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Genome-Wide Analysis and Characterization of the *PIF* Gene Family Under Salt and Drought Stress in Common Beans (*Phaseolus vulgaris* L.)

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Abstract: The purpose of this study was to identify and describe members of the phytochrome-interacting factors (*PIF*s) gene family including the basic helix loop helix (bHLH) binding site in *Phaseolus vulgaris* plants, as well as to investigate their responses to salt and drought stress. Various tools of in silico approaches were used to identify five *Pvul-PIF* gene families in the *P. vulgaris* genome. This gene family contained 324 to 726 amino acids and has molecular weights ranging from 35.11 kDa to 77.67 kDa. The theoretical isoelectric points range from 6.03 (*Pvul-PIF-3.3*) to 8.30 (*Pvul-PIF-3.2*). *Pvul-PIF* proteins were shown to be clustered in three main groups with *Arabidopsis thaliana*, *Populus trichocarpa*, *Solanum lycopersicum*, *Zea mays*, *Arachis hypogaea* L., *Oryza sativa, Vitis vinifera, Glycine max*, and *Phaseolus vulgaris* species as a result of the phylogenetic study. Segmental duplication was detected between *Pvul-PIF-3.2*, *Pvul-PIF-3.3* and *Pvul-PIF-3.1* genes, *Pvul-PIF-4.1* and *Pvul-PIF-4.2* genes and *Pvul-PIF-3.3* and *Pvul-PIF-3.1* genes. When the expression patterns of the *Pvul-PIF* genes were examined, it was observed that they had different levels of expression under salt and drought stress and that they may be involved in specific biological and molecular processes in response to different abiotic and biotic stresses. The results of this research, which were established for the first time in response to salt and drought stress in *P. vulgaris* of the *PIF* gene family, will be a valuable source of knowledge and additional information in the fields of plant biotechnology, agricultural biotechnology, and molecular biology.

Keywords: Basic helix loop helix, gene structure, in silico analysis, phylogenetic analysis, phytochrome, transcription factor

1. Introduction

Phaseolus vulgaris L. (common beans) belonging to the Fabaceae (legume) family plays a key part in supplying the nutritional requirements of the world's growing population. It is also a significant protein source in human nutrition (De Ron et al., 2015). Legumes are the world's third biggest plant family, with 640 genera divided into 40 orders (Büyük, 2014). Of all major food crops, the plant with the most variability in the growing medium, seed quality, and maturity period is common beans. Due to its adaptability, it could be cultivated in a variety of agricultural systems and ecosystems, including Europe, Africa, China, the Americas, and the Middle East (Blair et al., 2010). Although beans can be grown as fresh broad beans and grains, they are mostly produced and consumed as dried grains. Because of their health benefits and potential to prevent illnesses in humans, beans have lately acquired favor as a functional meal. When included in diets, it has also been linked to a decreased risk of cardiac disease, diabetes, obesity, and breast, intestinal, and prostate cancers (Correa, 1981; Hangen and Bennink, 2002; Thompson et al., 2009). The high fiber and starch content, ability to handle glycemia and digestive function and antioxidant characteristics supplied by phenolic compounds and proteins are all factors that

contribute to these health benefits (De Ron et al., 2015).

Drought occurrences are expected to cause considerable losses in atmospheric evaporation, which are expected to grow significantly due to changing climatic conditions (Teuling et al., 2013). Due to the obvious inability to deliver the water required for agricultural productivity growth and development, yield losses will occur. In this context, it is critical to conduct urgent investigations on the drought tolerance of plants that are intended to be used in agricultural areas. This issue has been examined by many researchers (Carnicer et al., 2011; Peng et al., 2011; Park Williams et al., 2013; Vicente-Serrano et al., 2014).

Plants adapt to physiological and metabolic changes in the least harmful way feasible depending on the abiotic stress (Kalefetoğlu and Ekmekçi, 2005). However, despite the fact that these changes under abiotic stress conditions have been studied for many years, the processes behind them are still unknown. To better understand how plants respond to drought stress and enhance the tolerance of agriculturally important plants to diverse pressures, more research and creative approaches are needed (Örs and Ekinci, 2015).

At both cellular and plant levels, the tolerance of plants to salt stress is extremely complicated. Numerous morphological, physiological, and metabolic changes take place throughout the defense process (Ashraf and Harris, 2004). Especially plant physiology is negatively impacted by salinity in three main ways. As a result of the elevated sodium ion concentration, protein synthesis, enzyme activity, photosynthesis, and respiration are all inhibited as well as cellular organelles are damaged. Second, salt inhibits the uptake of nutrients from the soil, leading to a nutrient imbalance. Third, salinity lowers the osmotic potential of soil and hinders roots from absorbing water, which causes a physiological drought in the plant (Ruiz-Lozano et al., 2012).

Light not only acts as a signal playing a significant role in plant development, but it also generates energy for photosynthesis and regulates a wide range of photomorphogenesis and shadow aversion including physiological processes (Casal et al., 2014; Xu et al., 2015). Numerous major elements participating in light signal transmission channels have been discovered during the last two decades utilizing genetic and molecular techniques (Pham et al., 2018). Phytochromes function similarly to photoreceptors, receiving and transmitting red and far-red light signals to govern a variety of plant growth and developmental processes such as seed de-etiolation, seed germination, and flowering (Shin et al., 2016; Han et al., 2017). Phytochromes are generated in the cytoplasm and transported to the nucleus after being exposed to red light, where they interact with numerous phytochrome-interacting proteins to activate light signaling cascades (Paik and Huq, 2019).

Phytochrome-interacting factor (*PIF*), a member of the basic helix loop helix transcription factor (bHLH TF) subgroup, has been demonstrated to be a major transcriptional controller in response to environmental and light changes (Hao et al., 2021). Because of the rapid development of high-throughput sequencing methods and the increasing number of available genome sequences, bHLH TF has been discovered in genes of various *PIF* family members ranging from low plants to higher plants. For instance, one *MpPIF* gene in *Marchantia polymorpha* (Inoue et al., 2016), and seven different *ZmPIF* genes in maize (Gao et al., 2019; Wu et al., 2019) have been characterized. In *A. thaliana*, 8 *AtPIF* genes were discovered (Lee and Choi, 2017), and these genes were observed to be more studied than *PIF* genes discovered and defined in other plants. Extensive research and investigations have indicated that the members of the PIF gene family in *A. thaliana* play many key roles in the physiological phases of plants and that plants may adapt to changing environmental conditions via different signaling pathways, along with light, hormones, and abiotic stresses like cold, drought, and salt (de Lucas and Prat, 2014; Lin et al., 2018; Xu, 2018).

Regardless of the fact that the *PIF* gene family is vital in light signaling pathways, the role of *PIF* in beans under drought and salinity stresses has not been functionally described. Therefore, this study aimed to identify and describe members of the *PIF*s gene family including the bHLH binding site in *P. vulgaris* plants, as well as to investigate their responses to salt and drought stress.

