Genomic and molecular analysis of conserved and unique features of soybean PIF4

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Phytochrome-interacting factor 4 (PIF4) participates in light signaling by interacting with photoreceptors, phytochromes, and cryptochromes. Although well characterized in Arabidopsis, PIF4’s role in crop plants is unknown. Here we performed the first integrated genomics, transcriptomics, and molecular characterization of PIF4 in soybean (Glycine max) plants. Fifteen identified Glycine max PIFs (GmPIFs) grouped into PIF3, PIF4, and PIF8 subfamilies based on their phylogenetic relationships. The GmPIF4 subfamily formed two distinct clades (GmPIF4 I and GmPIF4 II) with different amino acid sequences in the conserved bHLH region. Quantitative transcriptional analysis of soybean plants exposed to different photoperiods and temperatures indicated that all PIF4 I clade GmPIF4s conserved PIF4-like expression. Three out of four GmPIF4 transcripts of the GmPIF4 I clade increased at 35 °C compared to 25 °C under short day conditions. RNA sequencing of soybeans undergoing floral transition showed differential regulation of GmPIF4b, and ectopic GmPIF4b expression in wild type Arabidopsis resulted in an early flowering phenotype. Complementation of GmPIF4b in Arabidopsis pif4-101 mutants partially rescued the mutant phenotype. PIF4 protein levels peaked before dawn, and a GmPIF4b protein variant was observed in soybean plants treated at high temperatures.

Environmental factors such as light and temperature have a profound effect on plant physiology and development; not only their presence but also the duration of exposure. The photoperiod (light and dark phase length) influences molecular signaling, with the circadian clock synchronizing these environmental signals with endogenous rhythms to ensure optimal development and reproduction.

High-throughput sequencing and genetic analyses have revealed that phytochrome interacting factors (PIFs), a class of basic helix-loop-helix (bHLH) transcription factors, play crucial roles in integrating photoperiodic signals through photoreceptor, phytochrome and cryptochrome, interactions. In the model plant Arabidopsis thaliana, PIFs belong to the bHLH superfamily of proteins, with the PIF subfamily consisting of PIF1, PIF3, PIF4, PIF5, PIF6, PIF7, and PIF8. The bHLH domain contains a stretch of 50–60 amino acids that comprises two segments: a stretch of around 40 amino acids forming two amphipathic α-helices separated by a variable length loop and a 10–15 basic amino acid domain with DNA-binding capacity.

PIF proteins have predominately been studied in Arabidopsis shade avoidance responses. PIFs interact with the light-activated form of phytochromes (Pfr) through their highly-conserved active phytochrome-binding (APB) motifs. PIFs typically accumulate in the dark, peak at dawn, and then degrade in the presence of light by interacting with PIFs and ubiquitin-proteasome degradation. PIF transcription is regulated by the evening circadian clock complex, with the ELF3-ELF4-LUX complex directly binding to PIF4 and PIF5 promoters to suppress their expression and regulate circadian responses. It has recently been suggested that PIF4 acts as an integrating hub for light and temperature-related signals and the evening circadian clock-expressed factor TOC1 to regulate thermoresponsive plant growth. PIF4 is also a central phytochrome regulator during Arabidopsis flowering under short day conditions through control of hormonal networks. In Arabidopsis, PIF4 also controls auxin (indole acetic acid, IAA) signaling by modulating the expression of SMALL AUXIN-UP RNA (SAUR) genes at high temperatures. PIF4 interacts with the blue light receptor CYTOCHROME 1 (CRY1) to regulate high temperature-mediated hypocotyl elongation by increasing IAA concentrations through stimulation of YUC8 (YUCCA8) and Tryptophan Aminotransferase of Arabidopsis 1 (TAA1) gene expression. PIF4 and PIF5 together play a crucial role in leaf senescence, activating ETHYLENE INSENSITIVE 3 (EIN3),...
ABSCISIC ACID INSENSITIVE 5 (ABI5), and ENHANCED EM LEVEL (EEL) gene expression to produce the senescence hormones ethylene and abscisic acid. Clearly, PIFs have pleiotropic roles in model plants, but their roles in other plants of commercial value is less well characterized.

