT-10*	0,056855	0,202275	0,281078	Purifying Selection	Tandem Duplication	33,16
PhvulVIT-8	*PhvulVIT-6*	0,316047	1,32E+15	0,239951	Purifying Selection	Tandem Duplication	2,16E+17
PhvulVIT-10	*PhvulVIT-11*	0,348542	0,810983	0,429778	Purifying Selection	Tandem Duplication	132,95

3.4. Phylogenetic Analysis of VITs

The analysis of multiple sequence alignments for PhvulVIT protein sequences demonstrates the presence of highly conserved regions characterized by specific amino acid sequences. A phylogenetic tree was constructed using the VIT protein sequences from *A. thaliana* and *G. max*, elucidating the relationships among PhvulVIT proteins. Phylogenetic tree analysis of 36 VIT proteins from these three plant species was performed utilizing the NJ approach in MEGA v11 software, with a bootstrap value of 1000 repetitions. The 36 *VIT* genes were classified into three clades based on their homology percentage. Clade A consisted of 3 PhvulVIT, 1 Arabidopsis, and 5 soybean members; Clade B had 3 PhvulVIT, 2 Arabidopsis, and 6 soybean members; and Clade C had 5 PhvulVIT, 3 Arabidopsis, and 8 soybean members (Figure 4). Combining the gene structure with the phylogenetic tree revealed that the exon numbers of *PhvulVIT* genes in various subgroups were related to their classification. For example, *PhvulVIT-6* and *PhvulVIT-10* genes in Clade B and *PhvulVIT-2* and *PhvulVIT-9* genes in Clade C were found to have 1 and 4 exons, respectively, and were subdivided in parallel with their exon numbers. The phylogenetic analysis divided 114 *VIT* genes from 14 different plant species into seven groups based on their intron-exon structure and the presence of different motifs (Cao, 2019).

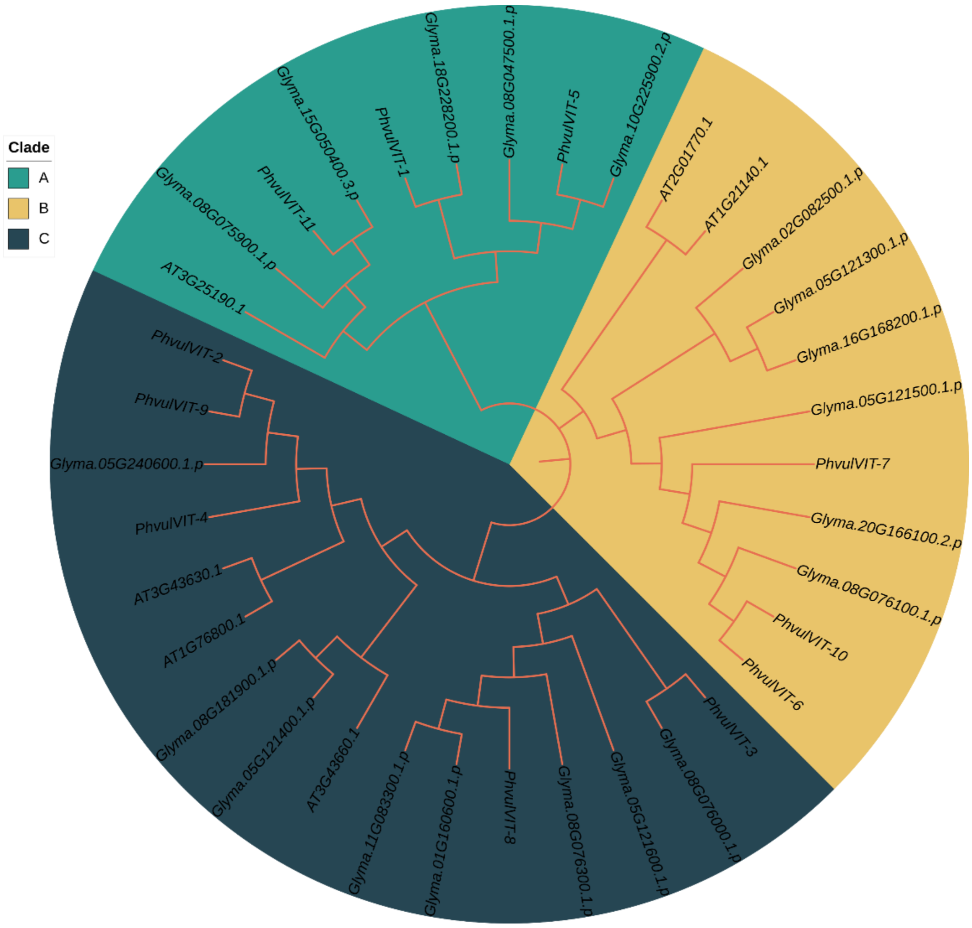


Figure 4. Phylogenetic relationships among 36 VIT proteins in bean, *Arabidopsis*, and soybean. The full-length sequences of bean VIT proteins were used to build the phylogenetic tree using the MEGA 11 software.

3.5. Synteny Analysis of VIT Protein in *P.vulgaris*, *A.thaliana* and *G. max*

The study demonstrated orthologous relationships of *VIT* genes among *P. vulgaris, A. thaliana* and *G. max* genomes. One syntenic relationship was determined among *P. vulgaris* and *A. thaliana VIT* genes, while fifteen syntenic relationships were determined among *P. vulgaris* and *G. max VIT* genes (Figure 5). Orthology was found between *PhvulVIT-2* and *PAC:19642412* genes in bean and Arabidopsis. Moreover, orthology was identified between the *PhvulVIT- 1 /Glyma.18G228200.1; PhvulVIT-2* and *Glyma.05G240600.1-Glyma.08G047500.1; PhvulVIT-3* and *Glyma.01G160600.1-Glyma.11G083300.1; PhvulVIT-5* and *Glyma.02G082500.1-Glyma.10G225900.2-Glyma.16G168200.1-Glyma.20G166100.2; PhvulVIT-6* and *Glyma.05G121300.1; PhvulVIT-7* and *Glyma.02G082500.1-Glyma.16G168200.1*, *PhvulVIT-8* and *Glyma.05G121500.1-Glyma.08G076000.1*, and *PhvulVIT-11* and *Glyma.08G075900.1* genes. There was no collinearity between the *PhvulVIT-4*, *PhvulVIT-9*, and *PhvulVIT-10* genes and *A. thaliana* or *G. max* which suggests that the homologous gene pairs formed after the species split up (Dey et al., 2023).