2. Materials and Methods

2.1. Identification, sequence alignment, and phylogenetic analysis of *PIF* proteins in the bean genome

The protein sequences of the *PIF* gene family in the *P. vulgaris* genome (Schmutz et al., 2014) were received from the Phytozome database v13 (Anonymous, 2021a) with the Pfam Accession Number (PF00010) from the Pfam database (Anonymous, 2021b). *P. vulgaris*, *Populus trichocarpa* (Tuskan et al., 2006), *Oryza sativa* (Ouyang et al., 2007), *Vitis vinifera* (Jaillon et al., 2007), *Arabidopsis thaliana* (Lamesch et al., 2012), *Glycine max* (Valliyodan et al., 2019), *Arachis*

hypogaea (Bertioli et al., 2019) *Solanum lycopersicum* (Hosmani et al., 2019), and *Zea mays* (Bornowski et al., 2021) were used in both the blastp in the Phytozome database v13 and the Hidden Markov Model (HMM) (Anonymous, 2021c) to discover all potential *PIF* proteins. Using the HMMER database (Anonymous, 2021d), the existence of the *PIF* domain in the related sequences was explored. The ProtParam tool (Anonymous, 2021e) was used to calculate the molecular weight, amino acid number, and isoelectric point (pI) of the obtained *PIF* proteins.

After alignment of the *Pvul-PIF* protein sequences with ClustalW (Thompson et al., 1997), the phylogenetic tree was generated in MEGA v11 (Tamura et al., 2021) using the Neighbor-Joining tree (1000 repeated bootstrap value and Poisson model) method. The Interactive Tree of Life (iTOL) v6 interface was used to visualize the phylogenetic tree (Letunic and Bork, 2011).

2.2. Structure of *Pvul-PIF* genes, physical locations, gene duplications, comparative mapping, and identification of conserved motifs

Pvul-PIF proteins exon-intron regions were investigated using Gene Structure Display Server v2.0 (Hu et al., 2015).

The Phytozome database v13 was used to find the chromosomal locations of the *PIF* gene. *Pvul-PIF* genes were identified and mapped on all *P. vulgaris* chromosomes using MapChart. (Voorrips, 2002). Gene duplications between *P. vulgaris*, *G. max*, and *A. thaliana* were identified using the MCScanX Toolkit (Wang et al., 2012). The substitution rates between duplicate pairs of *Pvul-PIF* genes for non-homologous (Ka), homologous (Ks), and non-homologous to homologous (Ka/Ks) were calculated using the PAL2NAL (Suyama et al., 2006) interface tools in PAML software (Yang, 2007). A synteny map of the *PIF* genes discovered in *P. vulgaris*, *G. max*, and *A. thaliana* was created using the TBtools software (Chen et al., 2020). Then, the time of duplication and cleavage of each *PIF* gene (million years ago) was calculated according to the following Equation 1 (Yang and Nielsen, 2000; Lynch and Conery, 2003). The λ value used in Equation 1 was $6.56E^{-9}$.

$$T = Ks/2\lambda \quad (1)$$

The Multiple Em for Motif Elicitation (MEME) Suite was used to find conserved motifs in *Pvul-PIF* proteins (Bailey et al., 2006). The MEME Suite tool was adjusted site distribution, motif count, motif sites, and, motif width to any number of repeats, 10, the most 300 sites and, between 6-50 wide, respectively. The detected motifs were scanned using the InterProScan with default parameters (Quevillon et al., 2005). In addition, using the WebLogo online web tool, sequence logo analysis of *PIF* domains for conserved area sequence analysis was created (Crooks et al., 2004).

2.3. Promoter analysis and subcellular localization of the bean *PIF* gene family

Using the PlantCARE (Lescot et al., 2002) web interface, cis-acting element analysis was performed for each gene separately after reaching the 2000 bp (base pair) upstream regions of the *Pvul-PIF* genes using the Phytozome database v13. TBTools (Chen et al., 2020) were used to build the phenogram. WoLF PSORT was used to determine their subcellular localizations (Horton et al., 2007).

2.4. Homology modeling of *PIF* proteins in common bean

The *PIF* protein sequences were found and uploaded into the Phyre2 database, and all *Pvul-PIF* proteins were estimated by 3D modeling (Kelley et al., 2015). Protein models with a confidence level of more than 95% were visualized.

2.5. *Pvul-PIF* protein-protein interactions (PPI)

The STRING database (Anonymous, 2021f) was utilized to determine protein-protein interactions at a physical, functional, and experimental level. The Cytoscape (Shannon et al., 2003) program was used to classify and show the data obtained.

2.6. In silico gene expression analysis

The Sequence Read Archive (SRA) data library in The National Center for Biotechnology Information (NCBI) database was used to obtain Illumina RNA-seq data. To find relevant RNA-seq data, we used accession numbers for salt and drought stress. Salt stress-treated leaf (SRR957668), leaf salt control (SRR958469) (Hiz et al., 2014), drought stress-treated leaf (SRR8284481), and leaf drought control (SRR8284480) (Anonymous, 2021g) were used. The Read per Kilobase (RPKM) method was used to normalize gene expression levels (Mortazavi et al., 2008). A heatmap was drawn using the CIMMiner tool (Anonymous, 2021h).

3. Results and Discussion

3.1. *PIF* gene family characteristics discovered in the *P. vulgaris* genome

The members of the *PIF* gene family were searched in the *P. vulgaris* genome found in the Phytozome database v13 utilizing the PFAM

accession number (PF00010). As an outcome of this research, 5 *PIF* genes were discovered in the common bean genome. The chromosome locations of the *Pvul-PIF* genes, as well as their start and end position, protein lengths, isoelectric points, molecular weights, instability indices, and intracellular localization was given in Table 1. In the bean genome, the identified *Pvul-PIF* genes have been located on chromosomes 1, 6, 7, and 8 (Figure 1). *PIF* proteins have a length of 324-726 amino acids. With 726 residues, *Pvul-PIF-3.3* has the most amino acids, whereas *Pvul-PIF-3.1* has the lowest, with 324 residues. Furthermore, all of the discovered genes were found to be unstable, with instability indices ranging from 45.38 to 62.93. Except for *Pvul-PIF-3.1* and *-3.2*, theoretical isoelectric points of all genes were found to be in the acidic range, and it is ranging from 6.04 to 8.30. *PIF* proteins have a molecular weight of 35.11-77.67 kDa. *Pvul-PIF-3.3* had the largest molecular weight as 77.67 kDa, while *Pvul-PIF-3.1* had the lowest as 35.11 kDa. As seen in Table 1, the subcellular locations of *PIF* genes were detected in different cell components such as the chloroplast, cell membrane, and cytoskeleton, according to data obtained from the WoLF PSORT database. In the *PIF* gene family, which has been characterized and identified genome-wide in different species, 8 in *S. lycopersicum* (Rosada et al., 2016), 8 in *A. thaliana* (Lee and Choi, 2017), 4 in *V. vinifera* (Zhang et al., 2018), 15 in *Z. mays* (Shi et al., 2018), 14 in *G. max* (Arya et al., 2018), 30 in *Brassica napus*, 12 in *Brassica rapa*, and 18 in *Brassica oleracea* (Li et al., 2021), and 14 *PIF* genes in *Arachis hypogaea* (Wang et al., 2021) were identified.

