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Genome-Wide Identification of Phytochrome-Interacting Factor (PIF) Gene Family in Potatoes and Functional Characterization of *StPIF3* in Regulating Shade-Avoidance Syndrome

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Abstract: The phytochrome-interacting factor (PIF) proteins are part of a subfamily of basic helix–loop–helix (bHLH) transcription factors that integrate with phytochromes (PHYs) and are known to play important roles in adaptive changes in plant architecture. However, the characterization and function of *PIFs* in potatoes are currently poorly understood. In this study, we identified seven *PIF* members in potatoes and named them *StPIF01-1*, *StPIF01-2*, *StPIF03*, *StPIF06-1*, *StPIF06-2*, *StPIF07*, and *StPIF09* based on their location in potato chromosomes. The chromosomal location, gene structures, physicochemical characteristics, phylogenetic tree, and tissue-specific expression of *StPIFs* were also analyzed. RT-qPCR analysis revealed that the *StPIF3* gene was highly induced by shade and may play a crucial regulatory role in potato responses to shade stress. Also, multiple cis-regulatory elements involved in light response were detected in the promoter of the *StPIF* genes. Subcellular localization analysis indicated that the StPIF3-encoding protein is mainly localized in the nucleus. Transgenic overexpression of *StPIF3* in potatoes increased stem length, chlorophyll accumulation, and enhanced shade-avoidance symptoms, whereas the *StPIF3*-interfering lines had a lower plant height and more chlorophyll accumulation. These findings enhance our comprehension of *StPIF* gene roles, potentially advancing potato yield and quality research. This study provides detailed information about *StPIFs* and identifies the function of *StPIF3*, which is involved in shade-avoidance syndrome.

Keywords: phytochrome-interacting factor; *Solanum tuberosum*; bioinformatics analysis; *StPIF3*

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1. Introduction

Light is one of the most important environmental factors for supporting plant life and not only provides energy for plant photosynthesis but also a critical environmental signal to modulate plant growth and development [1]. Plants have developed a refined control system that responds to environmental stimuli by sensing changes in the light environment [2]. Photoreceptors have been discovered that play a crucial role in external light signal reception in plants, including phytochromes (PHYs), cryptochromes (CRYs), the UV-B photoreceptor (UVR8), and phototropin [3]. Among these photoreceptors, the PHYs are involved in a series of downstream physiological and biochemical processes in plants, such as plant morphogenesis, abiotic stress, and defense responses [4–6]. The PHY proteins can bind the bilin of a chromophore, which absorbs light and triggers protein conformational changes that incur the first step of signal transduction [5]. However, PHY proteins interact with multiple partners to modulate the transcription of downstream target genes to control the light-regulated modulation of plant growth and development [6]. The phytochrome-interacting factor (PIF) family is one of the key interacting partners and acts as a key regulator of the transition from skotomorphogenesis to photomorphogenesis [7].

PIF proteins are encoded by a subfamily of bHLH transcription factors with two conserved domains, bHLH and APB (active phyB-binding) [8], that are widely distributed from bryophytes to higher plants and have been identified from bryophytes to angiosperms, such as Arabidopsis (*Arabidopsis thaliana*, eight members) [9], rice (*Oryza sativa*, six members) [10], maize (*Zea mays*, eight members) [11], tomatoes (*Solanum lycopersicum*, eight members) [12], apples (*Malus domestica*, eight members) [13], grapes (*Vitis vinifera*, four members) [14], *Brachypodium distachyon* (eight members) [15], *Marchantia polymorpha* (one member) [16], and *Physcomitrella patens* (six members) [17]. Previous studies have demonstrated that *PIFs* are diffusely involved in many processes of plant development and growth, such as seed germination, embryo shaft elongation, chlorophyll biosynthesis, shade-avoidance responses, circadian clock, and so on [18].

PIF proteins can bind to the G-box core motif (CACGTG) to enhance or inhibit the expression of downstream genes [9]. The primary function of PIFs has been demonstrated to be an attenuator within the phototransduction cascade that can suppress photomorphogenesis and facilitate skotomorphogenesis under dark conditions. In *Arabidopsis*, PIFs promote skotomorphogenesis mainly by enhancing hypocotyl elongation; for example, AtPIL5 impedes seed germination, and inhibits hypocotyl negative geotropism and hypocotyl elongation [19]. The ETHYLENE-INSENSI-TIVE 3 (EIN3) activation of AtPIF3 is essential for ethylene-mediated hypocotyl growth. AtPIF4 has been found to regulate stomatal development, chlorophyll degradation, and leaf senescence in the absence of light and can enhance the cold resistance of the plant [19]. AtPIF5 inhibits red-light-induced anthocyanin production in Arabidopsis seedlings [19]. AtPIF6 is strongly induced during seed development, which exists in two splice variants, including an alpha form and a beta form. Elevated AtPIF6 expression diminishes seed dormancy, while the suppression of AtPIF6 enhances it [20]. PHYB, as the primary shade detector, indirectly modulates growth through the dephosphorylation of PIF7 in shaded conditions, leading to an upregulation of auxin biosynthetic genes [9].

The potato (*Solanum tuberosum* L.), ranking fourth globally after rice, maize, and wheat, is pivotal to the agricultural economy [21]. However, the *PIF* family in the potato has not been well characterized, especially the functions of *StPIFs*, which are still unclear. In our study, a global analysis of the *PIF* gene family in potatoes was carried out, and seven *StPIFs* were identified. The chromosomal location, gene structures, physicochemical characteristics, phylogenetic tree, and tissue-specific expression patterns of *StPIFs* were also analyzed. Furthermore, we found that *StPIF3* plays a crucial regulatory role in potato responses to shade. The overexpression of *StPIF3* in potatoes increased stem length and chlorophyll accumulation and enhanced shade-avoidance symptoms. Consequently, this study provides detailed information about *StPIFs* and identifies the function of *StPIF3*, which is involved in shade-avoidance syndrome in potatoes.

2. Materials and Methods

2.1. Plant Materials and Processing Method

The potato cultivar "Atlantic" was used as the experimental material in our research. Potatoes were planted in test tubes under the following conditions: 22 ± 2 °C; 16 h light/8 h dark; humidity at 80%. Then, 20-day-old test-tube plants were randomly divided into control and treatment groups, treatment groups were subjected to shaded treatment by controlling the light intensity at 3500 Lx, and control groups were grown as usual (20,000 Lx). All the materials were separately harvested, immediately frozen in liquid nitrogen, and then stored at a −80 °C refrigerator for later use.

2.2. Genome-Wide Identification and Annotation of Potato PIF Genes

To identify the *PIF* family members in potatoes, we downloaded all protein sequences of AtPIF from the Arabidopsis Information Resource (TAIR) Database; all the downloaded sequences were used to search against Spud DB Potato Genomics Resources [22]. The INTERPRO [23] and SMART databases were used to further verify the candidates on the basis of integrity and the existence of the conserved domains of bHLH and APB [23]. The

ExPASy database was used to calculate the isoelectric points (pIs) and molecular weights (MWs) of the proteins of all the StPIFs.

2.3. Bioinformatics Analysis of StPIF Genes

The plant-mPLoc website was used to analyze the subcellular localization of StPIFs. We exploited GSDS to identify the number and location of the introns and exons of *StPIF* genes by blasting the CDS and genomic sequences of the *StPIF* genes. Chromosome localization of the *StPIFs* was achieved with TBtools (v2. 034). MEGA7 was used for multiple sequence alignment analysis, and the bootstrap test had 1000 replicates. We downloaded all protein sequences for PIFs from rice, tomato, and *Arabidopsis thaliana* genomic databases to construct the phylogenetic analysis. The MEME (v5. 5. 5) website was used to analyze conserved motifs; we set the motif number as 10 and chose motifs with E-values $\leq 1 \times 10^{-30}$. In order to reveal the potential regulatory patterns of *StPIFs*, PlantCare and TBtools were used to analyze the cis-acting elements in the promoter regions of the *StPIF* genes.

2.4. Gene Expression Analysis of StPIFs

Total RNA was extracted from the potato leaves using the CTAB method [24] and was then used for reverse transcription using kits offered by TaKaRa (Takara Biomedical Technology, Beijing, China). Quantitative real-time PCR (RT-PCR) was performed with the CFX96 Real-Time PCR Detection system (Roche, Switzerland). Each qPCR reaction was established with three technical replicates. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression levels of the *StPIFs* [25]. The *EF1α* gene served as an internal control.