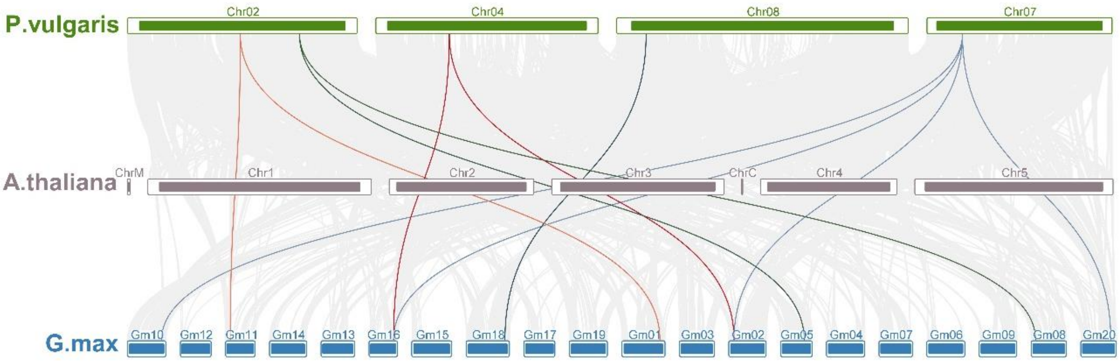


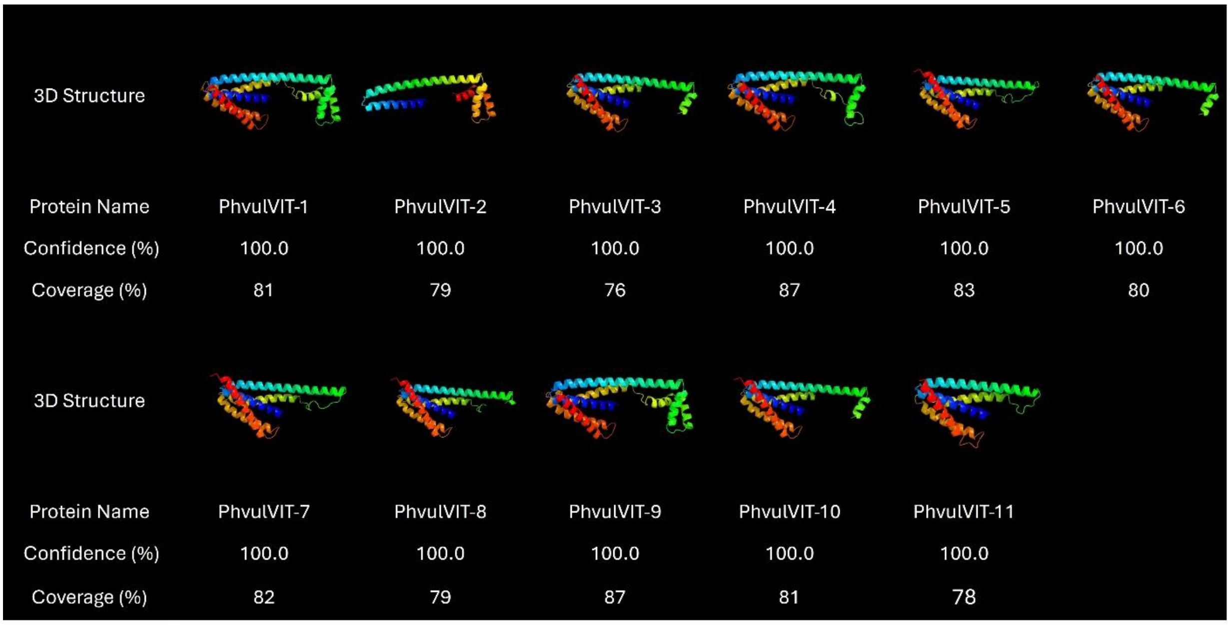
Figure 5. Synteny analysis of the *VIT* genes among the *P. vulgaris, A. thaliana.* and *G. max.* Different color lines delineate the syntenic *VIT* gene pairs.

3.6. Homology Modeling of Identified PhvulVITs

The proteins' structure and function were predicted using the Phyre 2 database. With a 100% modeling confidence level, the 3D structures of the 11 PhvulVIT proteins were predicted using homology modeling. Coverage values also ranged between 76-87%. The results showed that most of the secondary structures of PhvulVIT proteins consisted of disordered structures (Table 4). Specifically, a distinctive alpha (α) helix and TM-helix structure were observed in PhvulVIT proteins (Table 4). Specifically, 7 out of the 11 VIT proteins in *P. vulgaris* displayed similar proportions of alpha and TM-helix structures (Figure 6). The alpha helix is critical in signal transduction and dynamic motions in some proteins. For instance, G-proteins and receptor proteins use this structure in signal transmission (Palczewski et al., 2000). TM-helix, hydrophobic amino acids, facilitate protein passage through biological membranes, ensuring correct localization of membrane proteins by interacting with the hydrophobic region of the lipid bilayer (White & Wimley, 1999).

Table 4. The 3D structure of VIT proteins in bean.

Protein Name	Disordered Structures (%)	Alpha Helix (%)	TM-Helix (%)
PhvulVIT-1	25	73	39
PhvulVIT-2	26	68	23
PhvulVIT-3	34	69	43
PhvulVIT-4	28	73	43
PhvulVIT-5	35	68	44
PhvulVIT-6	35	69	45
PhvulVIT-7	35	67	44
PhvulVIT-8	38	65	44
PhvulVIT-9	22	70	44
PhvulVIT-10	34	68	45
PhvulVIT-11	35	67	46



3D Structure						
Protein Name	PhvulVIT-1	PhvulVIT-2	PhvulVIT-3	PhvulVIT-4	PhvulVIT-5	PhvulVIT-6
Confidence (%)	100.0	100.0	100.0	100.0	100.0	100.0
Coverage (%)	81	79	76	87	83	80

3D Structure					
Protein Name	PhvulVIT-7	PhvulVIT-8	PhvulVIT-9	PhvulVIT-10	PhvulVIT-11
Confidence (%)	100.0	100.0	100.0	100.0	100.0
Coverage (%)	82	79	87	81	78

Figure 6. Homology modeling of PhvulVIT proteins.

