Transcriptome Analysis to Identify Putative Genes Involved in Flowering Time Under Different Photoperiods in ‘Hong jin gou’ Common Bean

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ABSTRACT. Flowering time influences pod yield and quality of common bean (*Phaseolus vulgaris*); however, our knowledge of flowering time genes and flowering mechanisms in common bean remain limited. We performed RNA-sequencing (RNA-seq) analyses [long-day (LD) condition and short-day (SD) condition] to identify the flowering time genes and analyzed differentially expressed genes to examine their expression levels in relation to flowering time in ‘Hong Jin Gou’ common bean, a cultivar highly sensitive to photoperiod. The circadian patterns of related genes were identified using quantitative real-time polymerase chain reaction (qRT-PCR). Flowering time in ‘Hong Jin Gou’ was influenced by day length: SD conditions promoted flowering. A total of eight flowering time–related genes were identified, which were classified into photoperiod pathways. Homologs of *pseudo-response regulator 5*, *pseudo-response regulator 7*, and *gigantea* were more highly expressed under SD conditions than under LD conditions. Homologs of *late elongated hypocotyl* and *timing of cab expression 1* were differentially expressed under light and dark conditions. *Early flowering 3* is a key regulator of the pathway, which coordinates light and circadian clock inputs in leaves to trigger the expression of downstream genes. The present study provides critical information that could facilitate further investigations on the genetic mechanism of flowering time in common bean.

Environmental factors (daylength and temperature) and internal signals (gibberellins and autonomous pathways) simultaneously regulate flowering time in plants (Bhakta et al., 2017; Putterill et al., 2004). Higher plants detect fluctuations in daylength, which influence flowering time with changes in seasons. Common bean is a tropical facultative SD legume that is currently grown in tropical and temperate zones. The observation underscores how domestication and modern breeding practices can alter the adaptive phenology of a species (Moyses et al., 2018). During common bean domestication and dissemination from its centers of domestication, selection for photoperiod insensitivity allowed common bean to spread to higher latitudes (Gepts and Debouck, 1991). In addition, the determinate growth habit has been exploited in crop breeding activities to accelerate flowering and shorten the flowering period (Cober and Tanner, 1995).

In comparison with LD plants like *Arabidopsis thaliana*, much less is known about the genetic mechanisms that regulate flowering in SD plants, such as soybean (*Glycine max*), rice (*Oryza sativa*), and maize (*Zea mays*), among others; however, some progress has been made in such species through genetic analyses that have facilitated the identification of numerous genes. As a first step toward isolating the genes in common bean, we sought to use information obtained in model species such as *A. thaliana* using a candidate gene approach. Photoreceptors, circadian clock components, bio-clock, and light-regulated genes are key components for daylength detection using the external coincidence model. Among the clock and light-regulated genes, *constans* (*CO*) has been identified as a key gene participating in the integration of light and clock signals. The overexpression of the *CO* gene leads to early flowering in *A. thaliana* through the regulation of the expression of downstream genes, such as *flowering locust* (*FT*), *apetala1* (*API*), and *leafy* (*LFY*), regardless of the length of daylight (Aidyn et al., 2002; Suárez-López et al., 2001; Yanovsky and Kay, 2002).

Intriguingly, more detailed molecular genetics analyses have revealed that several quantitative trait locus (QTL) associated with time-to-flower in SD species are orthologs of genes that regulate flowering in *A. thaliana* (Lee and An, 2007; Simpson and Dean, 2002; Salome et al., 2011). For example, rice QTL, HD1, and HD3a were found to be orthologs of *A. thaliana CO* and *FT*, respectively (Kojima et al., 2002; Tamaki

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et al., 2007). Such analyses have also discovered some flowering QTL that do not have orthologs in *A. thaliana*, like the *early heading date 1* QTL in rice, a gene that regulates the expression of *FT* (Itoh et al., 2010). The observation suggests that additional mechanisms that control time-to-flower are likely to be discovered in SD plants. Buttressing this point is the discovery that setaria (*Setaria viridis*), a SD grass, has a secondary mechanism that operates under long days (Doust et al., 2017). RNA-seq technology facilitates the discernment of novel perspectives in transcriptome sequence analysis by providing comprehensive coverage of transcripts. In addition, RNA-seq could be used as an alternative to other transcript quantification approaches with the benefit of higher sensitivity and the potential to distinguish between very similar paralogs of a gene that differ based only on a few nucleotides. RNA-seq has been used to characterize transcriptional changes resulting from different flowering times (Kitae et al., 2017), and the differentially expressed genes (DEGs) identified were found to be useful for predicting differences in tolerance between common bean cultivars. Previous investigations have identified a dominant photoperiod-sensitive gene regulating flowering time in beans (Gu et al., 1998; Kornegay et al., 1993; Kwak et al., 2008; Wallace et al., 1991; White and Laing, 1989; White et al., 1996), but a comprehensive genetic analysis of the trait has not been carried out. In the present study, we used RNA-seq and quantitative reverse transcription PCR (qRT-PCR) to investigate potential changes in the common bean transcriptome in response to the changes in daylength. Known genes and novel predicted genes were identified and the functions of the molecules associated with the regulation of flowering time were evaluated. The findings could provide a theoretical basis for the enhancement of the adaptation of common bean cultivars to different photoperiods.