2.5. Tissue-Specific Analysis of Expression Levels

For inquiry on the expression patterns of the *StPIFs* in different organs and tissues of potatoes, we downloaded the RNA sequencing data of the *StPIFs* from the PGSC database [26], which were DM (double monoploid *S. tuberosum* group phureja DM-13). Tbtools was adopted to visualize the analysis of RNA-seq expression.

2.6. Subcellular Localization of StPIF3

To investigate the subcellular localization of the StPIF3 protein, the ORF sequences of the *StPIF3* gene were cloned into the pCAMBIA1300-35S-EGFP vector, and we constructed the fusion expression vector of pCAMBIA1300-EGFP-StPIF3. The primers were as follows: 5′-AGAACACGGGGGAC**GAGCTC**ATGCCTCTCTCTGAGTTCG-3′ (SacI site in bold); 5′-TGCTCACCAT**GTCGAC**GTTGGTCAACCTATTCGCC-3′ (SalI site in bold). These were then transformed into *Agrobacterium tumefaciens* GV3101. *Nicotiana benthamiana* foliage was infiltrated with StPIF3 GFP vectors. After two days of treatment in darkness, GFP fluorescence signals were visualized with a laser scanning confocal microscope (Leica, Weztlar, Germany).

2.7. Plasmid Construction and Generation of the Solanum tuberosum Transgenic Plant

We amplified the full-length sequence of StPIF3 from potatoes with the primes 5′-AGAACACGGGGGAC**GAGCTC**ATGCCTCTCTCTGAGTTCTG-3′ (SacI site in bold) and 5′-TGCTCACCAT**GTCGAC**TCAGTTGGTCAACCTATTCGCG-3′ (SalI site in bold); then, the amplified fragment was cloned into the pCAMBIA1300-35S-EGFP vector, and we constructed the overexpression vector of *StPIF3*. The interference vector of *StPIF3* was constructed with artificial miRNA technology according to a previously published method [27]. The recombinant construct was transformed into *Agrobacterium tumefaciens* strain GV3101. Sterile microtubes were employed for Agrobacterium-mediated potato transformation to generate potato transgenic plants following the method described by Si et al. [28]. The regenerated plantlets were selected from hygromycin-resistant genes and confirmed by genomic PCR analysis.

2.8. Shade-Avoidance Syndrome Analysis of Transgenic Potato Plants

Plant height and chlorophyll accumulation are typical syndromes under shade-avoidance responses. In order to understand the function of *StPIF3* in potato shade-avoidance responses, we measured the plant height and chlorophyll content of the transgenic plants. Thus, 25-day-old both wild-type and transgenic plants were used for shade-avoidance syndrome analysis. The chlorophyll content (Chl) analysis was performed according to the TYS-B live chlorophyll meter (Jisenpuer, Zhejiang, China) and the fully expanded leaves of the upper, middle, and lower parts of each plant were measured. The plant height assays of the wild-type and transgenic plants were performed after seven days of shade treatment.

3. Results

3.1. Identification of Potato PIFs

In this study, a total of seven candidate *StPIFs* were finally identified in potatoes and named *PIF01-1*, *PIF01-2*, *PIF03*, *PIF06-1*, *PIF06-2*, *PIF07*, and *PIF09* based on their locations on chromosomes; each of their encoded proteins contain bHLH and APB domains, while StPIF06-2 and, StPIF01-2 have APA domains. The CDS sequence of seven *StPIF* genes varying from 1254 to 2151 bp in length, encodes proteins varying from 417 to 716 amino acids in length; the theoretical isoelectric point (pI) value ranges from 5.15 to 8.71, and the molecular weight ranges from 41,285.79 to 76,772.66 Da. Detailed information on gene IDs, subfamilies, CDS length, protein length, molecular weight, theoretical pI, and domain regions are summarized in Table 1.

Table 1. Characteristics of phytochrome-interacting factor (*PIF*) genes in the potato.

Gene ID	Name	Subfamily	CDS Length	Protein Length	Molecular Weight	Theoretical pI	Characteristic Domains
Soltu.DM.01G041140.1	PIF01-2	PIF3	2151	716	76,772.66	7.26	bHLH (460–508)
Soltu.DM.06G002140.1	PIF06-2	PIF1	1548	515	56,408.13	5.13	bHLH (311–359)
Soltu.DM.07G014300.2	PIF07	PIF4	1647	548	61,114.45	6.93	bHLH (372–412)
Soltu.DM.09G018570.7	PIF09	PIF4-RELATED	1737	578	62,943.67	6.42	bHLH (347–393)
Soltu.DM.06G025680.1	PIF06-1	PIF7	1254	417	46,935.82	8.71	bHLH (198–246)
Soltu.DM.01G031430.2	PIF01-1	PIF8	1395	464	50,747.96	7.62	bHLH (286–331)
Soltu.DM.02G028560.1	PIF02	NOT NAMED	1131	376	41,285.79	5.04	bHLH (151–197)
Soltu.DM.03G029660.1	PIF03	PIF7	1314	437	48,174.88	6.79	bHLH (237–283)

3.2. Subcellular Localization, Chromosome Localization, and Gene Structure Analysis of Potato PIFs

The results of subcellular localization prediction showed that seven *StPIF* genes were located in the nucleus, indicating that they were typical transcription factors and could regulate the expression of downstream genes. Seven *StPIFs* were mapped into five chromosomes of the potato (Chr 1, Chr3, Chr6, Chr7, and Chr9) (Figure 1). Gene structure analysis showed that all seven *StPIF* genes had intron and exon structures; *PIF01-2*, *PIF06-2*, and *PIF09* had six introns; and the rest had five introns (Figure 2).

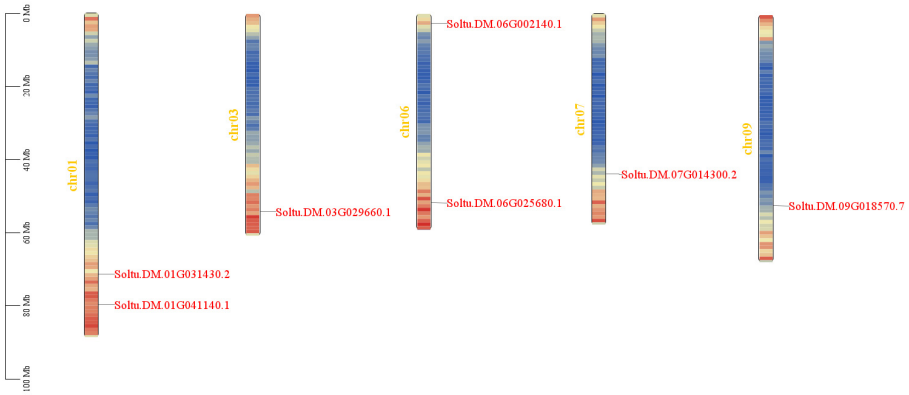

Figure 1. Chromosome localization analysis of *StPIFs*. The different colors represent the density information of the genes, red represents higher gene density, blue is the opposite.

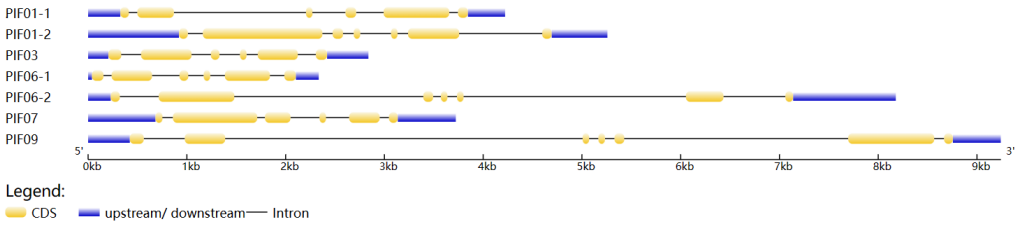

Figure 2. Gene structure characteristics of *StPIFs*. The blue and yellow boxes represent UTR and CDS, and the black lines represent introns.

3.3. Multiple Sequence Alignment and Phylogenetic Analysis

The bHLH and APB domains were universally identified across all AtPIF proteins, showcasing their highly conservative. The same results were obtained for seven StPIF proteins, and StPIF02 was removed due to a lack of an APB domain [29]. Our study employed phylogenetic analysis utilizing an unrooted tree with 1000 bootstrap replicates of full-length amino acid sequences to analyze the evolutionary trajectories of potato PIF proteins alongside those from diverse plant species (Figure 3). Our phylogenetic investigation revealed six distinct clades, with StPIFs predominantly pairing with their orthologous counterparts within subgroups in *Solanum lycopersicum* or *Arabidopsis thaliana*. For example, StPIF01-2 shared high homology with SlPIF3. StPIF01-1 clustered with SlPIF8a. StPIF07 showed a close relationship with SlPIF4. According to Figure 4, StPIFs are more closely related to SlPIFs than OsPIFs and AtPIFs.