3.7. Cis-Elements Analysis in the Promoter Regions of *PhvulVIT* Genes

Cis-acting elements are distinct motifs in each gene's promoter region that regulates gene transcription (Buttanri et al., 2024). The 2000 bp upstream region of the *PhvulVIT* gene promoter was examined to identify key cis-acting elements in the 11 *PhvulVIT* gene. The analyses conducted in the PlantCARE database indicated the cis-regularity elements in the *PhvulVIT* gene sequences using the TBtools (Figure 7). The promoter sequences of bean *VIT* genes show a wealth of potential cis-acting elements that play a critical role in mediating stress responses, phytohormone synthesis, plant growth and development, and adaptations to anaerobic conditions (Figure 7). This demonstrates the significant regulatory pathways involved in providing plant resilience and adaptation. All PhvulVIT includes the promoter elements MYB, MYC, TCT-motif, and Box 4 (Figure 7).

The 172 elements shown to be linked with environmental stress included MYC, MBS, and MYB (drought-related regulatory), DRE core (dehydration-responsive element), W-box and box S (pathogen response elements), LTR (low-temperature responsiveness), WUN-motif (wound-responsive element), WRE3 (high-temperature elements), TC-rich repeats, and STRE (defense and stress-responsive element) (Figure 7). J. Li et al. (2020) identified ten cis-elements associated with abiotic and biotic stresses, including DRE, MYB, MBS, MYC, LTR, STRE, TC-rich repeats, W box, WUN motif, and WRE3.

The 72 cis-acting elements related to phytohormones primarily include the ABRE (abscisic acid-responsive elements), ERE (estrogen-responsive element), AuxRR-core and TGA- element (auxin-responsive element), and TCA-element (salicylic acid responsiveness) (Figure 7). Rasool et al. (2023) found cis elements connected to hormones, including the ABRE, TCA element, GARE motif, P and TATC boxes, TGA element, and AuxRR core. The ARE (regulatory anaerobic induction) cis element was present in 4 *PhvulVIT* genes and was revealed to be responsible for anaerobic induction. In PhvulVIT, light sensitivity was associated with 127 cis-elements, e.g., the TCT motif, Box 4, MRE, G-Box, AE-Box, ACE, LS7, and L-Box (Figure 7). These findings suggest that PhvulVIT proteins are crucial in signaling pathways during plant growth and development and in responding to different abiotic stresses.

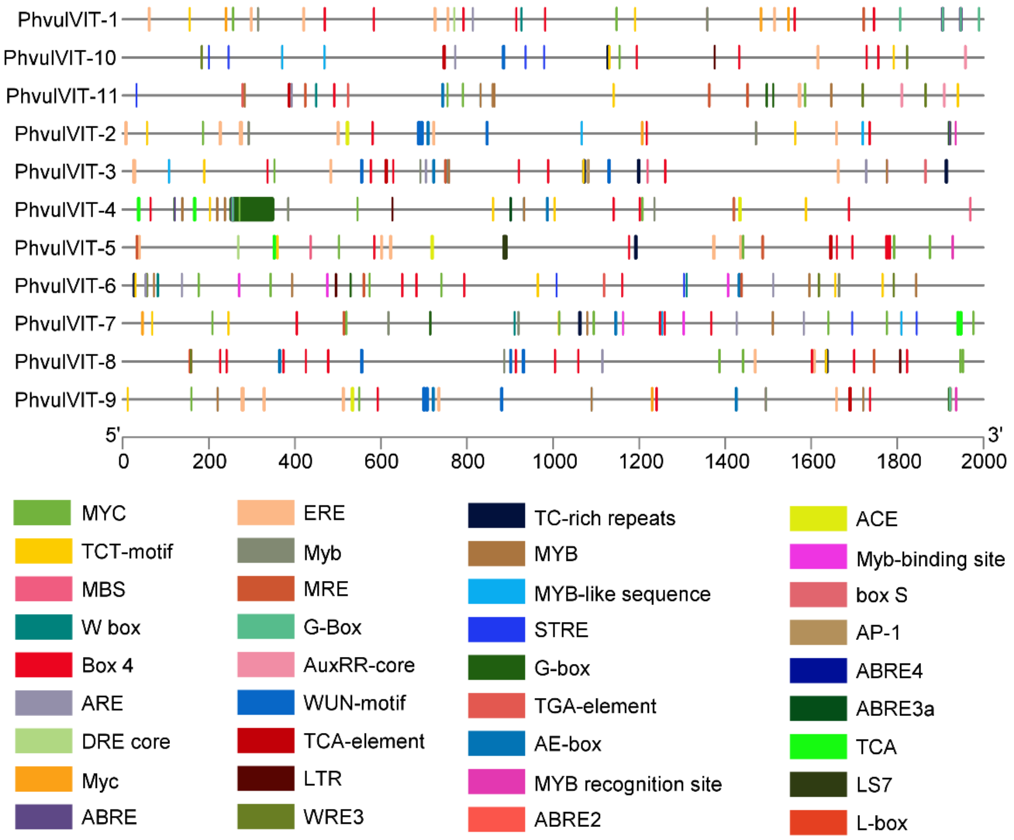


Figure 7. Analysis of cis-acting elements in promoter regions of bean *VIT* genes.

3.8. PhvulVIT Protein–Protein Interactions

Protein-protein interactions (PPIs) provide information about the role of proteins in biological processes and molecular functions (Peng et al., 2017). Figure 8 presents a graphical representation of protein-protein interactions among PhvulVIT proteins using the STRING database (Szklarczyk et al., 2023). PhvulVIT-2 and PhvulVIT-11 interact with other proteins more than any other molecules. PhvulVIT-2 interacts with PhvulVIT-3, PhvulVIT-5, PhvulVIT-6, PhvulVIT-7, and PhvulVIT-8 proteins, while PhvulVIT-11 interacts with PhvulVIT-2, PhvulVIT-4, and PhvulVIT-9 proteins (Figure 8). This suggests that they may play a part in building complex networks and interacting with many different molecules inside cells. V7ASI1_PHAVU, V7CXI9_PHAVU, and V7CX76_PHAVU proteins contain glutamine amidotransferase type-2. The V7B1N1_PHAVU and V7AT93_PHAVU proteins are uncharacterized.