Table 1. The 5 *Pvul-PIF* identified in *Phaseolus vulgaris* L. and their sequence characteristics

Gene name	Phytozome ID	Chn	Start	End	Strand	AA length	MW (kDa)	pI	Instability index	(WolfPsort)*
Pvul-PIF-3.2	Phvul.001G218800	Chr01	47432918	47435728	Forward	549	61.25	8.30	56.04	nucl: 9, chlo: 3, plas: 1, cysk: 1
Pvul-PIF-4.1	Phvul.006G028500	Chr06	10725080	10730961	Forward	550	60.92	6.21	57.98	nucl: 14
Pvul-PIF-3.1	Phvul.007G208500	Chr07	33081018	33083312	Reverse	324	35.11	8.17	45.38	nucl: 14
Pvul-PIF-3.3	Phvul.007G206000	Chr07	32844497	32849427	Forward	726	77.67	6.04	58.24	nucl: 14
Pvul-PIF-4.2	Phvul.008G196800	Chr08	54108236	54113590	Forward	549	60.64	6.15	62.93	nucl: 14

*WoLF PSORT predictions: chlo: Chloroplast, nucl: Nucleus, plas: Plasma membrane, cysk: Cytoskeleton, Chn: Chromosome number, AA: Aminoacid, MW: Molecular weight, pI: Theoretical isoelectric point

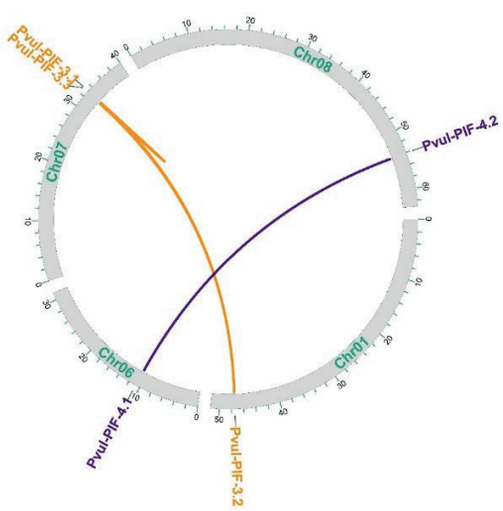

Figure 1. Chromosomal distributions of *Pvul-PIF* genes

Purple and orange colors indicate segmental duplication between *Pvul-PIF* genes

As a result of gene duplication analysis, *Pvul-PIF3.2*/*Pvul-PIF3.3*, *Pvul-PIF3.2*/*Pvul-PIF3.1*, *Pvul-PIF4.1*/*Pvul-PIF4.2*, and *Pvul-PIF3.3*/*Pvul-PIF3.1* were identified as segmentally-duplicated gene pairs and Ka, Ks and Ka/Ks ratios are shown in Table 2. Positive selection in the evolutionary process is indicated by a Ka/Ks value larger than 1, purifying selection is indicated by a Ka/Ks value less than 1, and natural selection in duplication events is indicated by a Ka/Ks value equal to 1 (Juretic et al., 2005; Kasapoğlu et al., 2020).

3.2. Interspecies phylogenetic analysis of Pvul-PIF proteins, conserved motif, and gene structure display

PIF protein sequences from *P. vulgaris*, *A. thaliana*, *G. max*, *P. trichocarpa*, *V. vinifera*, *Z. mays*, *O. sativa*, *S. lycopersicum*, and *A. hypogaea* species were utilized to create a phylogenetic tree to analyze the evolutionary relationship of *PIF* gene family. The phylogenetic

Table 2. Gene duplications of *Pvul-PIF* genes

Gene 1	Gene 2	Ka	Ks	Ka/Ks	MYA	Selection pressure	Duplication type
Pvul-PIF-3.2	Pvul-PIF-3.3	0.82	9.77	0.08	577.8	purifying	segmental
Pvul-PIF-3.2	Pvul-PIF-3.1	0.38	1.37	0.28	81.24	purifying	segmental
Pvul-PIF-4.1	Pvul-PIF-4.2	0.22	0.53	0.42	31.43	purifying	segmental
Pvul-PIF-3.3	Pvul-PIF-3.1	0.66	0.66	0.14	265.6	purifying	segmental

MYA: Million years ago

tree of 43 *PIF* proteins from nine plant species was analyzed using the MEGA 11 software and the Neighbor-Joining: NJ method. *PIF* proteins were divided into three groups, as indicated in Figure 2. A close relationship between *A. thaliana*, *Z. mays*, *G. max*, *A. hypogaea*, and *Pvul-PIF-3.1* and *Pvul-PIF-3.2* proteins in Group A was observed. *Pvul-PIF-3.3* and *S. lycopersicum*, *A. hypogaea*, *P. trichocarpa*, *V. vinifera*, *G. max* proteins showed orthology in Group B. It was observed to be a close relationship between *Z. mays* and *O. sativa* proteins and *Pvul-PIF-4.1 and Pvul-PIF-4.2* proteins in Group C separately from other groups. The 14 *AhPIFs* genes identified in the phylogenetic tree classification of the peanut (*A. hypogaea*) were divided into four groups according to Wang et al. (2021), and the genes associated with *PIF*-3 and *PIF*-4 were closely related to different plants in different groups. *PIF* genes are commonly divided into three or four groups in terms of evolution, and these genes are mainly related to *A. thaliana* and *G. max* species, according to this information.

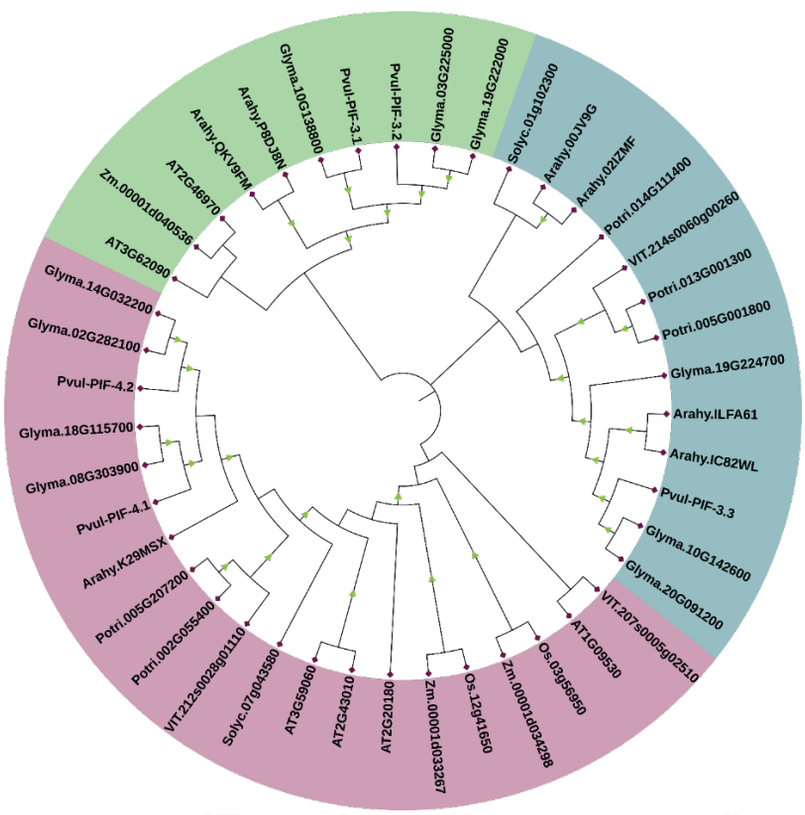

Figure 2. The phylogenetic tree of PIF genes of *P. vulgaris*, *P. trichocarpa*, *O. sativa*, *V. vinifera*, *A. thaliana*, *G. max*, *A. hypogaea*, *S. lycopersicum,* and *Z. mays*

The phylogenetic tree was drawn with PIF proteins from nine plant species. PIF full-length amino acid sequences from *P. vulgaris* and 9 other plant species were aligned with ClustalW and the phylogenetic tree was constructed using the MEGA v11 software by the Neighbor-Joining (NJ) method with 1000 bootstrap. Pvul-PIF subfamilies, groups A, B, and C, are marked green, blue, and pink, respectively.