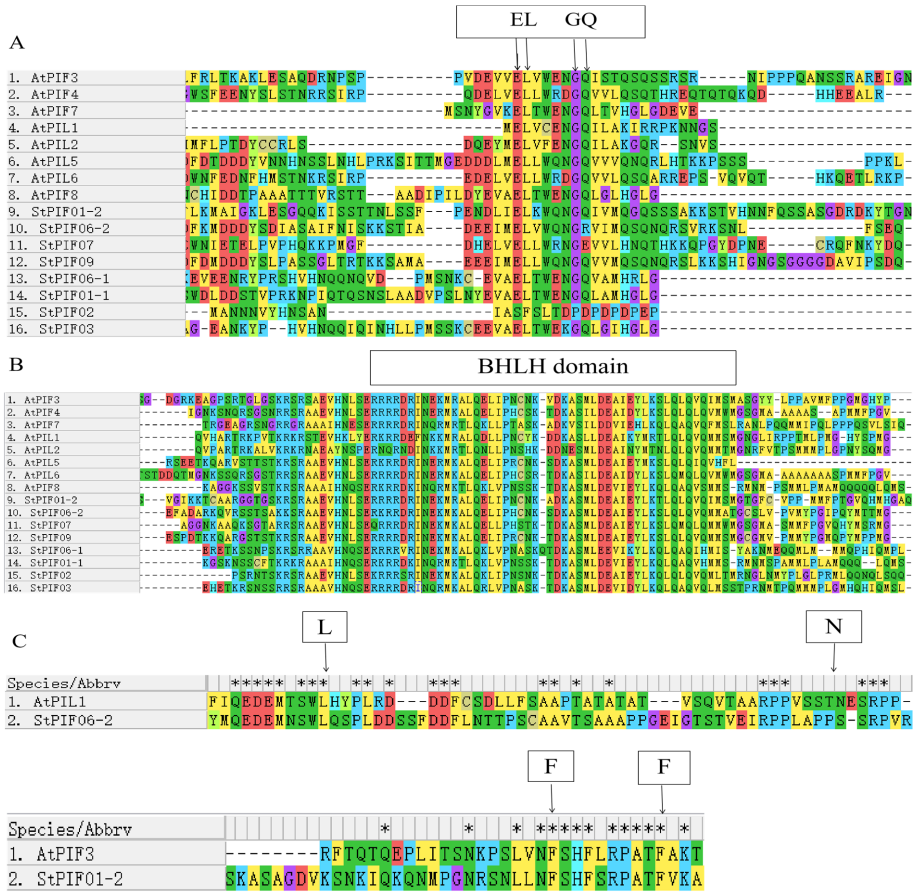

Figure 3. The multiple alignment of deduced amino acid sequences of StPIFs. (**A**) Comparison of active PHYB binding (APB). (**B**) Comparison of BHLH binding. (**C**) Comparison of active PHYA binding (APA). * represents identical amino acid residue. EL, GQ represent conserved amino acid residues in the APB domain. L, N represent conserved amino acid residues of the APA domain in PIF1. F, F represent conserved amino acid residues of the APA domain in PIF3.

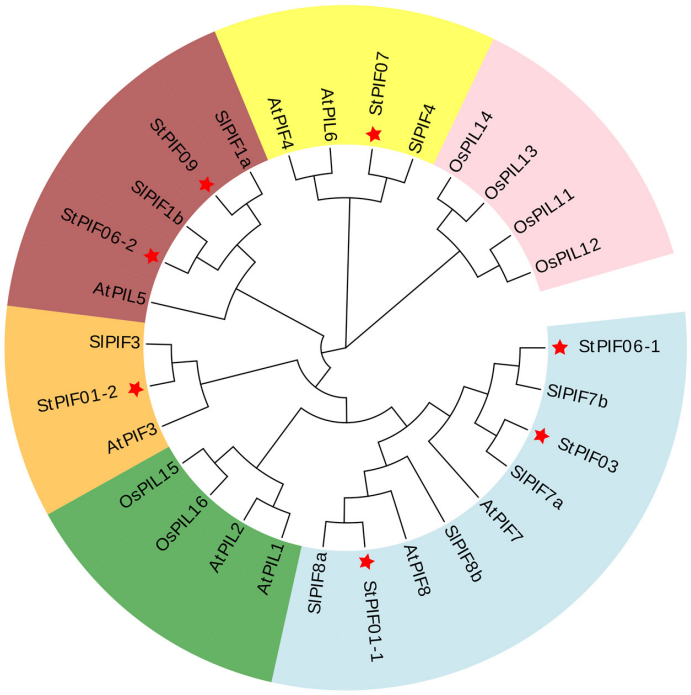

Figure 4. Unrooted phylogenetic tree of PIF proteins from *Solanum tuberosum* L., *Solanum lycopersicum*, *Arabidopsis thaliana*, and *Oryza sativa*. Each color represents one group. The red stars represent the highlighting of the StPIFs.

3.4. Conserved Motif and Cis-Acting Element Analysis of PIF Family

To investigate the structural diversity of StPIF-encoded proteins, we detected the motif compositions of these StPIFs. A total of 10 motifs were found among the *StPIF* gene family (Figure 5), named Motifs 1 to 10. In detail, Motif 1 and Motif 2 existed in all StPIF-encoded proteins and were inferred from the nucleotide composition of the bHLH domain. All StPIFs had Motif 3 (the APB domain). Moreover, StPIF06-2 and StPIF09 had the same kinds of motifs and were on a branch of the phylogenetic tree. StPIF06-1 and PIF03 had the same condition.

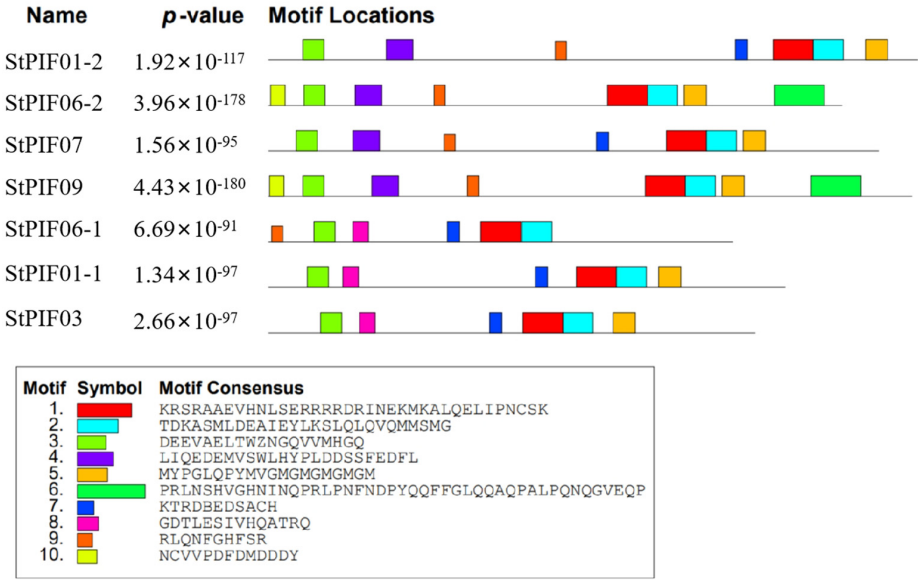

Figure 5. Conserved motifs of *PIF* genes in potatoes. The MEME software (V5. 5. 4) facilitated the prediction of conserved motifs, with motif quantity fixed at ten. One color box corresponds to one motif.