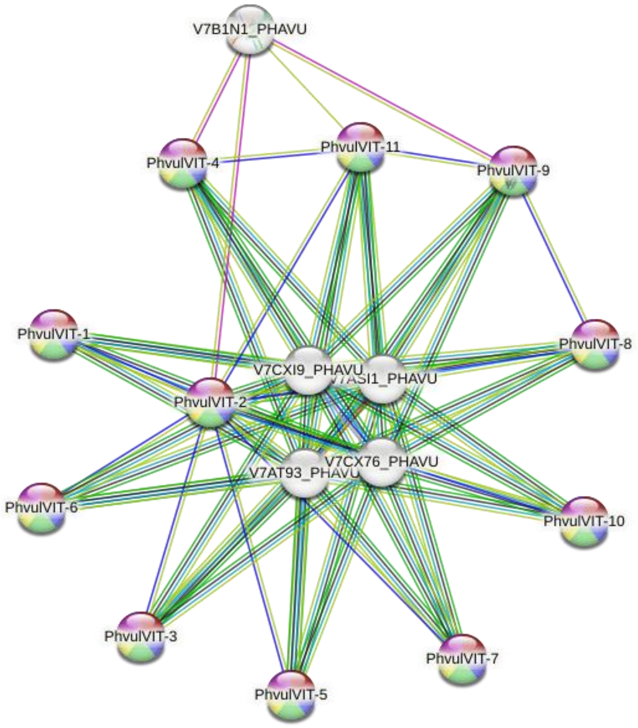


Figure 8. Protein–protein interactions schematic demonstration found in *PhvulVIT* genes. The different colors in the circle show biological functions (red: cellular manganese ion homeostasis, purple: intracellular sequestering of iron ion, green: manganese ion transmembrane transport) and molecular functions (yellow: iron ion transmembrane transporter activity, pink: manganese ion transmembrane transporter activity).

According to the results of PPI analysis, all PhvulVIT proteins are involved in biological processes such as cellular manganese ion homeostasis, intracellular sequestration of iron ion, and manganese ion transmembrane transport (Figure 9A). Moreover, PhvulVIT proteins have molecular functions such as iron and manganese ion transmembrane transporter activity (Figure 9B). Iron-excess-dependent transporters may facilitate iron detoxification or sequestration for homeostasis in rice and wheat (Krishna et al., 2023).

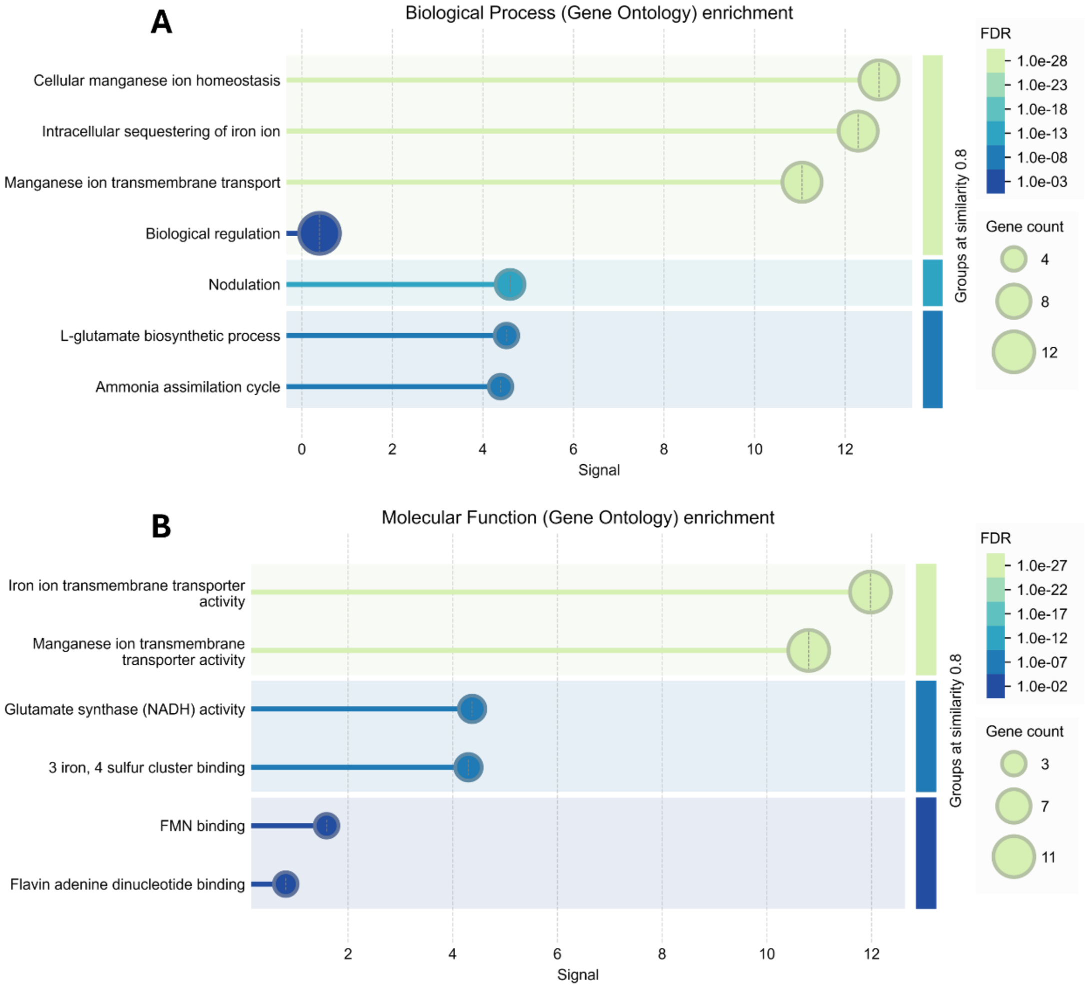


Figure 9. Functional enrichment visualization of PhvulVIT proteins covering biological processes (A) and molecular functions (B) visualized employing the STRING database.