With conserved motif analyses of *Pvul-PIF* proteins, 10 conserved motifs were discovered. The length of the identified motifs was found to be between 15 to 50 amino acids (Figure 3). *Pvul-PIF-4.1* and *Pvul-PIF-4.2* (9 motifs) were determined to have the most motifs, whereas *Pvul-PIF-3.1* and *Pvul-PIF-3.3* had the lowest motifs (5 motifs). The WEBLOGO was used to determine the conserved domain sequences and motif logos of *PIF* gene families from *P. vulgaris* species (Figure 4). Furthermore, based on the best possible match data, *Pvul-PIF* motifs were found to have the best match with motifs 1 and 10 (Table 3).

The structural analysis of *Pvul-PIF* genes using the GSDS v2.0 database revealed intron numbers and exon sizes (bp). *Pvul-PIF* has 24 introns and 29 exons, according to the results of the study (Figure 5). In terms of exon numbers, *Pvul-PIF-4.1* had the most with 8, while *Pvul-PIF-4.2* had the lowest with 3 exons. In contrast to Wang et al. (2021) work, *Pvul-PIF* genes in the same group had varied intron-exon distribution patterns. They also discovered that *PIF4* members from peanut (*A. hypogaea* L.) had 7 to 8 introns with similar lengths and positions.

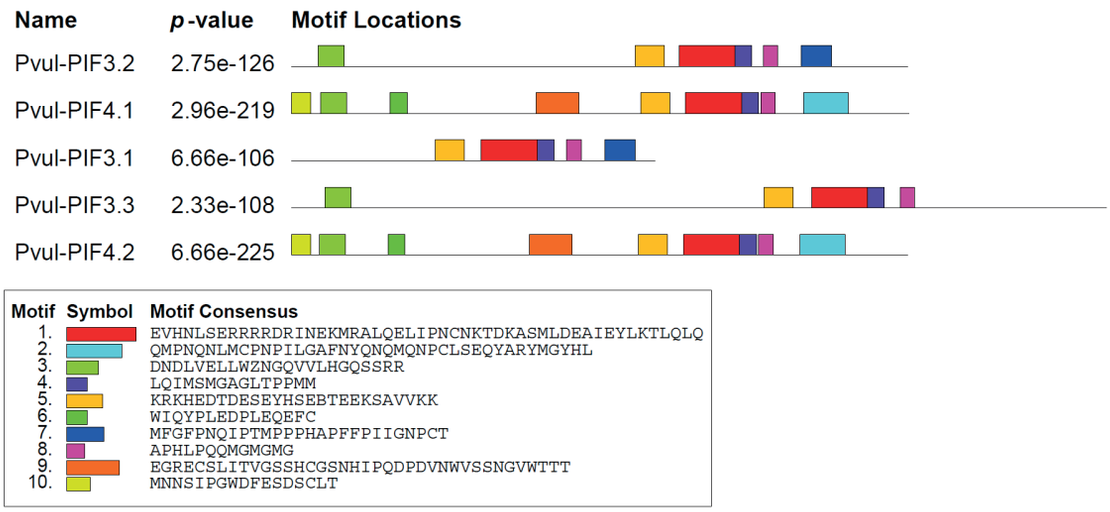

Figure 3. Predicted motif distribution in *Pvul-PIF genes*

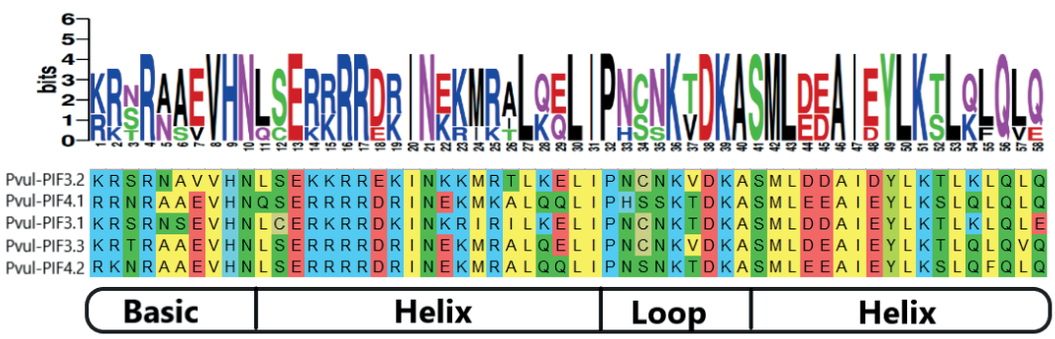

Figure 4. Conserved Basic-Helix-Loop-Helix of *Pvul-PIF* genes domains

Table 3. Estimated best-possible match information in *Pvul-PIF* genes

Motif id	Width	Possible best match	Contains domain
1	50	EVHNLSERRRRDRINEKMRALQELIPNCNKTDKASMLDEAIEYLKTLQLQ	*PIF*-3-like transcription factor
2	40	QMPNQNMMCQNPILGAFNYQNQMQNPCLSEQYARYMGYHL	N/A
3	23	DQDLVELVWQNGQVVVHGQSHRR	N/A
4	15	LQIMWMGAGMWPPMF	N/A
5	26	KRKHKDIDYSEYHSEDTEEKEAVVKK	N/A
6	15	WIQYPLDDPLEQEFC	N/A
7	27	MFGFPNQIPTMHMPHAPFFPIIGNPCT	N/A
8	13	IPHYPQQMGMGMG	N/A
9	38	EGRECSVITVGSSYCGSNHIPQDQDVNWVSSNGVWTTT	N/A
10	17	MNNTIPDWDFESDTCVT	*PIF*-3-like transcription factor

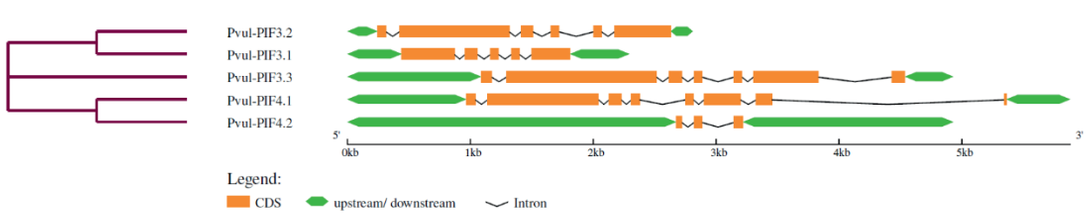

Figure 5. Exon and intron number, length, and position in *Pvul-PIF* genes

3.3. Comparative and synteny analyses of the *Pvul-PIF* gene family

A synteny map was created using *PIF* proteins from *P. vulgaris*, *A. thaliana*, and *G. max* plants. A relationship was found between *P. vulgaris* and *A. thaliana* (Figure 6), and between *P. vulgaris* and *G. max* (Figure 7), in the synteny analysis. Seven syntenic relationships were detected between *P. vulgaris* and *A. thaliana PIF* genes. Orthology was detected between the *At-PIF3.2-Pvul-PIF3.2*, *At-PIF3.4-Pvul-PIF3.2*, *At-PIF3.1-Pvul-PIF3.3*, *At-PIF3.2-Pvul-PIF3.1*, *At-PIF3.2-Pvul-PIF3.3*, *At-PIF3.4-Pvul-PIF3.1*, and *At-PIF4.1-Pvul-PIF4.2* genes. Ten syntenic relationships were defined between *P. vulgaris* and *G. max PIF* genes. Orthology was detected between *Gm-PIF3.2-Pvul-PIF3.2*, *Gm-PIF3.3-Pvul-PIF3.2*, *Gm-PIF3.1-Pvul-PIF3.2*, *Gm-PIF4.3-Pvul-PIF4.1*, *Gm-PIF4.4-Pvul-PIF4.1*, *Gm-PIF4.1-Pvul-PIF4.1*, *Gm-PIF4.2-Pvul-PIF4.1*, *Gm-PIF3.2-Pvul-PIF3.1*, *Gm-PIF3.3-Pvul-PIF3.1*, and *Gm-PIF3.1-Pvul-PIF3.1* genes.