We analyzed the cis-elements of *PIF* genes to clarify the gene function and transcriptional regulation mechanism. All *StPIFs* had TATA-box and CAAT-box. The predicted cis-elements could be divided into three classes; these are the cis-elements related to photoreaction, phytohormones, and adverse environmental conditions. The cis-elements related to photoreaction (GT1-motif, GA-motif, TCT-motif, Box 4, AE-box, GATA-motif, ACE, I-box, AT1-motif, Sp1, G-box, TCCC-motif, and CAG-motif) were more abundant than the remaining two classes and varied from three to nine. The cis-elements related to phytohormones included cis-acting elements involved in abscisic acid, jasmonic acid, gibberellin, salicylic acid, and the auxin response. *StPIF03* and *StPIF07* had low-temperature stress responsiveness (LTR) elements. Drought-inducibility (MBS)-related elements were found in the promoters of *StPIF03*, *StPIF06-1*, and *StPIF09*. Circadian control (circadian: CANNNNATC) elements were detected in the promoters of *StPIF01-1*, *StPIF06-1*, and *StPIF09*. *StPIF01-1* had a heat stress responsiveness element (an AT-rich element). Anaerobic induction (ARE)-related elements were detected in the promoters of *StPIF01-2*, *StPIF06-1*, and *StPIF07*; the promoters of *StPIF06-2*, *StPIF06-1*, and *StPIF07* had cis-acting regulatory elements of meristem expression (CAT-box). The cis-acting elements related to zein metabolism were detected in the promoters of *StPIF06-2* and *StPIF06-1*. The promoters of *StPIF06-1* had the MYBHv1 (CCAAT-box) cis-acting element. Additionally, cis-acting elements involved in defense and stress responsiveness (TC-rich repeats) were also found in the promoters of *StPIF01-2*; *StPIF09* had the cis-acting elements of seed-specific expression (GCN4-motif), and the MYB binding site involved in light responsiveness (MRE) was found in the promoters of *StPIF06-2* (Figure 6). These results indicated that *StPIFs* play diverse roles in regulating polymorphism, hormone metabolism, and responses to various stresses.

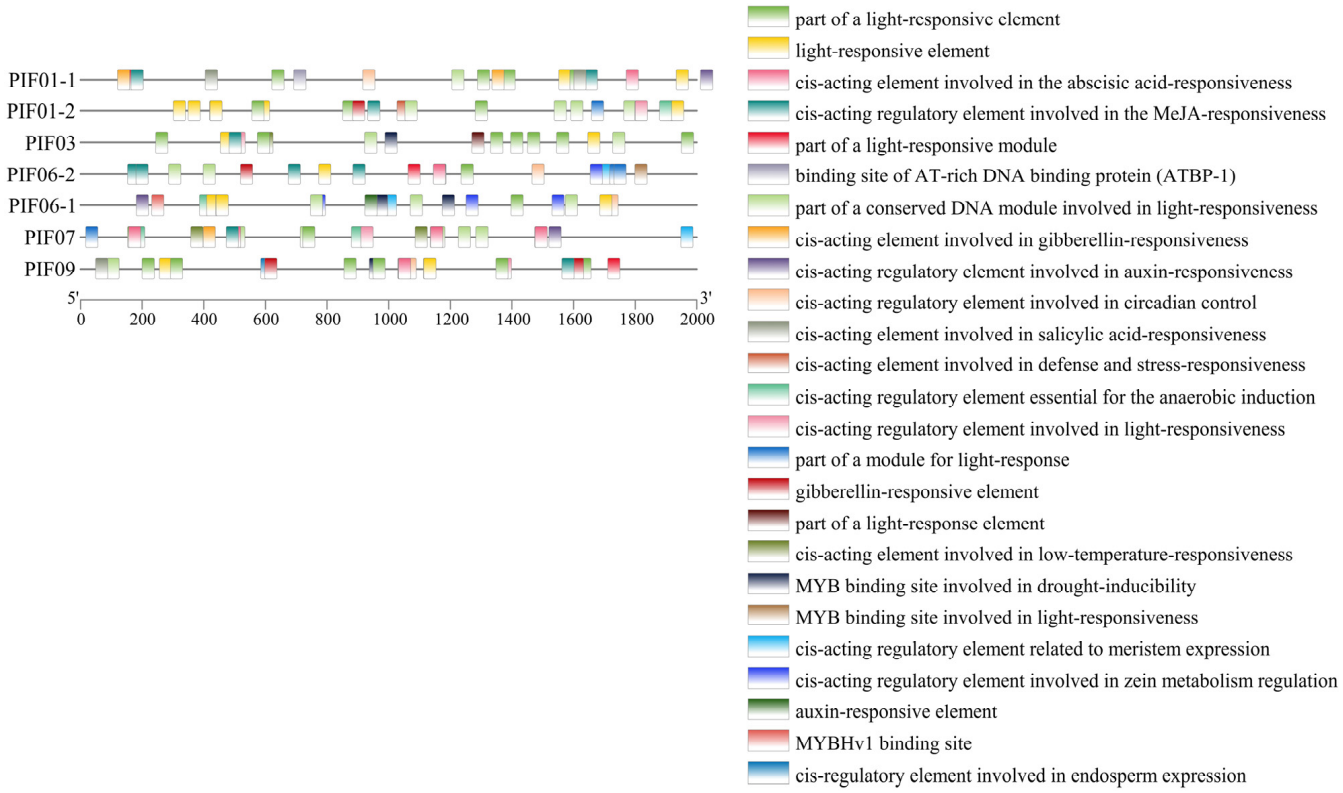

Figure 6. The cis-elements in the promoters of potato *PIF* genes. The PlantCare website was used to analyze and present the results with TBtools. The 2000 bp upstream of the *StPIFs* was used to perform cis-elements analysis.

3.5. QRT-PCR Analysis of StPIF Genes under Shaded Stress

By combining cis-acting element analysis and previous report results, we found that the *PIF* gene family is involved in shade-avoidance responses and regulates multiple shade-

avoidance syndromes. To investigate the expression pattern of the seven *StPIF* genes under shading conditions, the expression levels of these *StPIFs* under shade were analyzed by RT-qPCR (Figure 7). The results showed that the expression level of *StPIF01-2* was obviously increased after shade treatment; the expression level of *StPIF06-1* was decreased after shade treatment; the expression levels of *StPIF06-2* and *StPIF03* were increased after 5 days of shade treatment and decreased after 10 days of shade treatment; the expression level of *StPIF01-1* was decreased after 5 days of shade treatment and increased after 10 days of shade treatment; and the expression of *StPIF07* was not detected in this experiment. Based on the results above, the expression level of *StPIF01-2* was found to be significantly induced under shade conditions, and it was renamed *StPIF3* for future functional identification.

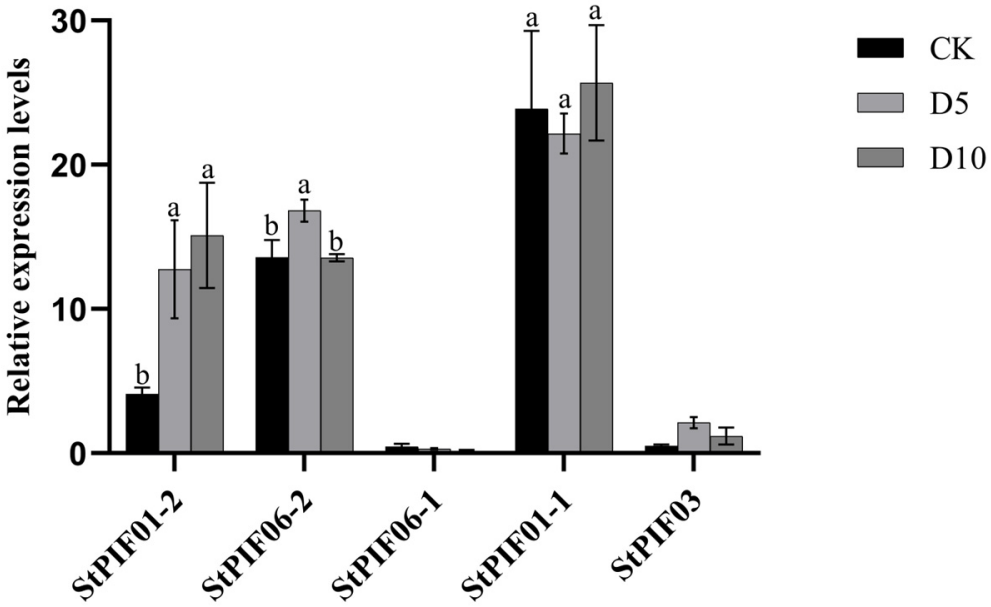

Figure 7. Relative expression of *StPIF* genes under shade. Black squares represent the control group. Gray squares represent the shading treatment on the fifth day. Dark gray squares represent the shading treatment on the tenth day. Significant differences are shown using different alphabets when assessed using Duncan's multiple range test ($p < 0.05$, $n = 3$).