3.9. In Silico Expression Profiles *PhvulVIT* Genes Under Heavy Metal Stress

VIT genes are crucial in plant metal homeostasis and stress responses. They primarily sequester iron into vacuoles, thereby controlling its availability within the cell (Shekhawat et al., 2023). Recent investigations have expanded our understanding of *VIT* genes, revealing their involvement in transporting and detoxifying various heavy metals beyond Fe (T. Li et al., 2023; Sharma et al., 2020).

The expression profiles of *PhvulVIT* genes in the root tissues of bean were examined utilizing SRA data under heavy metal stress conditions (Control, Cu, Co, Zn, and Mn). It is observed that the expression levels of *PhvulVIT* genes in root tissue vary according to different heavy metals (Figure 10). When Cu treatment was compared with the control, it was determined that the expression of *PhvulVIT-1* and *PhvulVIT-3* genes increased significantly, the expression of *PhvulVIT-4* and *PhvulVIT-7* genes did not change much, and the expression levels of *PhvulVIT-2*, *PhvulVIT-6*, *PhvulVIT-9*, and *PhvulVIT-10* genes decreased significantly. In roots exposed to Co metal, the *PhvulVIT-7* gene was significantly up-regulated compared to the control, and the *PhvulVIT-2*, *PhvulVIT-6*, and *PhvulVIT-9* genes were significantly down-regulated. In response to Zn stress, the expression levels of the *PhvulVIT-7*, *PhvulVIT-10*, and *PhvulVIT-11* genes were remarkably increased compared to the control. Conversely, the expression levels of the *PhvulVIT-2* and *PhvulVIT-9* genes showed a significant decline. On the other hand, when Mn treatment was evaluated, *PhvulVIT-6*, *PhvulVIT-7*, *PhvulVIT-10*, and *PhvulVIT-11* genes were found to have higher expression compared to the control, while *PhvulVIT-2* and *PhvulVIT-9* genes had significantly decreased expression. When all treatments were evaluated, a significant decrease was observed in the expression profiles of *PhvulVIT-2* and *PhvulVIT-9* genes compared to the control. The *PhvulVIT-10* gene demonstrates a notable increase, particularly when exposed to Zn and Mn stress, outperforming other treatment conditions. This highlights its potential significance in stress response mechanisms (Figure 10).

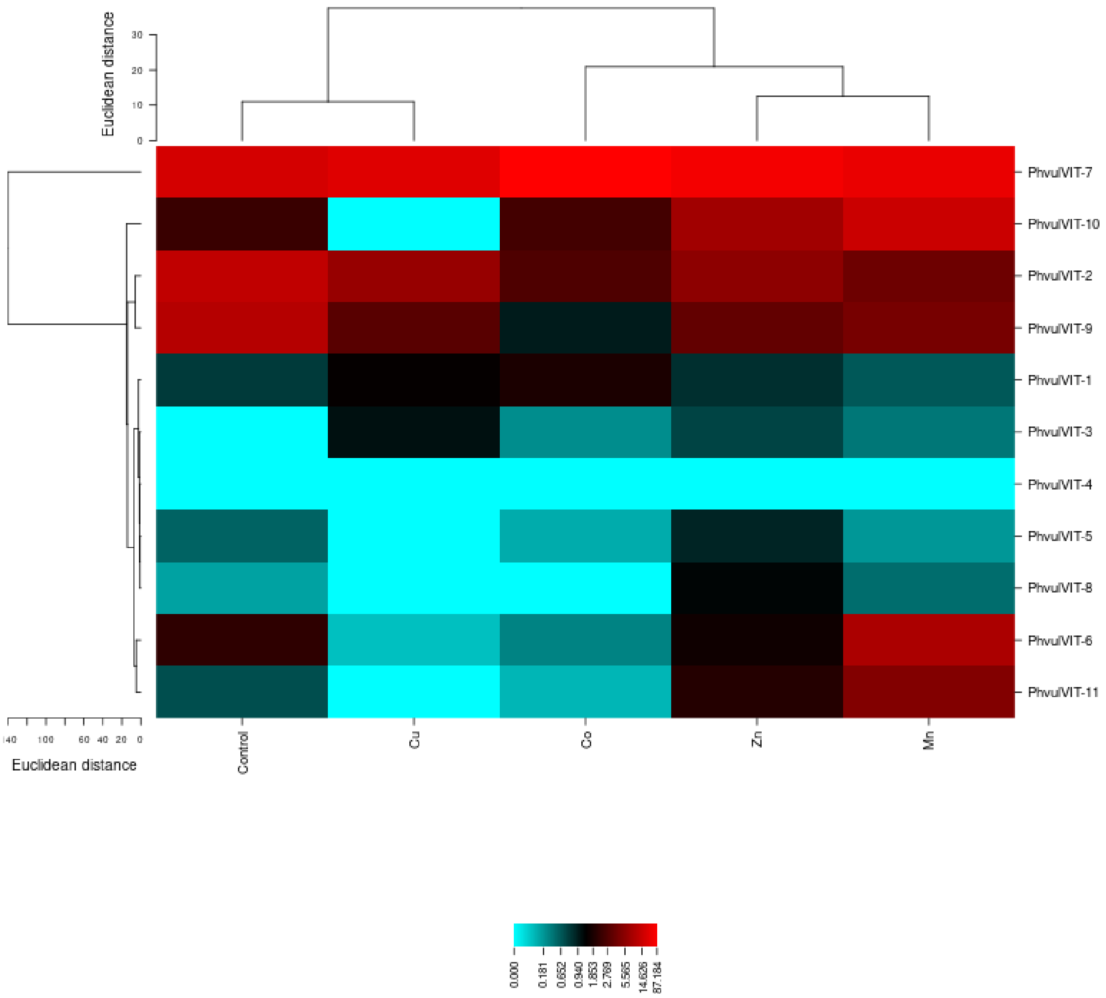


Figure 10. Figure 10. Expression profiles of *PhvulVIT* genes in *P. vulgaris* roots under heavy metal stress conditions. Each row corresponds to a specific *PhvulVIT* gene, while the columns represent different treatment conditions (Control, Cu, Co, Zn, and Mn). The color gradient indicates the expression levels of the *PhvulVIT* genes, ranging from blue (indicating low expression) to red (indicating high expression).