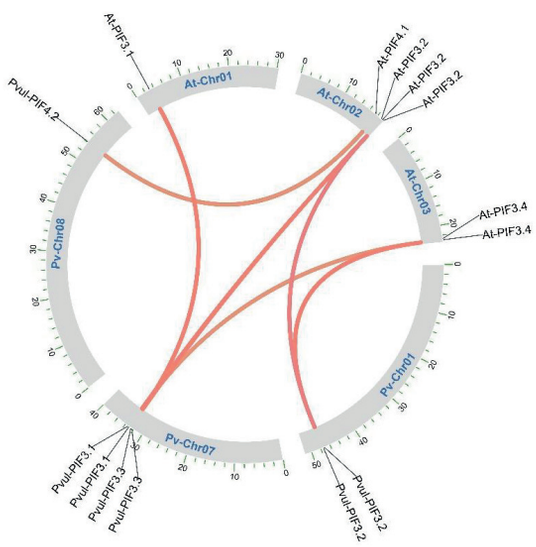

Figure 6. Syntenic relationship between *P. vulgaris* and *A. thaliana* genes

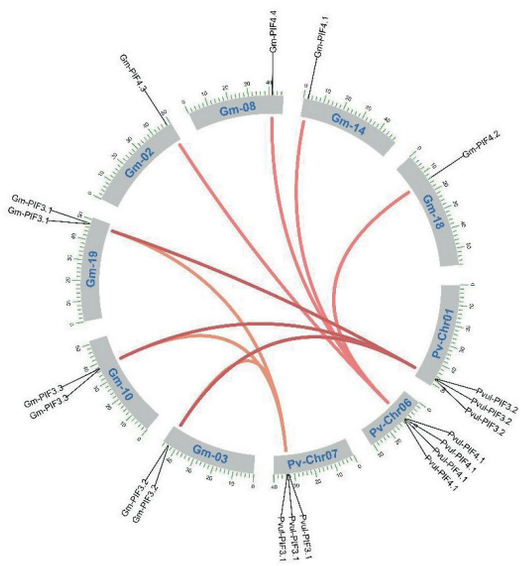

Figure 7. Syntenic relationship between *P. vulgaris* and *G. max* genes

3.4. Promotor analysis of *Pvul-PIF* genes

The sequences acquired from the PIF genes 2000 bp upstream region were investigated. The promoter regions of the PIF genes were discovered to be beneficial in plant growth, the molecular response to abiotic stresses, and adaptability to environmental conditions. The cis-acting elements in the sequences of the *Pvul-PIF* genes were determined as a result of the analyzes performed in the PlantCARE database and visualized with the TBTools software (Figure 8). It was found that the *Pvul-PIF* genes contained 65 cis-acting elements based on the data. The data showed that elements related to photosensitive elements, such as the AE-box, G-box, I-box, and Box 4, were found in all *Pvul-PIF* genes. In addition, elements associated

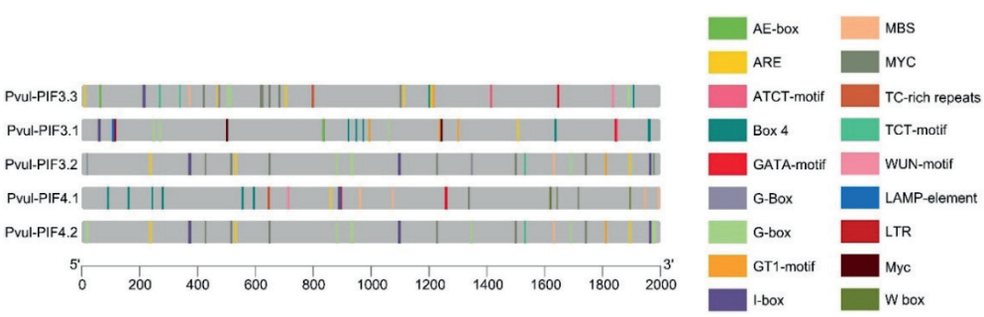

Figure 8. Promotor regions of *Pvul-PIF* genes

The promoter sequences (-2000 bp) of 5 *Pvul-PIF* genes were analyzed with the help of the PlantCARE database. The scale indicates the length of the upstream along with the translation codon. Different colored boxes indicate different cis-acting elements.

with abiotic and biotic stresses, such as MBS, ARE, W box, LTR, and TC-rich repeats, were identified in all *Pvul-PIF* genes. The predicted cis-acting elements in *V. vinifera* differed between species and genes according to Zhang et al. (2018). In this context, they found that light-sensitive cis-acting elements are the most common elements in plants in general. In the same study, they determined that there are 9 cis-acting elements in the *VvPIF* gene.

3.5. 3D homology modeling of *Pvul-PIF* genes and protein-protein interactions

BLASTP was used to scan *PIF* proteins from the Protein Data Bank (PDB), and the 3D homology modeling of these proteins was visualized in the Phyre2 database, which is used to collect information about the structure and functions of *PIF* proteins. The 3D homology patterns of the *PIF* proteins identified in this research were shown in Figure 9. As a result of the data obtained from the STRING database, the protein-protein interactions of the identified *PIF* proteins were visualized in Figure 10 using the Cytoscape tool.