3.6. Tissue-Specific Analysis of Expression Levels of StPIFs in Potatoes

The expression levels of the seven *StPIFs* were investigated thoroughly using a transcriptome analysis procedure based on the public transcriptomic data of 18 different tissues of potatoes, including leaves, roots, shoots, leaf-wounding secondary tissue, callus, tuber 1, tuber 2, sepals, stamens, stolon, whole mature flowers, petioles, petals, carpels, leaf-wounding primary tissue, mature whole fruit, immature whole fruit, and the insides of the fruit (mesocarp and endocarp) (Figure 8). The results indicated that the seven *StPIF* genes exhibited constitutive expression in all tissues. In leaf-wounding secondary tissue, *StPIF01-1* had the highest expression compared with the other tissues. Some *StPIFs* were predominantly expressed in different organs and tubers, such as *StPIF01-1*, which had a higher expression in leaf-wounding secondary tissue, sepals, leaves, petioles, and leaf-wounding primary tissue; *StPIF06-2* had a higher expression in shoots and showed a slight expression in roots, stolons, tuber 2, petals, and leaves. *StPIF01-2* in mature whole fruit, roots, the callus, and sepals presented with higher expression than the other tissues. *StPIF03* in leaf-wounding primary tissue had higher expression than the other tested tissues. *StPIF09* in roots and *StPIF06-1* in leaf-wounding primary tissue showed slight expression. *StPIF07* was almost not expressed, and the expression level was the lowest compared with other members.

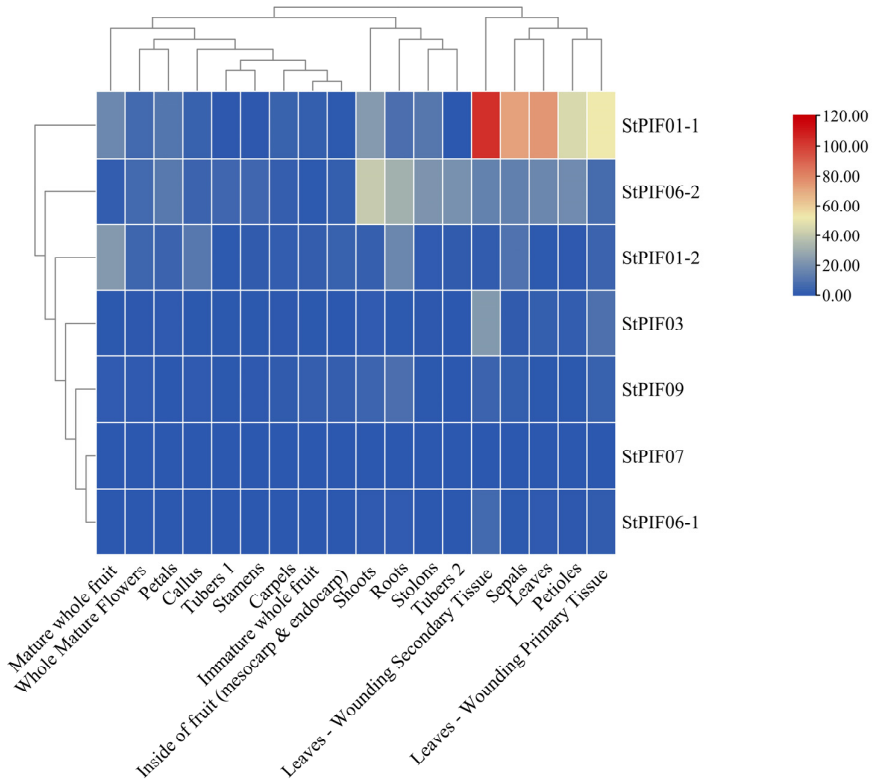

Figure 8. Expression levels of *StPIFs* in different tissues of potatoes.

3.7. Subcellular Localization of StPIF3

To better understand the role of *StPIF3* in potatoes, subcellular localization analysis was used to trace the intracellular proteins of StPIF3, and we constructed a fusion plasmid StPIF3-GFP. the recombinant plasmid was transiently transfected into tobacco leaf cells, and subsequent confocal laser scanning microscopy revealed the nuclear localization of StPIF-GFP fusion protein fluorescence, while GFP in the controls was distributed throughout the cytoplasm and nucleus (Figure 9). These results indicated that StPIF3 is localized in the nucleus. This shows that it is a typical transcription factor.

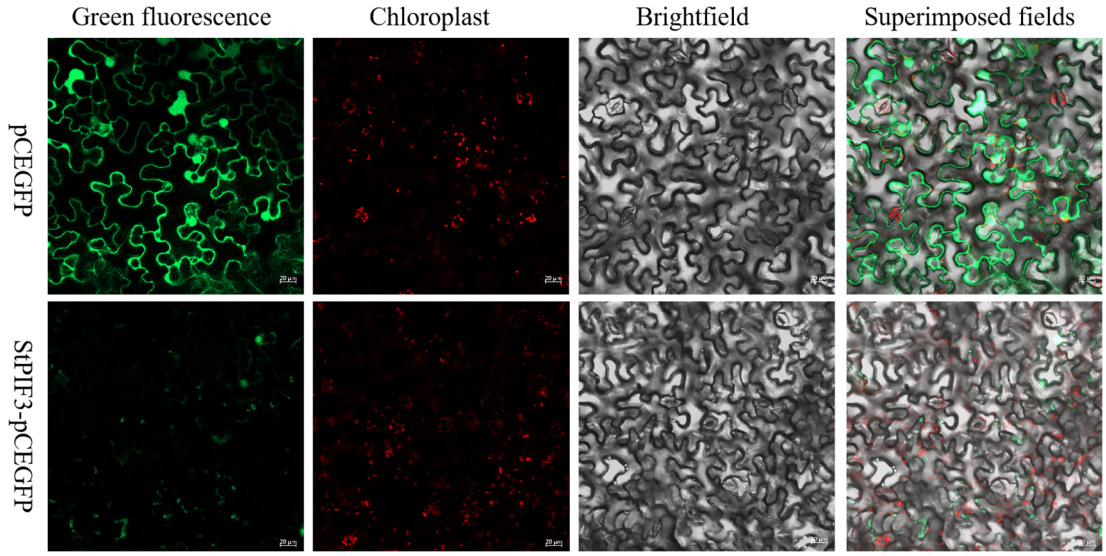

Figure 9. Subcellular localization of StPIF3 protein. Scale bar = 20 μm. Green represents green fluorescent protein and red represents red fluorescent protein, a pair of marker proteins that are often used.

3.8. Potato Transformation and Identification

To elucidate the potential function of *StPIF3* in potato shade-avoidance responses, *StPIF3* overexpression (OE) and knockdown (RNAi) transgenic potato lines were obtained. In total, six independent transformants were detected as transgenic potato lines by genomic PCR (Figure 10). QRT-PCR analysis revealed that the expression level of *StPIF3* in the leaves of the overexpression transgenic lines was significantly higher than that of the non-transgenic lines (Figure 10A), while the relative expression levels of *StPIF3* were significantly lower in RNAi lines than non-transformed lines (Figure 10B), indicating that we successfully achieved *StPIF3* overexpression and the suppression of transgenic potato lines. Based on the qRT-PCR results, three independent overexpression lines (StPIF3-OE1, StPIF3-OE2, and StPIF3-OE3) and three suppression transgenic lines (StPIF3-RNAi1, StPIF3-RNAi2, abd StPIF3-RNAi3) were severally selected for further analysis.

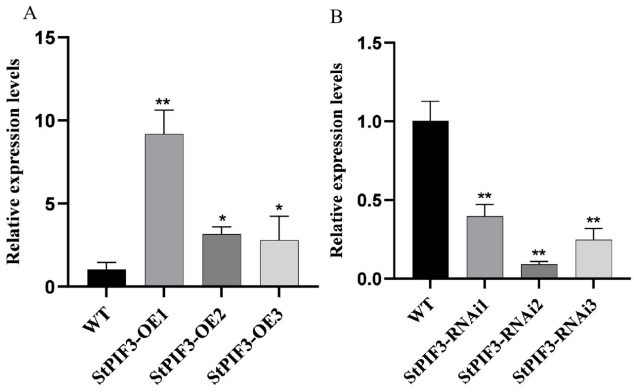

Figure 10. Expression analysis of *StPIF3* in WT and transgenic strains. *StEf1α* (elongation factor 1α) was used as an internal control. (**A**) Comparison of the expression levels of overexpressed *StPIF3* lines and WT controls. (**B**) Comparison of the expression levels of interfering expression lines and WT controls. Asterisks denote significant differences from control (*, $p < 0.05$; **, $p < 0.01$).

3.9. StPIF3 Regulates Shade-Avoidance Syndrome by Coordinating Chlorophyll Accumulation

Previous studies have shown that a shade-induced decrease in chlorophyll content is a typical phenotype of shade-avoidance syndrome in plants. Chlorophyll content indicators can be used to assess plant nutritional status, photosynthesis efficiency, and growth and development levels. To examine the effects of *StPIF3* on potato chlorophyll content, we measured chlorophyll content among *StPIF3* overexpression and knockdown transgenic lines and wild-type potatoes (Figure 11). The results showed that the chlorophyll content was higher in the *StPIF3* knockdown plants than the wild-type plants, while the chlorophyll content was lowest in the *StPIF3* overexpression plants between wild-type and knockdown lines. These results reveal that *StPIF3* negatively regulates chlorophyll content and then enhances shade-avoidance syndrome.