Soybean (*Glycine max* (L.) Merrill) is a leguminous crop that is mainly used as a source of protein and vegetable oil and that can fix atmospheric nitrogen via a symbiotic relationship with soil-borne microorganisms. The soybean genome is complex due to two genome duplication events estimated to have occurred 59 and 13 million years ago. The paleopolyploid soybean genome presents the exciting opportunity to explore evolutionary diversification in gene function occurring due to chromosomal rearrangements during duplication. The presence of multiple forms/copies of a gene is often linked to the acquisition of new functions (neo-functionalization) or division of labor to divide the function (sub-functionalization) in a species. These gene diversification events lay the foundation for phenotypic variability and adaptability in plants. Soybean flowering and pod set is dependent on the photoperiod. Hence, soybean cultivars are divided into different maturity groups depending on day length requirements, and some of the quantitative trait loci that affect soybean flowering have recently been reported.

The roles of PIFs and their interactions with phytochromes during soybean flowering have yet to be investigated. Moreover, the functions of genes related to temperature and light perception in soybean are unknown. The recent sequencing of the soybean genome has provided the means to examine the genes participating in soybean flowering pathways. To explore PIF4's roles in soybean plants, especially short day-specific signaling in soybean flowering, we studied all the *GmPIF* sequences present in the soybean genome. Phylogeny, conserved protein motifs, and expression profiles of these genes were comprehensively analyzed using bioinformatics approaches. Further, gene expression patterns under flowering non-inductive (long day) and flowering inductive (short day) light conditions and at elevated temperatures were quantitatively analyzed. The function of differentially regulated *GmPIF4* (*GmPIF4b*) was studied by ectopic expression in *Arabidopsis* Col-0 and in *pif4-101* mutants. We reveal structural and functional divergence in soybean PIF4 genes and proteins.

**Results**

**Identification, phylogeny, and subcellular localization of *GmPIF* genes.** Systematic and comprehensive database searches of the available genome sequences of leguminous plants revealed multiple PIF family members. To investigate the phylogenetic relationship between different PIFs and their evolutionary conservation, four leguminous plants with sequenced genomes were considered. The Phytozome search and phylogenetic analysis grouped fifteen PIF-like sequences into PIF4, PIF3, and PIF8 clades. There is strong evidence that soybean has undergone two whole genome duplication events during evolution. Based on the chromosomal evidence, soybean's recent lineage-specific palaeotetraploidization was probably an allotetraploidy event preceded by an early legume duplication event occurring near the origins of the papilionoid lineage. Recently, the Legume Family Working Group (LPWG) refined the classification of the Leguminosae family into six subfamilies: *Caesalpinioidae*, *Cercidioideae*, *Detarioideae*, *Dialioideae*, *Dnaparquetoidae*, and *Faboideae*, with soybean assigned to the family *Faboideae*.

To establish the phylogenetic relatedness of legume PIF proteins, soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), barrel clover (*Medicago truncatula*), and peanut (*Arachis duranensis*) sequences were extracted. All these plants belong to the *Faboideae* family, with the soybean, common bean, and peanut short day plants and *Medicago* a long day plant. Phylogenetic analysis using the neighbor-joining algorithm revealed that different soybean PIFs group into different clades (PIF4, PIF3, and PIF8; Fig. 1A) and include the signature PIF4 sequence of *Arabidopsis*. Soybean PIF4s grouped into two clades, *GmPIF4* I and *GmPIF4* II (marked with asterisks in Fig. 1A), with *GmPIF4a*, *GmPIF4b*, *GmPIF4c*, *GmPIF4d* grouping into *GmPIF4* I and *GmPIF4e*, *GmPIF4f*, and *GmPIF4g* grouping into *GmPIF4* II. Similarly, PIF3 and PIF8 were classified based on their relatedness to the *GmPIF3* and *GmPIF8* sequences. Their position in the tree indicated that these multiple PIF copies in soybean may have evolved at different evolutionary points. Some of the PIF4s in soybean retain family-specific relatedness because of the early legume genome duplication event, while the other PIFs arose more recently due to a soybean-specific duplication event. PIFs grouped more closely to the common bean PIFs compared to *Medicago* and peanut, consistent with the common bean being a closer relative.