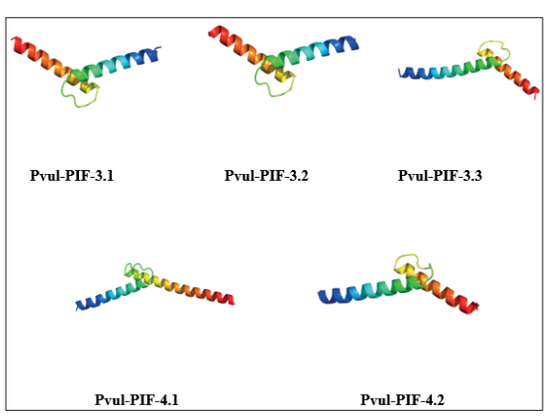

Figure 9. 3D structure modeling of Pvul-PIF proteins

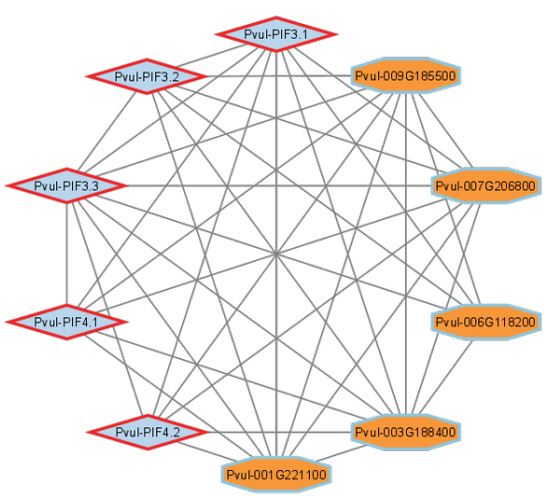

Figure 10. Protein-protein interactions (PPI) of the identified PIF proteins

3.6. In silico expression analysis of *Pvul-PIF* genes in stress conditions

For in silico expression analysis of the common bean *PIF* genes for salt and drought stress, NCBI SRA database (Sequences Read Archive) RNAseq data from SRR957668 (salt stress treated leaf), SRR958469 (leaf salt control), SRR8284481 (drought stress treated leaf), and SRR8284480 (leaf drought control) were used. The expression levels of the *Pvul-PIF* genes under salt and drought stresses were visualized in Figure 11 according to the heatmap generated by the log2 transformation of the RPKM values based on the results obtained from the RNAseq data. In this context, *Pvul-PIF-3.3*, *Pvul-PIF-4.1*, and *Pvul-PIF-4.2* were the genes in which expression levels increased in response to salt stress. On the other hand, *Pvul-PIF-3.1* was the gene of which expression levels decreased in response to drought stress.

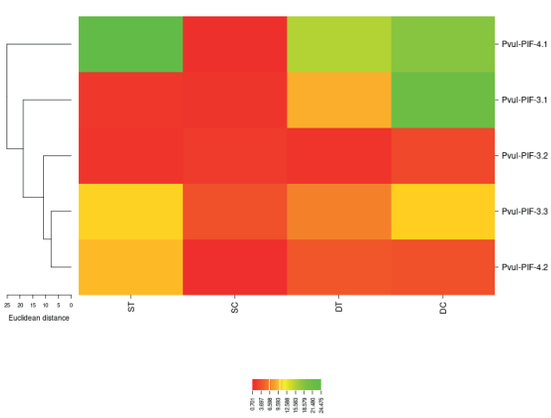

Figure 11. Heat map of differentially expressed *Pvul-PIF* genes in leaf tissue under salt and drought stress

C: Control, T: Treatment, S: Salt, D: Drought. The expression levels were represented according to the color bar

In a study conducted on *B. napus*, Li et al. (2021) found that three *BnaPIF4* genes (*BnaC03g23970D*, *BnaA03g19970D*, and *BnaC04g48630D*) were expressed at higher levels in the outer parts of the flower than in other organs and tissues. They also reported that two Bna*PIF5* (*BnaC08g29580D* and *BnaA09g37540D*) and three *BnaPIF2* (*BnaC04g52060D*, *BnaA03g21570D*, and *BnaA05g00920D*) genes were predominantly expressed in leaves and stems.

4. Conclusions

The common bean is a major leguminous crop used to supply significant nutritional requirements and a source of protein. There is a growing demand for the creation of economically important high yielding and adaptable to changing environmental circumstances types. It is vital to understand plant growth, as well as their reactions to salt and drought

stress produced by global warming, to enhance agricultural output.

This study provided a thorough examination of the *PIF* gene family in common beans (*P. vulgaris* L.). We identified 5 *PIF* genes in the *P. vulgaris* genome. These members were spread across four separate chromosomes. The gene structure, motif, and Ka/Ks findings suggest that the majority of *Pvul-PIF* were substantially conserved. According to evolutionary and synteny analysis, the majority of the genes exhibited a one-to-one homologous relationship between the *A. thaliana* and *G. max* genomes. At the time of segmental duplication and purifying selection, *PIF*s may have aided common bean species' growth and development.

This study focused on the PIF gene family, which is regulated by phytochromes and is necessary for plant photomorphogenesis. Furthermore, the salt and drought stress responses of determined gene family members were studied. We believe that this study, which was conducted for the first time on *P. vulgaris* species, will be a valuable resource for scientists conducting research in the fields of agricultural biotechnology, plant biotechnology, and molecular biology.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

References

Anonymous, 2021a. Phytozome Database. (https://phytozome-next.jgi.doe.gov/), (Accessed: 20.11.2021).

Anonymous, 2021b. Pfam Database. (http://pfam.xfam.org/family/PF00010), (Accessed: 21.11.2021).

Anonymous, 2021c. Hidden Markov Model (HMM). (http://www.ebi.ac.uk), (Accessed: 21.11.2021).

Anonymous, 2021d. HMMER. (http://www.ebi.ac.uk), (Accessed: 21.11.2021).

Anonymous, 2021e. The ProtParam Tool. (https://web.expasy.org/protparam/), (Accessed: 22.11.2021).

Anonymous, 2021f. String Database. (https://string-db.org/), (Accessed: 20.12.2021).

Anonymous, 2021g. Sequence Read Archive. (https://www.ncbi.nlm.nih.gov/bioproject-/PRJNA508605), (Accessed: 21.12.2021).

Anonymous, 2021h. CIMMiner. (http://discover.nci.nih.gov/cimminer), (Accessed: 25.12.2021).

Arya, H., Singh, M.B., Bhalla, P.L., 2018. Genomic and molecular analysis of conserved and unique features of soybean PIF4. *Scientific Reports*, 8: 12569.

Ashraf, M.P.J.C., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166(1): 3-16.

Bailey, T.L., Williams, N., Misleh, C., Li, W.W., 2006. MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Research*, 34: W369-W373.

Bertioli, D.J., Jenkins, J., Clevenger, J., Dudchenko, O., Gao, D., Seijo, G., Leal-Bertioli, S.C.M., Ren, L., Farmer, A.D., Pandey, M.K., Samoluk, S.S., Abernathy, B., Agarwal, G., Ballén-Taborda, C., Cameron, C., Campbell, J., Chavarro, C., Chitikineni, A., Chu, Y., Dash, S., El Baidouri, M., Guo, B., Huang, W., Kim, K.D., Korani, W., Lanciano, S., Lui, C.G., Mirouze, M., Moretzsohn, M.C., Pham, M., Shin, J.H., Shirasawa, K., Sinharoy, S., Sreedasyam, A., Weeks, N.T., Zhang, X., Zheng, Z., Sun, Z., Froenicke, L., Aiden, E.L., Michelmore, R., Varshney, R.K., Holbrook, C.C., Cannon, E.K.S., Scheffler, B.E., Grimwood, J., Ozias-Akins, P., Cannon, S.B., Jackson, S.A., Schmutz, J., 2019. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nature Genetics*, 51(5): 877-884.

Blair, M.W., González, L.F., Kimani, P.M., Butare, L., 2010. Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. *Theoretical and Applied Genetic*, 121(2): 237-248.

Bornowski, N., Michel, K.J., Hamilton, J.P., Ou, S., Seetharam, A.S., Jenkins, J., Grimwood, J., Plott, C., Shu, S., Talag, J., Kennedy, M., Hundley, H., Singan, V.R., Barry, K., Daum, C., Yoshinaga, Y., Schmutz, J., Hirsch, C.N., Hufford, M.B., de Leon, N., Kaeppler, S.M., Buell, C.R., 2021. Genomic variation within the maize stiff-stalk heterotic germplasm pool. *The Plant Genome*, 14: e20114.

Büyük, I., 2014. Investigation of Lea-3 Gene mRNA expression levels in different bean (*Phaseolus vulgaris* L.) cultivars developed under salt and drought stress by quantitative Real-Time PCR method. PhD thesis, Ankara University (Unpublished), Ankara, Türkiye. (In Turkish).

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences*, 108(4): 1474-1478.