Sharma et al. (2020) expression study results indicate that *VTL* genes may play a role in giving tolerance to high Fe and Zn levels in soil. Furthermore, under Ni, Cd and Co stress, down-regulation of the majority of *TaVTL* genes was shown in roots, and up-regulation of the majority of *TaVTL* genes occurred in Co stress. A transgenic study characterizes *OsVIT1* and *OsVIT2* in rice, orthologs of *Arabidopsis VIT1*, as vacuolar transporters for Fe, Zn, and Mn. Disrupting *OsVIT1* and *OsVIT2* increased Fe/Zn accumulation in rice seeds while reducing levels in flag leaves, enhancing nutrient translocation (Zhang et al., 2012). Consequently, these results emphasize the crucial role of VIT/VTL proteins in maintaining Fe, Mn, and Zn homeostasis, as their altered expression profiles under stress conditions reveal differential gene responses, highlighting their potential in heavy metal stress adaptation and suggesting that VIT gene modulation could be a promising strategy for biofortifying staple crops.

4. Conclusion

This study provides a comprehensive genome-wide analysis of the vacuolar iron transporter (*VIT*) gene family in *Phaseolus vulgaris*, highlighting their critical roles in heavy metal stress tolerance. A total of 11 *PhvulVIT* genes were determined and systematically characterized, revealing significant insights into their structural features, regulatory mechanisms, and evolutionary relationships. It was also found that *PhvulVIT* genes have conserved Ccc1 domains, which are necessary for them to function as metal ion transporters. Protein-protein interaction networks revealed the involvement of PhvulVIT proteins in complicated pathways regulating homeostasis and stress responses. Homology modeling confirmed conserved 3D structures, with alpha-helices and transmembrane regions enabling efficient ion transport across vacuolar membranes. Differential expression profiles of *PhvulVIT* genes under various metal stress conditions in bean root tissues suggest their different roles in metal stress adaptation. *PhvulVIT-10* appears as a key player in responding to Zn and Mn stress, while *PhvulVIT-2* and *PhvulVIT-9* consistently exhibit decreased expression across all treatments. The study highlights the *PhvulVIT* gene family's role in enhancing stress resilience in *Phaseolus vulgaris*, paving the way for future research in biofortification, environmental stress management, and sustainable agriculture.

Conflict of Interest

The author has no financial or non-financial interests that could impact this study's research.

References

Aizaz, M., Khan, I., Lubna, Asaf, S., Bilal, S., Jan, R., Khan, A. L., Kim, K. M., & AL-Harrasi, A. (2023). Enhanced physiological and biochemical performance of mung bean and maize under saline and heavy metal stress through application of endophytic fungal strain SL3 and exogenous IAA. *Cells*, *12*(15), 1960. https://doi.org/10.3390/cells12151960

Aygören, A. S., Güneş, E., Muslu, S., Kasapoğlu, A. G., Yiğider, E., Aydın, M., Büyük I., & İlhan, E. (2023). Genome-wide analysis and characterization of *SABATH* gene family in *Phaseolus vulgaris* genotypes subject to melatonin under drought and salinity stresses. *Plant Molecular Biology Reporter*, *41*, 242-259. https://doi.org/10.1007/s11105-022-01363-5

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., Ren, J., Li, W. W., & Noble, W. S. (2009). MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Research*, *37*(suppl_2), W202-W208. https://doi.org/10.1093/nar/gkp335

Bano, Y., Khan, R., Sharma, J., & Shrivastav, A. (2019). Changes in activities of nitrogen metabolism enzymes in arsenic stressed *Phaseolus vulgaris*. *International Journal of Scientific Research and Review*, *8*(4), 336-341.

Buttanri, A., Kasapoğlu, A. G., Öner, B. M., Aygören, A. S., Muslu, S., İlhan, E., Yildirim, E., & Aydin, M. (2024). Predicting the role of *β-GAL* genes in bean under abiotic stress and genome-wide characterization of *β-GAL* gene family members. *Protoplasma*, *262*, 365-383. https://doi.org/10.1007/s00709-024-01998-z

Cao, J. (2019). Molecular evolution of the *Vacuolar Iron Transporter (VIT)* family genes in 14 plant species. *Genes*, *10*(2), 144. https://doi.org/10.3390/genes10020144

Chen, C., Wu, Y., Li, J., Wang, X., Zeng, Z., Xu, J., Liu, Y., Feng, J., Chen, H., & He, Y., (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Molecular Plant*, *16*(11), 1733-1742. https://doi.org/10.1016/j.molp.2023.09.010

Chen, X., Zhao, Y., Zhong, Y., Chen, J., & Qi, X. (2023). Deciphering the functional roles of transporter proteins in subcellular metal transportation of plants. *Planta*, *258*, 17. https://doi.org/10.1007/s00425-023-04170-8

Connorton, J. M., Jones, E. R., Rodríguez-Ramiro, I., Fairweather-Tait, S., Uauy, C., & Balk, J. (2017). Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. *Plant Physiology*, *174*(4), 2434-2444. https://doi.org/10.1104/pp.17.00672

Cui, Y., Zhao, Q., Hu, S., & Jiang, L. (2020). Vacuole biogenesis in plants: How many vacuoles, how many models? *Trends in Plant Science*, *25*(6), 538-548. https://doi.org/10.1016/j.tplants.2020.01.008

Daszkiewicz, T. (2022). Food production in the context of global developmental challenges. *Agriculture*, *12*(6), 832. https://doi.org/10.3390/agriculture12060832

Dey, S., Malviya, R., Pandey, A., Banavath, H. N., Muthamilarasan, M., & Gayen, D. (2023). Identification and expression analysis of the FtsH protein family in chickpea in response to drought stress. *ResearchSquare*, 1-34. https://doi.org/10.21203/rs.3.rs-3505392/v1

Fang, P., Hu, Y., Xia, W., Wu, X., Sun, T., Pandey, A. K., Ning, K., Zhu, C., & Xu, P. (2022). Transcriptome dynamics of common bean roots exposed to various heavy metals reveal valuable target genes and promoters for genetic engineering. *Journal of Agricultural and Food Chemistry*, *71*(1), 223-233. https://doi.org/10.1021/acs.jafc.2c06301

Gollhofer, J., Schläwicke, C., Jungnick, N., Schmidt, W., & Buckhout, T. J. (2011). Members of a small family of nodulin-like genes are regulated under iron deficiency in roots of *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *49*(5), 557-564. https://doi.org/10.1016/j.plaphy.2011.02.011