**Analysis of *GmPIF* protein sequence motifs.** Ten motifs were identified and designated motifs 1–10 (Supplementary Figure 1). Motif 5, 7, and 9 mainly distinguished *GmPIF4* I from *GmPIF4* II proteins (Fig. 1A). A lack of motif 9 and 5 and the presence of motif 8 was a characteristic feature of PIF3s. Furthermore, motifs 3, 5, 8, and 9 were absent in PIF8s. Motif patterns help to distinguish sequences, as motif location and frequency are important for protein folding during translation. Motifs also act as recognition sequences for molecules involved in important processes such as post-translation modifications, subcellular transport and localization, and translation start and termination.

**Two whole genome duplications contributed to *GmPIF* gene family expansion.** The genomic survey showed an uneven distribution of fifteen *GmPIF* genes on 11 soybean chromosomes (Fig. 1B). Chromosome 3 and 19 had two genes each, chromosome 10 had three genes, and the other nine genes were located on chromosomes 1, 2, 8, 13, 14, 18, and 20. Two main gene duplication types occur during evolution: tandem duplication, resulting in gene clusters; and segmental duplication, which gives rise to members scattered across the genome. 5,671 putative soybean transcription factor genes have been identified, of which 9.5% show tandem duplication. Detailed analysis of *GmPIF* genes revealed that two gene pairs of the PIF3 subfamily were tandemly duplicated (*GmPIF3a*-*GmPIF3c* and *GmPIF3b*-*GmPIF3f*; Fig. 1B).

We next estimated the possible duplication time according to their pairwise distances (Ks values) based on previous soybean studies. Ks values of 0.06–0.39 correspond to the 13 million years ago (Mya) *Glycine* lineage-specific genome duplication, Ks values of 0.40–0.80 correspond to the 59 Mya early legume whole genome duplication. Some of the PIF3s in soybean retain family-specific relatedness because of the early legume genome duplication event, while the other PIFs arose more recently due to a soybean-specific duplication event. PIFs grouped more closely to the common bean PIFs compared to *Medicago* and peanut, consistent with the common bean being a closer relative.
transcript abundance significantly declined at 8 h compared to 0 h. Differential response during the day and night. During light periods, GmPIF4b was the only transcript observed in seeds, and no transcript was observed at late stages in young pods. GmPIF4f, GmPIF4g, and GmPIF3a were abundantly expressed in the SAM, while GmPIF4a-e were expressed in the leaves. GmPIF4a, b, and f were previously shown to be highly regulated in leaves. Tissue-specific expression analysis showed that GmPIF4b, GmPIF4b, GmPIF4c, GmPIF4d were expressed in leaves, while GmPIF4a, GmPIF4g, GmPIF4f, GmPIF3a were present in leaves, flower, and young pods. GmPIF3f was the only transcript observed in seeds, and no GmPIF transcript was observed at late developmental stages (Fig. 3). Long day specific diurnal rhythm of GmPIF4 transcripts. Soybean leaves were sampled every four hours to examine whether soybean PIFs were diurnally regulated. Over long days, all transcripts showed differential responses during the day and night. During light periods, GmPIF4b transcript abundance significantly declined at 8 h compared to 0 h. GmPIF4c and GmPIF4g showed significant decreases at 12 h. However, these
transcripts were re-expressed in the last four hours of the day, i.e., between 12 and 16 h (Fig. 4A–F), consistent with the long day behavior of *Arabidopsis thaliana* PIF4 transcripts, which re-accumulate on prolonged exposure to light and indicating that decreases in PIF4 levels upon light exposure are transient 6.