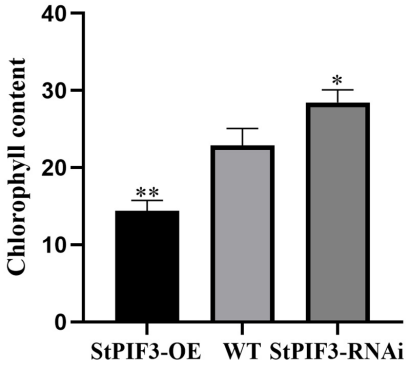

Figure 11. Accumulation of chlorophyll in StPIF3-OE, WT, and StPIF3-RNAi plants. (*, $p < 0.05$; **, $p < 0.01$).

3.10. StPIF3 Regulates Shade-Avoidance Syndrome by Regulating Plant Heights

In order to identify the role of *StPIF3* in potato shade-avoidance responses, we constructed *StPIF3* overexpression and knockdown transgenic lines and measured the plant height of transgenic potato lines and a wild-type control. The results showed that there was a significant difference in plant height between the transgenic lines and the wild-type potato plants (Figure 12A,C). The plant height was obviously increased in the *StPIF3* overexpression transgenic lines, whereas the plant height was significantly shortened in *StPIF3* knockdown transgenic lines. As expected, the plant height of both the transgenic and wild-type plants significantly increased after shade treatment; however, there was a significant difference in the increase rate between the transgenic and wild lines (Figure 12B,D). Taken together, these results imply the crucial role of *StPIF3* in regulating plant height and shade-avoidance responses.

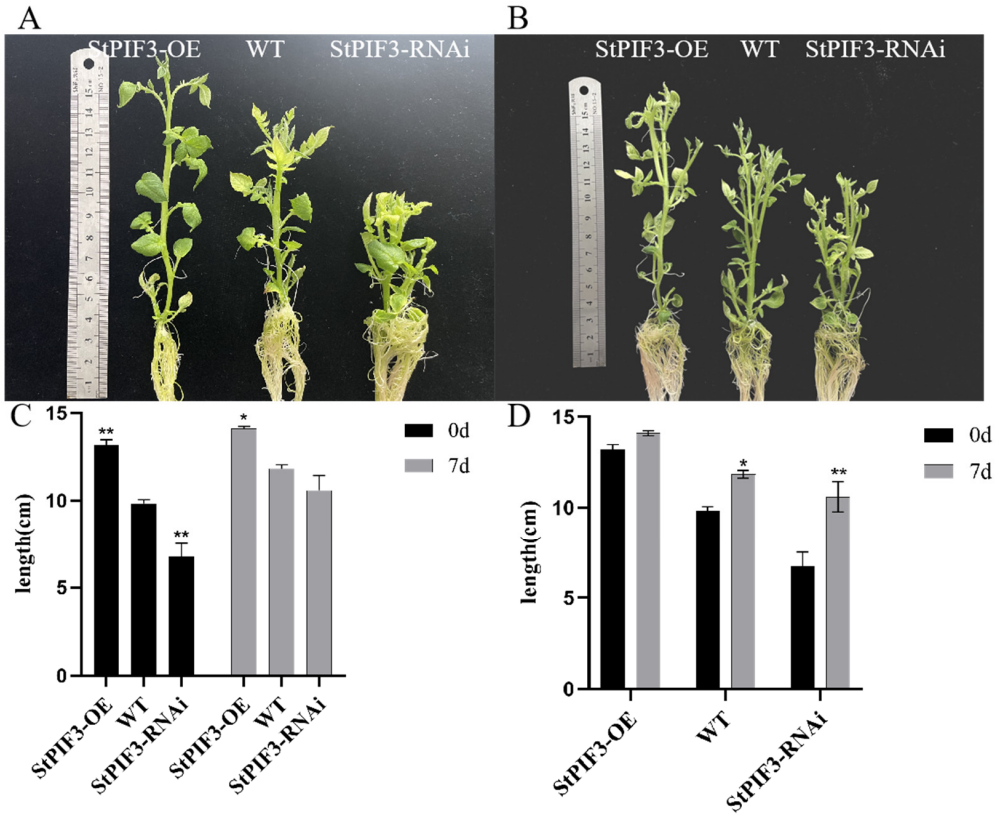

Figure 12. Physiological indicator determination of transgenic and wild-type plants before and after shading treatment. (**A**,**B**) Pictures of transgenic plants before and after low-light treatment. (**C**,**D**) Comparison of plant height between transgenic and wild-type plants after seven days of low-light treatment. (*, $p < 0.05$; **, $p < 0.01$).

4. Discussion

4.1. StPIF Identification and Evolution in Potatoes

Phytochrome-interacting factors have been discovered as regulatory hubs that integrate environmental cues into transcriptional scenarios, and functional characterizations have shown that PIF proteins are widely involved in plant growth, development, and stress response biological processes [30,31]. Several *PIF* genes have been identified in many species; for example, eight candidate *PIF* genes have been reported in Arabidopsis [9], tomatoes [12], maize [11,32], apples [13,33], and *Brachypodium distachyon* [15]; six have been reported in rice [10] and *Physcomitrella patens* [17]; and only one has been reported in *Marchantia polymorpha* [16]. However, the *PIF* genes have been insufficiently identified in potatoes to date. In this study, we identified seven candidate *StPIFs* in potatoes; StPIF02 was removed due to a lack of an APB domain (Figure 3). The number of *StPIFs* was

similar to most plants, probably due to the evolutionary conservation of the gene family. Physicochemical property analysis showed that there were differences in the theoretical isoelectric point and the location of the characteristic domains of the *StPIFs*; this showed that their structures were quite different, and we can speculate that they perform different functions (Table 1). The APB and APA domains have been reported to be necessary factors for identifying PIF family members apart from bHLH domains. PIF family members regulate the morphogenesis of plants via the APB domain, combined with the phyB or APA domain, combined with phyA [7]. In our study, all the gene family members had the APB domain; only StPIF06-2 and StPIF01-2 had both the APA domain and the APB domain, similar to AtPIF1 and AtPIF3, suggesting that they may physically interact with phyA and phyB, so these StPIFs might be close to AtPIF1 and AtPIF3 on an evolutionary scale [34] (Figure 3). A comparative phylogenetic investigation revealed high orthology between the StPIFs and their *Solanum lycopersicum* PIF counterparts (Figure 4).

4.2. PIF Transcription Factors Exhibit Phylogenetic Conservation across Plantae

Evolutionary analysis showed that the phylogenetic trees of *StPIFs* can be divided into six groups and clustered with tomatoes: SlPIF1a/1b, SlPIF3, SlPIF4, SlPIF7a/7b, and SlPIF8a/8b (Figure 4). Further analysis of the conserved motifs of the *StPIF* family members using the MEME software revealed that the majority of *StPIFs* in the same group had similar motifs, and those genes containing similar motifs were likely generated by gene amplification in the same group (Figure 5) [35]. We found motifs that were conserved across all groups; for example, Motifs 1, 2, and 8 contained the bHLH domain, and Motif 3 contained the APB domain. Different groups of *StPIFs* showed some differences in motifs. Although *StPIF03* was missing Motif 4 in a group and had one more Motif 7 compared with *StPIF06-1*, this may reflect the diversity of *StPIF* protein functions. The number of exons and introns of the *StPIF06-2* and *StPIF07* was similar to that of *A. thaliana* and *Vitis vinifera* L., implying that *PIF* genes can be functionality conserved across plant species [9,14]. *StPIF06-2, StPIF09*, *StPIF06-1*, *StPIF03*, and *StPIF01-1* shared the same number of exons and introns; we also found that they were from the same group (Figure 2).