Gu, D., Andreev, K., & Dupre, M. E. (2021). Major trends in population growth around the world. *China CDC Weekly*, *3*(28), 604-613. https://doi.org/10.46234/ccdcw2021.160

Hall, C., Dawson, T. P., Macdiarmid, J. I., Matthews, R. B., & Smith, P. (2017). The impact of population growth and climate change on food security in Africa: Looking ahead to 2050. *International Journal of Agricultural Sustainability*, *15*(2), 124-135. https://doi.org/10.1080/14735903.2017.1293929

Hammami, H., Parsa, M., Bayat, H., & Aminifard, M. H. (2022). The behavior of heavy metals in relation to their influence on the common bean (*Phaseolus vulgaris*) symbiosis. *Environmental and Experimental Botany*, *193*, 104670. https://doi.org/10.1016/j.envexpbot.2021.104670

Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., & Nakai, K. (2007). WoLF PSORT: Protein localization predictor. *Nucleic Acids*

Research, *35*(suppl_2), W585-W587. https://doi.org/10.1093/nar/gkm259

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. (2015). GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics*, *31*(8), 1296-1297. https://doi.org/10.1093/bioinformatics/btu817

Inal, B., Muslu, S., Yigider, E., Kasapoglu, A., Ilhan, E., Ciltas, A., Yildirim, E., & Aydin, M., (2024). In silico analysis of *Phaseolus vulgaris* L. metalloprotease *FtsH* gene: Characterization and expression in drought and salt stress. *Genetic Resources and Crop Evolution*, *72*, 1065-1088. https://doi.org/10.1007/s10722-024-02031-1

Isıyel, M., İlhan, E., Kasapoğlu, A. G., Muslu, S., Öner, B. M., Aygören, A. S., Yigider, E., Aydin, M., & Yıldırım, E. (2024). Identification and characterization of *Phaseolus vulgaris CHS* genes in response to salt and drought stress. *Genetic Resources and Crop Evolution*, *72*, 271-293. https://doi.org/10.1007/s10722-024-01980-x

Kaur, R., Das, S., Bansal, S., Singh, G., Sardar, S., Dhar, H., & Ram, H. (2021). Heavy metal stress in rice: Uptake, transport, signaling, and tolerance mechanisms. *Physiologia Plantarum*, *173*(1), 430-448. https://doi.org/10.1111/ppl.13491

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*, 845-858. https://doi.org/10.1038/nprot.2015.053

Khalil, R., Haroun, S., Bassyoini, F., Nagah, A., & Yusuf, M. (2021). Salicylic acid in combination with kinetin or calcium ameliorates heavy metal stress in *Phaseolus vulgaris* plant. *Journal of Agriculture and Food Research*, *5*, 100182. https://doi.org/10.1016/j.jafr.2021.100182

Khoudi, H. (2021). Significance of vacuolar proton pumps and metal/H+ antiporters in plant heavy metal tolerance. *Physiologia Plantarum*, *173*(1), 384-393. https://doi.org/10.1111/ppl.13447

Kim, S. A., Punshon, T., Lanzirotti, A., Li, L., Alonso, J. M., Ecker, J. R., Kaplan, J., & Guerinot, M. L. (2006). Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. *Science*, *314*(5803), 1295-1298. https://doi.org/10.1126/science.1132563

Kosakivska, I. V., Babenko, L. M., Romanenko, K. O., Korotka, I. Y., & Potters, G. (2021). Molecular mechanisms of plant adaptive responses to heavy metals stress. *Cell Biology International*, *45*(2), 258-272. https://doi.org/10.1002/cbin.11503

Krishna, T. P. A., Ceasar, S. A., & Maharajan, T. (2023). Biofortification of crops to fight anemia: Role of vacuolar iron transporters. *Journal of Agricultural and Food Chemistry*, *71*(8), 3583-3598. https://doi.org/10.1021/acs.jafc.2c07727

Kumar, N., Sharma, V., Kaur, G., Lata, C., Dasila, H., Perveen, K., Khan, F., Gupta, V. K., & Khanam, M. N. (2023). Brassinosteroids as promoters of seedling growth and antioxidant activity under heavy metal zinc stress in mung bean (*Vigna radiata* L.). *Frontiers in Microbiology*, *14*, 1259103. https://doi.org/10.3389/fmicb.2023.1259103

Lapinskas, P. J., Lin, S. J., & Culotta, V. C. (1996). The role of the *Saccharomyces cerevisiae CCC1* gene in the homeostasis of manganese ions. *Molecular Microbiology*, *21*(3), 519-528. https://doi.org/10.1111/j.1365-2958.1996.tb02561.x

Letunic, I., & Bork, P. (2024). Interactive Tree of Life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research*, *52*(W1), W78-W82. https://doi.org/10.1093/nar/gkae268

Li, J., Zhang, M., Sun, J., Mao, X., Wang, J., Liu, H., Zheng, H., Li, X., & Zou, D. (2020). Heavy metal stress-associated proteins in rice and *Arabidopsis*: Genome-wide identification, phylogenetics, duplication, and expression profiles analysis. *Frontiers in Genetics*, *11*, 477. https://doi.org/10.3389/fgene.2020.00477

Li, T., Zhang, X. M., Gao, J. L., Wang, L., Si, L., Shu, Y. J., Guo, C. H., Lai, Y. C., Bi, Y. D., & Guo, D. L. (2023). Soybean *GmVIT1* gene confers plant tolerance to excess Fe/Mn stress. *Agronomy*, *13*(2), 384. https://doi.org/10.3390/agronomy13020384

Lisciani, S., Marconi, S., Le Donne, C., Camilli, E., Aguzzi, A., Gabrielli, P., Gambelli, L., Kunert, K., Marais, D., Vorster, B. J., Alvarado-Ramos, K., Reboul, E., Cominelli, E., Preite, C., Sparvoli, S., Losa, A., Sala, T., Botha, A. M., & Ferrari, M. (2024). Legumes and common beans in sustainable diets: Nutritional quality, environmental benefits, spread and use in food preparations. *Frontiers in Nutrition*, *11*, 1385232. https://doi.org/10.3389/fnut.2024.1385232