**GmPIF4a** and **GmPIF4d** did not show typical PIF4 like expression in long day photoperiod.

**Short day specific diurnal rhythm of GmPIF4 transcripts.** Samples were collected every 4 hours to study PIF4 transcription patterns after one short day treatment. One short day treatment was sufficient to alter the expression of **GmPIF4a**, **GmPIF4b**, and **GmPIF4c** (Fig. 4G–I), which accumulated during the dark (just before the day breaks), consistent with previous reports on the expression of PIF4 in *Arabidopsis* during short days 30. However, **GmPIF4f** and **GmPIF4g** showed no diurnal fluctuations under short day conditions (Fig. 4K,L). Often, duplication events silence the function of an ancient gene, with selective pressure giving rise to homologs with new functions 14.
A temperature-dependent role for PIF4 in flowering and blue light responses in Arabidopsis has been reported. To investigate how soybean PIF4 genes respond to temperature under flower-inducing short photoperiod conditions, the expression levels of soybean PIF4 transcripts were analyzed at 25 °C, 30 °C, and 35 °C under long and short-day conditions. There were no significant changes in transcript levels under long day conditions except for GmPIF4a and GmPIF4d, which showed an increase at 30 °C compared to at 25 °C (Fig. 4M–R). However, under short day conditions, GmPIF4a, GmPIF4c, GmPIF4d, and GmPIF4g transcripts significantly increased at 35 °C compared to 25 °C (Fig. 4M–R). According to the thermosensory activation model of flowering in Arabidopsis, PIF4 integrates short day photoperiod signals and combines them with the ambient temperature signal under the control of the endogenous clock. Kumar et al. proposed that, at higher temperatures, PIF4 directly interacts with flowering locus T (FT, florigen) to activate the flowering pathway in Arabidopsis. Further, temperature-based changes in PIF4 transcripts are rate limiting for the biological response, because H2A.Z nucleosomes decrease the accessibility of PIF4 to the FT promoter at cool temperatures. Since soybean is a warm climate plant requiring a temperature-based role for PIF4 in flowering, the increase in GmPIF4 transcript abundance at 35 °C (short day) indicates a possible role for soybean PIF4s in high temperature-mediated initiation of flowering.

Ectopic expression of GmPIF4b in Arabidopsis Col-0 plants. Analysis of RNA-seq data of soybean plants undergoing floral transition showed that GmPIF4b was differentially regulated in leaves. Hence, to further characterize gene function, GmPIF4b was expressed ectopically in Arabidopsis Col-0 plants and transgenic lines studied under long day 22 °C and short day 25 °C conditions. Transgenic lines had longer hypocotyls at SD-25 °C conditions, the expression levels of soybean PIF4 transcripts were analyzed at 25 °C, 30 °C, and 35 °C under long and short-day conditions.

Complementation of GmPIF4b in the Arabidopsis pif-101 mutant background. pif1-101 mutants have a T-DNA insertion in exon 5 of the Arabidopsis PIF4 gene. These mutant plants have shorter hypocotyls in the dark and a compact rosette (reduced petiole length) phenotype. We transformed the pif4-101 Arabidopsis mutant with the 35S::Gmpif4b::polyA construct for a gain-of-function analysis. Hypocotyl length was recorded in seedlings grown. Furthermore, petioles were also measured to assess rosette size. GmPIF4b partially rescued the mutant phenotype for both hypocotyl and petiole lengths under short day 25 °C conditions. Petiole length was 8 mm in wild-type, 2.6 mm in pif4-101, and 6 mm in complemented lines (Fig. 6) and hypocotyl length in complemented lines was 0.86 times of the WT (Fig. 6).