Casal, J.J., Candia, A.N., Sellaro, R., 2014. Light perception and signaling by phytochrome A. *Journal of Experimental Botany*, 65(11): 2835-2845.

Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., Xia, R., 2020. TBtools: An Integrative

Toolkit Developed for Interactive Analyzes of Big Biological Data. *Molecular Plant*, 13(8): 1194-1202.

Correa, P., 1981. Epidemiological correlations between diet and cancer frequency. *Cancer Research*, 41: 3685-3690.

Crooks, G.E., Hon, G., Chandonia, J.M., Brenner, S.E., 2004. WebLogo: A sequence logo generator. *Genome Research*, 14(6): 1188-1190.

de Lucas, M., Prat, S., 2014. PIFs get BRright: Phytochrome interacting factors as integrators of light and hormonal signals. *New Phytologist*, 202(4): 1126-1141.

De Ron, A.M., Papa, R., Bitocchi, E., González, A.M., Debouck, D.G., Brick, M.A., Fourie, D., Marsolais, F., Beaver, J., Geffroy, V., McClean, P., Santalla, M., Lozano, R., Yuste-Lisbona, F.J., Casquero, P.A., 2015. Common bean. In: A.M. De Ron (Eds.), *Grain Legumes*, Springer Science+Business Media, USA New York, pp. 1-36.

Gao, Y., Ren, X., Qian, J., Li, Q., Tao, H., Chen, J., 2019. The phytochrome-interacting family of transcription factors in maize (*Zea mays* L.): Identification, evolution, and expression analysis. *Acta Physiologiae Plantarum*, 41(1): 1-7.

Han, X., Tohge, T., Lalor, P., Dockery, P., Devaney, N., Esteves-Ferreira, A.A., Fernie, A.R., Sulpice, R., 2017. Phytochrome A and B regulate primary metabolism in Arabidopsis leaves in response to light. *Frontiers in Plant Science*, 8: 1394.

Hangen, L., Bennink, M.R., 2002. Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Nutrition and Cancer*, 44(1): 60-65.

Hao, Y., Zong, X., Ren, P., Qian, Y., Fu, A., 2021. Basic Helix-Loop-Helix (bHLH) transcription factors regulate a wide range of functions in Arabidopsis. *International Journal of Molecular Sciences*, 22(13): 7152.

Hiz, M.C., Canher, B., Niron, H., Turet, M., 2014. Transcriptome analysis of salt tolerant common bean (*Phaseolus vulgaris* L.) under saline conditions. *Plos One*, 9(3): e92598.

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.

Hosmani, P.S., Flores-Gonzalez, M., van de Geest, H., Maumus, F., Bakker, L.V., Schijlen, E., Haarst, J.V., Cordewener, J., Sanchez-Perez, G., Peters, S., Fei, Z., Giovannoni, J.J., Mueller, L.A., Saha, S., 2019. An improved de novo assembly and annotation of the tomato reference genome using single-molecule sequencing, Hi-C proximity ligation and optical maps. *BioRxiv*, 767764.

Hu, B., Jin, J., Guo, A.Y., Zhang, H., Luo, J., Gao, G., 2015. GSDS 2.0: an upgraded again feature visualization server. *Bioinformatics*, 31(8): 1296-1297.

Inoue, K., Nishihama, R., Kataoka, H., Hosaka, M., Manabe, R., Nomoto, M., Tada, Y., Ishizaki, K., Kohchi, T., 2016. Phytochrome signaling is mediated by phytochrome interacting factor in the liverwort *Marchantia polymorpha*. *The Plant Cell*, 28(6): 1406-1421.

Jaillon, O., Aury, J.M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F,, Pè, M.E., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A.F., Weissenbach, J., Quétier, F., Wincker, P., 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, 449: 463-467.

Juretic, N., Hoen, D.R., Huynh, M.L., Harrison, P.M., Bureau, T.E., 2005. The evolutionary fate of MULE-mediated duplications of host gene fragments in rice. *Genome Research*, 15(9): 1292-1297.

Kalefetoğlu, T., Ekmekçi, Y., 2005. The effects of drought on plants and tolerance mechanisms. *Gazi University Journal of Science*, 18(4): 723-740. (In Turkish).

Kasapoğlu, A.G., İlhan, E., Kızılkaya, D., Hossein Pour, A., Haliloğlu, K., 2020. Genome-wide analysis of BES1 transcription factor family in sorghum [*Sorghum bicolor* (L.) Moench] genome. *Turkish Journal of Agricultural Research*, 7(1): 85-95. (In Turkish).

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J.E., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, 10(6): 845-858.

Lamesch, P., Berardini, T.Z., Li, D.H., Swarbreck, D., Wilk, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D.L., Garcia-Hernandez, M., Karthikeyan, A.S., Lee, C.H., Nelson, W.D., Ploetz, L., Singh, S., Wensel, A., Huala, E., 2012. The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Research*, 40(D1): D1202-D1210.

Lee, N., Choi, G., 2017. Phytochrome-interacting factor from Arabidopsis to liverwort. *Current Opinion in Plant Biology*, 35: 54-60.

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouze, P., Rombauts, S., 2002. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research*, 30(1): 325-327.

Letunic, I., Bork, P., 2011. Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research*, 39 (suppl_2): W475-W478.

Li, W., Liu, Y., Wang, W., Liu, J., Yao, M., Guan, M., He, X., 2021. Phytochrome-interacting factor (PIF) in

rapeseed (*Brassica napus* L.): Genome-wide identification, evolution and expression analyzes during abiotic stress, light quality and vernalization. *International Journal of Biological Macromolecules*, 180: 14-27.

Lin, L., Liu, X., Yin, R., 2018. PIF3 integrates light and low temperature signaling. *Trends in Plant Science*, 23(2): 93-95.

Lynch, M., Conery, J.S., 2003. The evolutionary demography of duplicate genes. *Journal of Structural and Functional Genomics*, 3: 35-44.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods*, 5(7): 621-628.

Ouyang, S., Zhu, W., Hamilton, J., Lin, H., Campbell, M., Childs, K., Thibaud-Nissen, F., Malek, R. L., Lee, Y., Zheng, L., Orvis, J., Haas, B., Wortman, J., Buell, C.R., 2007. The TIGR Rice Genome Annotation Resource: improvements and new features. *Nucleic Acids Research*, 35(Suppl_1): D883-D887.

Örs, S., Ekinci, M., 2015. Drought stress and plant physiology. *Derim*, 32(2): 237-250. (In Turkish).

Paik, I., Huq, E., 2019. Rapid examination of phytochrome-phytochrome interacting factor (PIF) interaction by in vitro coimmunoprecipitation assay. In: A. Hiltbrunner, (Eds.), *Phytochromes*, Methods in Molecular Biology, Humana, USA New York, NY, pp. 21-28.

Park Williams, A., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., Cook, E.R., Gangodagamage, C., Cai, M., McDowell, N.G., 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. *Nature Climate Change*, 3(3): 292-297.

Peng, C., Ma., Z., Lei, X., Zhu, Q., Chen, H., Wang, W., Liu, S., Li, W., Fang, X., Zhou, X., 2011. A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature Climate Change*, 1(9): 467-471.

Pham, V.N., Kathare, P.K., Huq, E., 2018. Phytochromes and phytochrome interacting factors. *Plant Physiology*, 176(2): 1025-1038.

Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R., Lopez, R., 2005. InterProScan: Protein domains identifier. *Nucleic Acids Research*, 33(Suppl_2): W116-W120.