Mansour, M. M. F. (2023). Role of vacuolar membrane transport systems in plant salinity tolerance. *Journal of Plant Growth Regulation*, *42*, 1364-1401. https://doi.org/10.1007/s00344-022-10655-9

Noor, I., Sohail, H., Sun, J., Nawaz, M. A., Li, G., Hasanuzzaman, M., & Liu, J. (2022). Heavy metal and metalloid toxicity in horticultural plants: Tolerance mechanism and remediation strategies. *Chemosphere*, *303*(Part 3), 135196. https://doi.org/10.1016/j.chemosphere.2022.135196

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Trong, I. L., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., & Miyano, M. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*, *289*(5480), 739-745. https://doi.org/10.1126/science.289.5480.739

Peng, X., Wang, J., Peng, W., Wu, F. X., & Pan, Y. (2017). Protein–protein interactions: Detection, reliability assessment and applications. *Briefings in*

Bioinformatics, *18*(5), 798-819. https://doi.org/10.1093/bib/bbw066

Qi, M., Wang, S., Li, N., Li, L., Zhang, Y., Xue, J., Wang, J., Wu, R., & Lian, N. (2023). Genome-wide analysis of *TPX2* gene family in *Populus trichocarpa* and its specific response genes under various abiotic stresses. *Frontier Plant Science*, *14*, 1159181. https://doi.org/10.3389/fpls.2023.1159181

Ram, H., Sardar, S., & Gandass, N. (2021). Vacuolar Iron Transporter (Like) proteins: Regulators of cellular iron accumulation in plants. *Physiologia Plantarum*, *171*(4), 823-832. https://doi.org/10.1111/ppl.13363

Rasool, A., Azeem, F., Ur-Rahman, M., Rizwan, M., Hussnain Siddique, M., Bay, D. H., Binothman, N., Al Kashgry, N. A.T., & Qari, S. H. (2023). Omics-assisted characterization of two-component system genes from *Gossypium raimondii* in response to salinity and molecular interaction with abscisic acid. *Frontiers in Plant Science*, *14*, 1138048. https://doi.org/10.3389/fpls.2023.1138048

Rombauts, S., Déhais, P., Van Montagu, M., & Rouzé, P. (1999). PlantCARE, a plant *cis*-acting regulatory element database. *Nucleic Acids Research*, *27*(1), 295-296. https://doi.org/10.1093/nar/27.1.295

Salam, A., Afridi, M. S., Khan, A. R., Azhar, W., Shuaiqi, Y., Ulhassan, Z., Qi J., Xuo N., Chunyan Y., Chen N., & Gan, Y. (2023). Cobalt induced toxicity and tolerance in plants: Insights from omics approaches. In M. A. Hossain, AKM. Z. Hossain, S. Bourgerie, M. Fujita, O. P. Dhankher & P. Haris (Eds.), *Heavy metal toxicity and tolerance in plants: A biological, omics, and genetic engineering approach* (pp. 207-229). John Wiley & Sons Ltd. https://doi.org/10.1002/9781119906506.ch10

Shahid, M., Shamshad, S., Farooq, A. B. U., Rafiq, M., Khalid, S., Dumat, C., Zhang, Y., Hussain, I., & Niazi, N. K. (2019). Comparative effect of organic amendments on physio-biochemical traits of young and old bean leaves grown under cadmium stress: a multivariate analysis. *Environmental Science and Pollution Research*, *26*, 11579-11590. https://doi.org/10.1007/s11356-018-2689-4

Sharma, S. S., Dietz, K. J., & Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant, Cell & Environment*, *39*(5), 1112-1126. https://doi.org/10.1111/pce.12706

Sharma, S., Kaur, G., Kumar, A., Meena, V., Ram, H., Kaur, J., & Pandey, A. K. (2020). Gene expression pattern of vacuolar-iron transporter-like (VTL) genes in hexaploid wheat during metal stress. *Plants*, *9*(2), 229. https://doi.org/10.1101/863084

Shekhawat, P. K., Sardar, S., Yadav, B., Salvi, P., Soni, P., & Ram, H. (2023). Meta-analysis of transcriptomics studies identifies novel attributes and set of genes involved in iron homeostasis in rice. *Functional & Integrative Genomics*, *23*, 336. https://doi.org/10.1007/s10142-023-01265-z

Silva-Gigante, M., Hinojosa-Reyes, L., Rosas-Castor, J. M., Quero-Jiménez, P. C., Pino-Sandoval, D. A., & Guzmán-Mar, J. L. (2023). Heavy metals and metalloids accumulation in common beans (*Phaseolus vulgaris* L.): A review. *Chemosphere*, *335*, 139010. https://doi.org/10.1016/j.chemosphere.2023.139010

Singh, P., & Mukhopadhyay, K. (2021). Comprehensive molecular dissection of TIFY Transcription factors reveal their dynamic responses to biotic and abiotic stress in wheat (*Triticum aestivum* L.). *Scientific Reports*, *11*, 9739. https://doi.org/10.1038/s41598-021-87722-w

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., & Pyysalo, S., (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, *51*(D1), 638-646. https://doi.org/10.1093/nar/gkac1000

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Voorrips, R. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1), 77-78. https://doi.org/10.1093/jhered/93.1.77

Wainaina, I., Wafula, E., Sila, D., Kyomugasho, C., Grauwet, T., Van Loey, A., & Hendrickx, M. (2021). Thermal treatment of common beans (*Phaseolus vulgaris* L.): Factors determining cooking time and its consequences for sensory and nutritional quality. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 3690-3718. https://doi.org/10.1111/1541-4337.12770

White, S. H., & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annual Review of Biophysics and Biomolecular Structure*, *28*, 319-365. https://doi.org/10.1146/annurev.biophys.28.1.319

Zhang, Y., Xu, Y. H., Yi, H. Y., & Gong, J. M. (2012). Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *The Plant Journal*, *72*(3), 400-410. https://doi.org/10.1111/j.1365-313x.2012.05088.x

Zhu, W., Zuo, R., Zhou, R., Huang, J., Tang, M., Cheng, X., Liu, Y., Tong, C., Xiang, Y., Dong, C., & Liu, S. (2016). Vacuolar iron transporter *BnMEB2* is involved in enhancing iron tolerance of *Brassica napus*. *Frontiers in Plant Science*, *7*, 1353. https://doi.org/10.3389/fpls.2016.01353