GmPIF4b protein levels peak four hours before dawn under both long and short-day conditions. To study the diurnal rhythm of PIF4 protein, protein expression was assessed every 4 h under long and short day conditions. GmPIF4b transcript was more abundant in the leaves of the plants grown under short day conditions.
Figure 5. Overexpression analysis of GmPIF4b in Col-0 Arabidopsis background. (A) Binary vector construct containing GmPIFb CDS driven by the 35s promoter. (B) Early flowering phenotype in GmPIF4b-ox lines at SD 25°C. (C) Hypocotyl length phenotype in GmPIF4b-ox lines at SD 25°C. (D) Measurement of hypocotyl length of different lines. (E) Mean days to flowering of different lines. (F) Expression levels of GmPIF4b in WT and GmPIF4b-ox lines normalized against Arabidopsis actin gene. The significant differences between data were calculated using Student's t-test. Significant differences are indicated with asterisks: (*) P < 0.05 and (**) P < 0.01. Error bars represent SD, n = 10. WT plants appear orange because they were observed in blue light.

Figure 6. Mutant complementation analysis of GmPIF4b in pif4-101 Arabidopsis background. (A) Comparison of hypocotyl lengths in WT, pif-101, and complemented lines. (B) Petiole length phenotype in WT, pif-101, and complemented lines at SD 25°C. (C) Rosette size phenotype in WT, pif-101, and complemented lines. (D) Measurement of petiole length in different lines. (E) Measurement of hypocotyl length in different lines. (F) Expression levels of GmPIF4b in WT, pif4-101, and complemented lines normalized against Arabidopsis actin gene. The significant differences between data were calculated using Student's t-test. Significant differences are indicated with asterisks: (*) P < 0.05 and (**) P < 0.01. Error bars represent SD, n = 10. WT plants appear orange when observed in blue light.
compared to long day conditions. However, GmPIF4b transcripts followed a strict diurnal rhythm under both photoperiod conditions. For both conditions, protein levels peaked four hours before dawn (Fig. 7). Arabidopsis PIF4 levels are known to peak during the night due to superimposition of the clock and photoperiodic pathways, and PIF4 is thought to be under the control of the evening complex. Further, the TOC1 component of the clock binds to PIF4 in the evening and inactivates it in Arabidopsis. Here, the GmPIF4b protein expression rhythm in soybean was similar to Arabidopsis. GmPIF4b protein also showed the highest expression in soybean leaves at SD-1, suggesting involvement in floral transition. RNA-seq studies have previously indicated major reprogramming during floral transition, especially when SAM converts from the vegetative to reproductive stage after 4–6 short day treatment.

GmPIF4b variant observed at elevated temperatures show unique temperature adaptations in soybean. Arabidopsis lines containing the 35S::PIF4:HA construct have been reported to contain slightly higher PIF4 protein levels at 27 °C than at 12 °C and 22 °C. To study the effect of temperature on PIF4 protein expression, soybean plants were treated with a range of temperatures (25 °C to 35 °C), reflecting soybean as a warm temperature crop with ambient temperatures for soybean growing at different latitudes often exceeding 30 °C. A different molecular weight variant form of GmPIF4b was observed following exposure to plants at higher temperature. (Figure 7C). Higher molecular weight variant observed in response to higher temperature might reflect a protein modification that merits further experimental evaluation.

Discussion
Soybean is a major leguminous crop used to produce a significant amount of vegetable oil and protein for human consumption and fodder for animals. Soy products are increasingly used as meat and milk substitutes globally. Hence, the demand for breeding high-yield varieties of this commercially important crop in our changing environment is increasing. To refine yields, a full understanding of the key regulators of flowering and development is essential. PIF4 is a bHLH transcription factor that is thought to act as an integrating hub for light and temperature signals in Arabidopsis. However, its role in important crops such as soybean, a paleopolyploid, has yet to be investigated.