4.3. PIFs Function as Key Nodes Conjoining Environmental and Hormonal Signals

Increasing evidence underscores the centrality of PIFs as regulatory nodes in various biological processes, including resistance to drought [36], salt [37], cold [38], chlorophyll biosynthesis [39], plant hormone signaling pathways (GA, BR, and auxin) [40], and the regulation of plant immune responses [41]. Promoter cis-acting element analysis is useful for predicting the potential functions of genes. A variety of cellular proteins, including transcription factors, can bind promoter cis-elements that regulate gene transcription and expression in response to environmental and hormonal signals. There are a total of 25 predicted promoter cis-acting elements, which roughly include hormone response elements, light response elements, response elements related to abiotic stress, and some special elements (Figure 6). For example, in the gibberellin signaling pathway, DELLA protein stabilization inhibits PIF4-driven cellular expansion, while gibberellins promote elongation by destabilizing DELLA, facilitating nuclear PIF4 accrual and target gene activation [42]. In *Cucumis sativus*, researchers delineate *CsPIF3*'s pivotal function in the orchestration of red/far-red and UV-B light signaling pathways that govern hypocotyl development. Specifically, the interaction between *CsPhyB* and *CsPIF3*, followed by downstream engagement with *CsGA20ox-2-DELLA* and *CsPIF3-CsARF18*, modulates hypocotyl elongation via gibberellin and auxin routes. *CsPIF3*'s transcriptional control is affected through its binding to G/E-box motifs within the *CsGA20ox-2* and *CsARF18* promoter regions [30]. Considering the central role of *PIFs* in light signal transduction, this study explored the effect of light intensity on *StPIF* expression levels. Our results indicated that the expression of six *StPIFs* was affected by the light intensity. Shading treatments can increase the expression level of *PIF06-2* and *StPIF03*, but the increase was limited with time. The expression level of *StPIF01-1* decreased obviously and then increased after shade; the expression of *StPIF06-1*

was induced by light-shading conditions (Figure 7). In grapes, shade treatment facilitates increased transcript levels of *VvPIFs* [14]. This is generally consistent with our results.

Transcriptomic profiling of *StPIFs* in different potato organs and tissues revealed the dynamics of gene expression, providing valuable insights for functional analysis. An expression weight heat map was obtained and revealed the spatial differences in the expression levels of *StPIFs* in different potato organs/tissues. *StPIF01-1* had the highest expression in leaf-wounding secondary tissue, *StPIF06-2* was highly expressed in shoots, and most of the *StPIFs* showed different expression patterns in different organs/tissues, suggesting that different *StPIFs* had different biological functions (Figure 8).

4.4. StPIF3 Regulates Shade-Avoidance Syndrome in Potatoes

Previous studies have shown that PIFs participate in regulating light-dependent growth and development processes. AtPIF3 is a positive regulator of PHYB signal transduction; in *Poc1* mutant plants, dark conditions attenuate PIF3 transcript levels, whereas red illumination upregulates expression concomitantly with abbreviated hypocotyl elongation, which has been interpreted as being associated with *PIF3* overexpression [43]. In *Arabidopsis thaliana*, researchers characterized PIF3's role in photomorphogenesis, revealing that overexpression lengthens hypocotyls and shrinks cotyledons under red light, with diminished cotyledon expansion under red and far-red spectra, contrasting the truncated phenotypes observed in *pif3* mutants [44]. In our study, *StPIF3*-overexpressed potato plants have higher plant heights compared with wild-type and interfering expressing plants, and the overexpression plants exhibited smaller cotyledons; this is consistent with previous reports. The key role of PIF3 in light signal transduction is demonstrated in Figure 12. Shade during the tuber expansion period can lead to an increase in chlorophyll content [45]. In *Arabidopsis thaliana*, *PIF3* overexpression lines exhibit elevated anthocyanin and diminished chlorophyll levels [44]. Our results showed that *StPIF3* overexpression significantly decreased the chlorophyll content compared with wild-type plants, which may be related to the fact that they have smaller cotyledons; this is basically consistent with previous research. Increased plant height is one of the typical shade-avoidance syndromes. The results of our experiment also confirmed that the overexpression of *StPIF3* can greatly improve the plant height of transgenic potatoes. In summary, we propose that StPIF3 plays a critical role in regulating shade-avoidance syndrome in potatoes.

5. Conclusions

This study identified seven *StPIF* genes, all putative amino acid sequences containing APB and bHLH conserved domains unique to PIFs. In evolutionary relationships, StPIFs can be divided into four groups, each group with similar conserved motifs. Tissue expression profiling indicated that most of these genes were highly expressed in the leaves. All seven *StPIFs* screened responded to light intensity changes, and shading was beneficial to improving the expression level of the *StPIFs*. *StPIF3* overexpression showed higher plant height and less chlorophyll accumulation in normal light; interfering expression was the opposite. Furthermore, *StPIF3* overexpression reduced sensitivity to low-light treatment. Our research will provide ideas for optical signal transduction networks of PIFs in potatoes.

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References

1. Bae, G.; Choi, G. Decoding of light signals by plant phytochromes and their interacting proteins. *Annu. Rev. Plant Biol.* **2008**, *59*, 281–311. [CrossRef] [PubMed]
2. Roeber, V.M.; Bajaj, I.; Rohde, M.; Schmulling, T.; Cortleven, A. Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant Cell Environ.* **2021**, *44*, 645–664. [CrossRef] [PubMed]
3. Franklin, K.A.; Larner, V.S.; Whitelam, G.C. The signal transducing photoreceptors of plants. *Int. J. Dev. Biol.* **2005**, *49*, 653–664. [CrossRef] [PubMed]
4. Chen, M.; Chory, J. Phytochrome signaling mechanisms and the control of plant development. *Trends Cell Biol.* **2011**, *21*, 664–671. [CrossRef] [PubMed]
5. Carvalho, R.F.; Campos, M.L.; Azevedo, R.A. The role of phytochrome in stress tolerance. *J. Integr. Plant Biol.* **2011**, *53*, 920–929. [CrossRef] [PubMed]
6. Ballare, C.L. Light regulation of plant defense. *Annu. Rev. Plant Biol.* **2014**, *65*, 335–363. [CrossRef] [PubMed]
7. Castillon, A.; Shen, H.; Huq, E. Phytochrome interacting factors: Central players in phytochrome-mediated light signaling networks. *Trends Plant Sci.* **2007**, *12*, 514–521. [CrossRef] [PubMed]
8. Leivar, P.; Quail, P.H. PIFs: Pivotal components in a cellular signaling hub. *Trends Plant Sci.* **2011**, *16*, 19–28. [CrossRef] [PubMed]
9. Lee, N.; Choi, G. Phytochrome-interacting factor from Arabidopsis to liverwort. *Curr. Opin. Plant Biol.* **2017**, *35*, 54–60. [CrossRef]
10. Nakamura, Y.; Kato, T.; Yamashino, T.; Murakami, M.; Mizuno, T. Characterization of a set of phytochrome-interacting factor-like bHLH proteins in Oryza sativa. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1183–1191. [CrossRef] [PubMed]
11. Zhang, T.; Lv, W.; Zhang, H.; Ma, L.; Li, P.; Ge, L. Genome-wide analysis of the basic Helix-Loop-Helix (bHLH) transcription factor family in maize. *BMC Plant Biol.* **2018**, *18*, 235. [CrossRef]
12. Rosado, D.; Gramegna, G.; Cruz, A.; Lira, B.S.; Freschi, L.; de Setta, N. Phytochrome interacting factors (PIFs) in Solanum lycopersicum: Diversity, evolutionary history and expression profiling during different developmental processes. *PLoS ONE* **2016**, *11*, e0165929. [CrossRef] [PubMed]
13. Zheng, P.F.; Wang, X.; Yang, Y.Y.; You, C.X.; Zhang, Z.L.; Hao, Y.J. Identification of phytochrome-interacting factor family members and functional analysis of MdPIF4 in Malus domestica. *Int. J. Mol. Sci.* **2020**, *21*, 7350. [CrossRef] [PubMed]
14. Zhang, K.K.; Zheng, T.; Zhu, X.D.; Jiu, S.T.; Liu, Z.J.; Guan, L.; Jia, H.F.; Fang, J.G. Genome-wide identification of PIFs in Grapes (*Vitis vinifera* L.) and their transcriptional analysis under lighting/shading conditions. *Genes* **2018**, *9*, 451. [CrossRef] [PubMed]
15. Jiang, M.; Wen, G.; Zhao, C. Phylogeny and evolution of plant Phytochrome Interacting Factors (PIFs) gene family and functional analyses of PIFs in Brachypodium distachyon. *Plant Cell Rep.* **2022**, *41*, 1209–1227. [CrossRef] [PubMed]
16. Inoue, K.; Nishihama, R.; Kataoka, H.; Hosaka, M.; Manabe, R.; Nomoto, M. Phytochrome signaling is mediated by phytochrome interacting factor in the liverwort Marchantia polymorpha. *Plant Cell* **2016**, *28*, 1406–1421. [CrossRef] [PubMed]
17. Possart, A.; Xu, T.; Paik, I.; Hanke, S.; Keim, S.; Hermann, H.M.; Wolf, L.; Hiß, M.; Becker, C.; Huq, E.; et al. Characterization of phytochrome interacting factors from the moss *Physcomitrella patens* illustrates conservation of phytochrome signaling modules in land plants. *Plant Cell* **2017**, *29*, 310–330. [CrossRef] [PubMed]
18. Leivar, P.; Monte, E. PIFs: Systems integrators in plant development. *Plant Cell* **2014**, *26*, 56–78. [CrossRef] [PubMed]
19. Liu, Z.; Zhang, Y.; Wang, J.; Li, P.; Zhao, C.; Chen, Y.; Bi, Y. Phytochrome-interacting factors *PIF4* and *PIF5* negatively regulate anthocyanin biosynthesis under red light in *Arabidopsis* seedlings. *Plant Sci.* **2015**, *238*, 64–72. [CrossRef] [PubMed]
20. Penfield, S.; Josse, E.M.; Halliday, K.J. A role for an alternative splice variant of *PIF6* in the control of *Arabidopsis* primary seed dormancy. *Plant Mol. Biol.* **2010**, *73*, 89–95. [CrossRef] [PubMed]
21. Camire, M.E.; Kubow, S.; Donnelly, D.J. Potatoes and human health. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 823–840. [CrossRef] [PubMed]
22. Gómez-Ocampo, G.; Cascales, J.; Medina-Fraga, A.L.; Ploschuk, E.L.; Mantese, A.I.; Crocco, C.D.; Matsusaka, D.; Sánchez, D.H.; Botto, J.F. Transcriptomic and physiological shade avoidance responses in potato (*Solanum tuberosum*) plants. *Physiol. Plant.* **2023**, *175*, e13991. [CrossRef] [PubMed]
23. Liu, W.; Tang, X.; Zhu, X.; Qi, X.; Zhang, N.; Si, H. Genome-wide identification and expression analysis of the E2 gene family in potato. *Mol. Biol. Rep.* **2019**, *46*, 777–791. [CrossRef] [PubMed]
24. Wang, C.; Han, J.; Shangguan, L.; Yang, G.; Kayesh, E.; Zhang, Y.; Leng, X.; Fang, J. Depiction of grapevine phenology by gene expression information and a test of its workability in guiding fertilization. *Plant Mol. Biol.* **2014**, *32*, 1070–1084. [CrossRef]
25. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
26. Xu, X.; Pan, S.; Cheng, S.; Zhang, B.; Mu, D.; Ni, P.; Zhang, G.; Yang, S.; Li, R.; Wang, J.; et al. Genome sequence and analysis of the tuber crop potato. *Nature* **2011**, *475*, 189–195. [CrossRef] [PubMed]
27. Pieczynski, M.; Marczewski, W.; Hennig, J.; Dolata, J.; Bielewicz, D.; Piontek, P.; Wyrzykowska, A.; Krusiewicz, D.; Strzelczyk-Zyta, D.; Konopka-Postupolska, D.; et al. Down-regulation of CBP80 gene expression as a strategy to engineer a drought-tolerant potato. *Plant Biotechnol. J.* **2013**, *11*, 459–469. [CrossRef] [PubMed]
28. Si, H.J.; Xie, C.H.; Liu, J. Optimization of agrobacterium-mediated genetic transformation system of potato in vitro potato and introduction of antisense class I. patatin gene. *Acta Agron. Sin.* **2003**, *29*, 801–805.
29. Al-Sady, B.; Ni, W.; Kircher, S.; Schäfer, E.; Quail, P.H. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. *Mol. Cell* **2006**, *23*, 439–446. [CrossRef] [PubMed]