Rosado, D., Gramegna, G., Cruz, A., Lira, B.S., Freschi, L., de Setta, N., Rossi, M., 2016. Phytochrome Interacting Factors (PIFs) in *Solanum lycopersicum*: Diversity, evolutionary history and expression profiling during different developmental processes. *PLoS One*, 11(11): e0165929.

Ruiz-Lozano, J.M., Porcel, R., Azcón, C., Aroca, R., 2012. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *Journal of Experimental Botany*, 63(11): 4033-4044.

Schmutz, J., McClean, P.E., Mamidi, S., Wu, G.A., Cannon, S.B., Grimwood, J., Jenkins, J., Shu, S., Song, Q., Chavarro, C., Torres-Torres, M., Geffroy, V., Moghaddam, S.M., Gao, D., Abernathy, B., Barry, K., Blair, M., Brick, M.A., Chovatia, M., Gepts, P., Goodstein, D.M., Gonzales, M., Hellsten, U., Hyten, D.L., Jia, G., Kelly, J.D., Kudrna, D., Lee, R., Richard, M.M., Miklas, P.N., Osorno, J.M., Rodrigues, J., Thareau, V., Urrea, C.A., Wang, M., Yu, Y., Zhang, M., Wing, R.A., Cregan, P.B., Rokhsar, D.S., Jackson, S.A., 2014. A reference genome for common bean and genome-wide analysis of dual domestications. *Nature Genetics*, 46(7): 707-713.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research*, 13(11): 2498-2504.

Shi, Q., Zhang, H., Song, X., Jiang, Y.E., Liang, R., Li, G., 2018. Functional characterization of the maize phytochrome-interacting factors PIF4 and PIF5. *Frontiers in Plant Science*, 8: 2273.

Shin, A.Y., Han, Y.J., Baek, A., Ahn, T., Kim, S.Y., Nguyen, T.S., Son, M., Lee, K.W., Shen, Y, Song, P.S., Kim, J.I., 2016. Evidence that phytochrome functions as a protein kinase in plant light signaling. *Nature Communications*, 7(1): 1-13.

Suyama, M., Torrents, D., Bork, P., 2006. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Research*, 34(Suppl_2): W609-W612.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7): 3022-3027.

Teuling, A.J., van Loon, A., Seneviratne, S.I., Lehner, I., Aubinet, M., Heinesch, B., Bernhofer, C., Grünwald, T., Prasse, H., Spank, U., 2013. Evapotranspiration amplifies European summer drought. *Geophysical Research Letters*, 40(10): 2071-2075.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25(24): 4876-4882.

Thompson, M.D., Brick, M.A., McGinley, J.N., Thompson, H.J., 2009. Chemical composition and mammary cancer inhibitory activity of dry bean. *Crop Science*, 49(1): 179-186.

Tuskan, G.A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., Bhalerao, R.R., Bhalerao, R.P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., Busov, V., Campbell, M., Carlson, J., Chalot, M., Chapman, J., Chen, G.L., Cooper, D., Coutinho, P.M., Couturier, J., Covert, S., Cronk, Q., Cunningham, R., Davis, J., Degroeve, S., Déjardin, A., Depamphilis, C., Detter, J., Dirks, B., Dubchak, I., Duplessis, S., Ehlting, J., Ellis, B., Gendler, K., Goodstein, D., Gribskov, M., Grimwood, J., Groover, A., Gunter, L., Hamberger, B., Heinze, B., Helariutta, Y., Henrissat, B., Holligan,

D., Holt, R., Huang, W., Islam-Faridi, N., Jones, S., Jones-Rhoades, M., Jorgensen, R., Joshi, C., Kangasjärvi, J., Karlsson, J., Kelleher, C., Kirkpatrick, R., Kirst, M., Kohler, A., Kalluri, U., Larimer, F., Leebens-Mack, J., Leplé, J.C., Locascio, P., Lou, Y., Lucas, S., Martin, F., Montanini, B., Napoli, C., Nelson, D.R., Nelson, C., Nieminen, K., Nilsson, O., Pereda, V., Peter, G., Philippe, R., Pilate, G., Poliakov, A., Razumovskaya, J., Richardson, P., Rinaldi, C., Ritland, K., Rouzé, P., Ryaboy, D., Schmutz, J., Schrader, J., Segerman, B., Shin, H., Siddiqui, A., Sterky, F., Terry, A., Tsai, C.J., Uberbacher, E., Unneberg, P., Vahala, J., Wall, K., Wessler, S., Yang, G., Yin, T., Douglas, C., Marra, M., Sandberg, G., Van de Peer, Y., Rokhsar, D., 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science*, 313(5793): 1596-1604.

Valliyodan, B., Cannon, S.B., Bayer, P.E., Shu, S., Brown, A.V., Ren, L., Jenkins, J., Chung, C.Y.L., Chan, T.F., Daum, C.G., Plott, C., Hastie, A., Baruch, K., Barry, K.W., Huang, W., Patil, G., Varshney, R.K., Hu, H., Batley, J., Yuan, Y., Song, Q., Stupar, R.M., Goodstein, D.M., Stacey, G., Lam, H.M., Jackson, S.A., Schmutz, J., Grimwood, J., Edwards, D., Nguyen, H.T., 2019. Construction and comparison of three reference-quality genome assemblies for soybean. *The Plant Journal*, 100(5): 1066-1082.

Vicente-Serrano, S.M., Lopez-Moreno, J., Beguería, S., Lorenzo-Lacruz, J., Sanchez-Lorenzo, A., García-Ruiz, J.M., Azorin-Molina, C., Morán-Tejeda, E., Revuelto, J., Trigo, R., Coelho, F., Espejo, F., 2014. Evidence of increasing drought severity caused by temperature rise in southern Europe. *Environmental Research Letters*, 9(4): 044001.

Voorrips, R.E., 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, 93(1): 77-78.

Wang, X., Liu, Y., Huai, D., Chen, Y., Jiang, Y., Ding, Y., Liao, B., 2021. Genome-wide identification of peanut PIF family genes and their potential roles in early pod development. *Gene*, 781: 145539.

Wang, Y., Tang, H., Debarry, J.D., Tan, X., Li, J., Wang, X., Lee, T.H., Jin, H., Marler, B., Guo, H., Kissinger, J.C., Paterson, A.H., 2012. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research*, 40(7): e49.

Wu, G., Zhao, Y., Shen, R., Wang, B., Xie, Y., Ma, X., Zheng, Z., Wang, H., 2019. Characterization of maize Phytochrome-Interacting Factors in light signaling and photomorphogenesis. *Plant Physiology*, 181(2): 789-803.

Xu, D., 2018. Multifaceted roles of PIF4 in plants. *Trends in Plant Science*, 23(9): 749-751.

Xu, X., Paik, I., Zhu, L., Huq, E., 2015. Illuminating progress in phytochrome-mediated light signaling pathways. *Trends in Plant Science*, 20(10): 641-650.

Yang, Z.H., 2007. PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24(8): 1586-1591.

Yang, Z.H., Nielsen, R., 2000. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Molecular Biology and Evolution*, 17(1): 32-43.

Zhang, K., Zheng, T., Zhu, X., Jiu, S., Liu, Z., Guan, L., Jia, H., Fang, J., 2018. Genome-wide identification of PIFs in grapes (*Vitis vinifera* L.) and their transcriptional analysis under lighting/shading conditions. *Genes*, 9(9): 451.

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