Two gene duplication events occurred in the soybean genome nearly 59 and 13 million years ago, which were followed by gene diversification, loss, and numerous chromosomal rearrangements leading to 75% of soybean genes being present as multiple copies. Here we extracted fifteen GmPIF transcription factor genes from the Phytozome database and compared their sequences at both the nucleotide and amino acid levels. GmPIFs could be grouped into three significant subfamily clades (GmPIF4, GmPIF3, and GmPIF8) based on their conserved protein sequences. GmPIF4 could be further divided into two groups, GmPIF4 I and GmPIF4 II, based on sequence motif organization. This sequence-level observation supports the hypothesis that these transcription factors have undergone significant changes during evolution. Overall, there are estimated to be 31,264 gene paralogs in soybean, which may have developed from substitution and transversion events.

PIF transcription factors use their bHLH domain to bind DNA and regulate their downstream targets. Our detailed comparison of this conserved domain for all the GmPIF protein sequences highlighted amino acid variations within the bHLH domains of these proteins. These variations in conserved domains suggest that it is likely that these transcription factors have different protein binding specificities.

Gene duplication analysis of the GmPIF family revealed that GmPIF genes expanded during both glycine lineage-specific and early legume duplication events nearly 13 Mya and 59 Mya, respectively. Synteny of GmPIFs...
with common bean (*Phaseolus vulgaris*) *PvPIFs* was also evaluated to study the selective pressure on these genes, which showed that the Ka/Ks ratios for all *Gm-Pv* gene pairs were below 1, confirming purifying selection pressure.

Gene duplication serves as a mechanism to increase functional diversity33. In a paleopolyploid plant such as the soybean, these duplication events often lead to divergent expression patterns of closely related genes34. We found that the expression of these transcription factors varied in response to photoperiod and temperature stimuli. In *Arabidopsis thaliana*, *PIF4* transcription has been studied under both short day and warm conditions35. Soybean is a facultative short-day plant requiring the warm temperatures for floral initiation. Hence, we focused on studying *GmPIF4* transcription under short day conditions, under which four *GmPIF4s* showed similar expression to *Arabidopsis PIF4*, i.e., peaking at the end of the night phase (at dawn). All four *GmPIF4s* belong to the *GmPIF4 I* group; however, two *GmPIF4s* belonging to the *GmPIF4 II* clade did not follow a typical diurnal rhythm. A coincidence model has been proposed to understand short day-specific flowering in *Arabidopsis*, where *PIF4* accumulates at the end of the night on short days due to coincidence between the internal (circadian rhythm) and external (photoperiod) cues5. During the light phase, *PIF4* interacts with phytochromes and is degraded to switch on phytochrome signaling-mediated downstream processes8. In soybean, short days promote a shift from the vegetative to reproductive phase and hence control flowering32. Our data on *GmPIF4 I* group transcription is consistent with the co-incidence model, thus pointing towards conservation of gene function. To confirm this, ectopic expression of *GmPIF4b*, differentially regulated during soybean floral transition (*GmPIF4b*) in *Arabidopsis* Col-0 plants resulted in longer hypocotyls and an early flowering phenotype under short day 25°C conditions, and partially recovered the phenotype of hypocotyl length and compact rosette in *Arabidopsis* *pf4-101* mutants.

Protein expression of *GmPIF4b* peaked four hours before dawn under both long photoperiod and short period conditions, indicating superimposition of the biological clock in controlling *GmPIF4* expression in soybean plants. A unique *GmPIF4* higher molecular weight variant was observed following treatment of soybean plants at higher temperatures, indicating involvement of post-translational modifications in regulating *GmPIF4b* protein levels at the high temperatures.

Hence, apart from the general functions of *PIF4* in plants, this protein may participate in novel legume-specific development and function in soybean plants. Further detailed interaction analyses and metabolomic and proteomic-based studies are needed. Functional analysis of individual *PIF4* genes would uncover their specific roles in soybean development. This study paves the way for future research into specific biological functions of *GmPIF4s* in soybean development and floral transition.