30. Zhao, J.; Bo, K.; Pan, Y.; Li, Y.; Yu, D.; Li, C.; Chang, J.; Wu, S.; Wang, Z.; Zhang, X.; et al. Phytochrome-interacting factor PIF3 integrates phytochrome B and UV-B signaling pathways to regulate gibberellin- and auxin-dependent growth in cucumber hypocotyls. *J. Exp. Bot.* **2023**, *74*, 4520–4539. [CrossRef] [PubMed]
31. Pham, V.N.; Kathare, P.K.; Huq, E. Phytochromes and phytochrome interacting factors. *Plant Physiol.* **2018**, *176*, 1025–1038. [CrossRef] [PubMed]
32. Wu, G.; Zhao, Y.; Shen, R.; Wang, B.; Xie, Y.; Ma, X.; Zheng, Z.; Wang, H. Characterization of maize phytochrome-interacting factors in light signaling and photomorphogenesis. *Plant Physiol.* **2019**, *181*, 789–803. [CrossRef] [PubMed]
33. Han, X.; Liu, K.; Yuan, G.; He, S.; Cong, P.; Zhang, C. Genome-wide identification and characterization of AINTEGUMENTA-LIKE (AIL) family genes in apple (Malus domestica Borkh.). *Genomics* **2022**, *114*, 110313. [CrossRef] [PubMed]
34. Shen, H.; Zhu, L.; Castillon, A.; Majee, M.; Downie, B.; Huq, E. Light-induced phosphorylation and degradation of the negative regulator PHYTOCHROME-INTERACTING FACTOR1 from *Arabidopsis* depend upon its direct physical interactions with photoactivated phytochromes. *Plant Cell* **2008**, *20*, 1586–1602. [CrossRef] [PubMed]
35. Freeling, M. Bias in plant gene content following different sorts of duplication: Tandem, whole-genome, segmental, or by transposition. *Annu. Rev. Plant Biol.* **2009**, *60*, 433–453. [CrossRef] [PubMed]
36. Gao, Y.; Wu, M.; Zhang, M.; Jiang, W.; Ren, X.; Liang, E.; Zhang, D.; Zhang, C.; Xiao, N.; Li, Y. A maize phytochrome-interacting factors protein ZmPIF1 enhances drought tolerance by inducing stomatal closure and improves grain yield in *Oryza sativa*. *Plant Biotechnol. J.* **2018**, *16*, 1375–1387. [CrossRef] [PubMed]
37. Gao, Y.; Jiang, W.; Dai, Y.; Xiao, N.; Zhang, C.; Li, H.; Lu, Y.; Wu, M.; Tao, X.; Deng, D. A maize phytochrome-interacting factor 3 improves drought and salt stress tolerance in rice. *Plant Mol. Biol.* **2015**, *87*, 413–428. [CrossRef] [PubMed]
38. Wang, F.; Chen, X.; Dong, S.; Jiang, X.; Wang, L.; Yu, J.; Zhou, Y. Crosstalk of PIF4 and DELLA modulates CBF transcript and hormone homeostasis in cold response in tomato. *Plant Biotechnol. J.* **2020**, *18*, 1041–1055. [CrossRef] [PubMed]
39. Huq, E.; Al-Sady, B.; Hudson, M.; Kim, C.; Apel, K.; Quail, P.H. Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. *Science* **2004**, *305*, 1937–1941. [CrossRef] [PubMed]
40. Choi, H.; Oh, E. PIF4 integrates multiple environmental and hormonal signals for plant growth regulation in *Arabidopsis*. *Mol. Cells* **2016**, *39*, 587–593. [CrossRef] [PubMed]
41. Gangappa, S.N.; Berriri, S.; Kumar, S.V. PIF4 coordinates thermosensory growth and immunity in *Arabidopsis*. *Curr. Biol.* **2017**, *27*, 243–249. [CrossRef] [PubMed]
42. De Lucas, M.; Davière, J.M.; Rodríguez-Falcón, M.; Pontin, M.; Iglesias-Pedraz, J.M.; Lorrain, S.; Fankhauser, C.; Blázquez, M.A.; Titarenko, E.; Prat, S. A molecular framework for light and gibberellin control of cell elongation. *Nature* **2008**, *451*, 480–484. Available online: https://www.nature.com/articles/nature06520#citeas (accessed on 6 March 2024). [CrossRef] [PubMed]
43. Halliday, K.J.; Hudson, M.; Ni, M.; Qin, M.; Quail, P.H. Poc1: An Arabidopsis mutant perturbed in phytochrome signaling because of a T DNA insertion in the promoter of PIF3, a gene encoding a phytochrome-interacting bHLH protein. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5832–5837. [CrossRef] [PubMed]
44. Kim, J.; Yi, H.; Choi, G.; Shin, B.; Song, P.S.; Choi, G. Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* **2003**, *15*, 2399–2407. [CrossRef] [PubMed]
45. Li, P.H.; Peng, X. Study on photosynthesis effect of potato shading light. *Chin. Agric. Sci. Bull.* **2007**, *23*, 220–227. Available online: https://www.casb.org.cn/CN/Y2007/V23/I4/220 (accessed on 6 March 2024).