**Methods**

**Identification, phylogenetic analysis and sub-cellular localization prediction of soybean PIF family.** *PIF* genes were searched by using the keywords of “PIF”, “Phytochrome Interacting factors”, and blast searches against *Arabidopsis* *PIFs* in the proteome database of the latest version of soybean genome (*Wm82.a2.v1*) in Phytozome. Subsequently, all the sequences with E-value below 0.01 were kept and checked for the presence of conserved basic helix loop helix (bHLH) by using Hidden Markov Model (HMM) profile (PF00010) in Pfam database, http://pfam.xfam.org/. Self-blast was performed on the resulting sequences list, and all the redundant sequences were removed. Similarly, *PIF* sequences for other legumes such as common bean (*Phaseolus Vulgaris*), barrel clover (*Medicago truncatula*) and peanut (*Arachis duranensis*) were also searched. The resulting sequences were listed in a table (Supplementary Table 1) and aligned using ClustalW program with default parameters in the alignment window of MEGAS7 software, http://www.megasoftware.net/ (Kumar, Stecher, and Tamura 2015). A phylogenetic tree was constructed using the *PIF* sequences of all the legumes and *Arabidopsis* using a neighbor-joining algorithm, JTT model, and partial deletion parameters. Based on the phylogenetic analysis, the putative soybean *PIFs* were named according to their respective clades. The subcellular localizations of *GmPIF4s* were predicted using LOCALIZER tool of the Commonwealth Scientific and Industrial Organization of Australia (CSIRO)35.

**Conserved protein motif search.** MEME search (http://meme-suite.org/tools/meme) was used for protein motif search comparison86. The length of the motif was fixed to 6–100 amino acids. To detect motifs ZOOPS model was used, which considers that the motif occurrence can be zero or 1 in a sequence. Maximum 10 motifs were searched.

**Analysis of chromosome distribution, gene duplication and synteny with common bean.** The chromosome distribution of soybean *PIF* genes was obtained from Phytozome, and duplicated genes were obtained from Plant genome duplication database (PGDD) (http://chibba.agtec.uga.edu/duplication/) by downloading the dataset of duplicated blocks in soybean genome37. Duplicated *PIF* gene pairs were searched in the dataset, and their nucleotide non-synonymous (*Ka*) to synonymous (*Ks*) ratios (*Ka/Ks*) was also calculated (Supplementary Table 2). *Ks* values were used to estimate the duplication time for soybean *PIFs*. Similarly, syntenic blocks between soybean and common bean were also searched. PGDD uses BLASTP to search for potential anchors (*E* < 1e-5, top 5 matches) between every possible pair of chromosomes in the genomes considered. Input for MCscan synteny search tool is the homologous pairs38. The built-in scoring scheme for MCscan is min (*−log*-*E*, 40) for every matching gene pairs and -1 for each 10 kb distance between anchors, similar to DAGChainer synteny tool39 and blocks that have scores >200 are kept. The resulting syntetic chains are evaluated using a procedure in CoLineanScan and *E*-value < 1e-10 as a significance cutoff. The data for duplicated *PIF* gene pairs within soybean and their putative orthologs in common bean is listed (Supplementary Table 2).
Multiple sequence alignment of the bHLH domain GmPIFs. The bHLH domain was identified after aligning the sequences of all 15 PIFs by using clustal alignment option in Jalview software. The logo of bHLH domain was obtained from Pfam database of protein HMMs.

RNA seq data analysis for soybean undergoing floral transition and expression in different tissues. The RNA seq data for the soybean undergoing floral transition was obtained from previously published research (Wong et al. 2013). RNA sequencing data for the expression of GmPIFs in different tissues was obtained from soybase https://www.soybase.org. The RNA sequencing reads have been listed in Supplementary Table 3. The heat maps were constructed using MORPHEUS tool of the Broad Institute (https://software.broadinstitute.org/morpheus/).

Plant material, Treatments and Expression analysis using qRT-PCR. For photoperiod-dependent expression analysis, two sets of (Glycine max [L.] Merill) cv. Bragg plants were grown for 10 long days (16hrs light, 8hrs dark) at 25 °C, 400 μM m⁻¹ s⁻¹ light intensity. On 11th day, one set of plants was subjected to one short-day (8hrs light, 16hrs dark) treatment. Leave samples (from three different plants within a set) from both sets were harvested every 4 hours for 24 hours.

For temperature dependent expression analysis, six sets of the plants were grown for 10 days under long day conditions. On the 11th day, three sets were subjected to long-day at 25 °C, 30 °C and 35 °C and the other three sets were subjected to short-day at 25 °C, 30 °C and 35 °C respectively. Samples were collected at the end of the night. All the expression analyses were performed using three biologically replicated experiments.

Total RNA was extracted from the leaves samples by using Trizol method and cDNA was synthesized by using SuperScript III Reverse transcriptase of Invitrogen. SYBR-Green master mix from Agilent Technologies was used. The expression data of GmPIF4a, GmPIF4b, GmPIF4c, GmPIF4d, and GmPIF4g transcripts was normalized against the expression of Glycine max Actin gene (Glyma.08G146500.1). Supplementary Figure 4 shows that this actin gene is not regulated diurnally or in response to heat treatment.

GmPIF4b over-expression construct, Arabidopsis transformation, and transgenic line analysis. Total RNA was extracted from Soybean's leaf tissue. The amplified DNA was cloned downstream of constitutive 35S promoter and resulting 35SS::GmPIF4b::ploA was used for plant transformation. Arabidopsis plants (wild type and mutants) were grown in soil, long day photoperiod and at 22 °C for 4 weeks (till flowering started). The first inflorescence was cut-off to promote flowering on lateral branches because we followed floral dip method for Arabidopsis transformation.

Arabidopsis seeds obtained from T0 generation were grown for 7 days and on 8th day, these were sprayed with the herbicide Glufosinate (Basta) to select transgenic lines. Strong YFP signal was observed in the surviving plants (observed in blue light). This generation (T1) was examined for the expression of GmPIF4b by qRT-PCR. Similarly, complemented lines were obtained by infecting Arabidopsis pif4-101 mutants with GmPIF4b over-expression construct. Hypocotyl lengths were analysis using Image J software (114 pixels were scaled to 1 cm).

Production of rabbit polyclonal antibody against the GmPIF4b protein, plant nuclear protein extraction, and immunoblotting. The codon optimized GmPIFb gene construct by the GenScript Services (Hong-Kong) was used to express this protein in E.coli. The recombinant protein was quantified on a BSA standard curve and used for immunization of rabbits. The antibody was purified from total sera (Supplementary Figure 2). Nuclear protein was extracted according to Haerizadeh et al. For immunoblotting, 1.5 μg of total nuclear protein from soybean, primary antibody (anti-GmPIF4b developed in our lab) and secondary antibody (anti-rabbit IgG) were used. The blots were imaged using Licor western blot imager (800 nm channel). Two independent experiments were performed to check the validity of the western blots. Dot blot analysis of Arabidopsis transgenic lines containing over-expressed GmPIF4b showed reactivity with GmPIF4b antibody, whereas wild type Arabidopsis (Col-0) showed no reactivity (Supplementary Figure 6).

Data availability. Data described in this study can be obtained from the corresponding author by request.

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Acknowledgements
This work was supported by The Australian Research Council Discovery Grant, ARC DP0988972.

Author Contributions
P.L.B. and M.B.S. conceived and designed the experiments; H.A. performed the experiments; H.A., M.B.S. and P.L.B. wrote the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-30043-2.

Competing Interests: The authors declare no competing interests.

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