Materials and Methods

PLANT MATERIALS AND LIGHT TREATMENTS. A total of 215 common bean cultivars were sown in Harbin [northeast China (lat. 44°30'24"N, long. 125°42'41"E)] and Sanya [south China (lat. 18°09'34"N, long. 108°56'30"E)] on 21 May and 1 Nov. 2017, respectively (Supplemental Table 1). The flowering times of all cultivars were recorded at both sites and the most photoperiod-sensitive cultivars were screened for use as material in the present study. To eliminate the impact of major environmental factors, the seeds of photoperiod-sensitive common bean cultivars, Hong Jin Gou [indeterminate climbing, yellow pod with red streak (developed at Heilongjiang University, Harbin, China)] were cultivated in climate chambers at 25 °C under LD conditions (16/8 h light/dark) with 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of white light. All of plants were cultivated in this climate chambers at first. Half of the plants were transferred to SD conditions (8/16 h light/dark) under a similar temperature regimen after the trifoliolate leaves emerged. The trifoliolate leaves at 0600 and 1400 HR under both conditions at 20 d after emergence were collected and immediately frozen in liquid nitrogen and stored at -80 °C. Samples were collected at 20 and 21 d after emergence at the same sampling time points, with leaf samples from the three independent biological replicates being collected at each sampling time. Samples from the same sampling time points during 2 d were mixed to minimize errors. A total of 12 RNA libraries were constructed, three for each

photoperiod $\times$ sampling time-point combination: LD-L (0600 HR), LD-D (1400 HR), SD-L (0600 HR), and SD-D (1400 HR).

RNA EXTRACTION, SEQUENCING, AND ASSEMBLY. Total RNA was extracted from the leaf tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Total RNA quantity and purity were analyzed using an automated electrophoresis tool (Bioanalyzer 2100; Agilent Technologies, Santa Clara, CA) and RNA 6000 Nano LabChip Kit (Agilent Technologies) with RNA integrity number >7.0. Following purification, the messenger RNA (mRNA) was fragmented into small pieces using divalent cations under elevated temperature conditions. The cleaved RNA fragments were then reverse-transcribed to create the final complementary DNA (cDNA) library according to the protocol of the mRNA-Seq sample preparation kit (Illumina, San Diego, CA), the

Fig. 1. 'Hong jin gou' common bean [indeterminate climbing, yellow pod with red streak (developed at Heilongjiang University, Harbin, China)] cultivated in climate chambers at 25 °C under long-day (LD) conditions (16/8 h light/dark) with 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of white light. All plants were cultivated in this climate chamber at first. Half of the plants were transferred to short-day (SD) conditions (8/16 h light/dark) under a similar temperature regimen after the trifoliolate leaves emerged. SD condition promoted flowering in 'Hong jin gou'. The date of flowering initiation was 33 d after sowing under the SD condition. (The arrows marks the position of flower.) In contrast, under the LD condition, the date of flowering initiation was delayed by $\approx$ 37 d.

Table 1. Sequencing and assembly statistics of two time points following long-day [LD (16/8 h light/dark)] and short-day [SD (8/16 h light/dark)] photoperiod treatment in common bean. Four pools of messenger RNA samples from SD and LD conditions were used to build libraries for high-throughput sequencing. More than 6 billion total nucleotides were obtained from each library, and all high-quality reads were selected and assembled. Each sample from the leaves of the three independent biological replicates for each of sampling times.

Sample identity ^a	Reads (×10 ⁶)	Base pairs (×10 ⁹)	Mapped reads (×10 ⁶)	Proportion mapped (%)
SD-D	38.14	5.72	24.10	63.22
SD-L	43.05	6.46	28.45	66.15
LD-D	46.59	6.97	27.53	59.12
LD-L	43.56	6.53	26.54	60.87

^aPhotoperiod × sampling time-point combinations: LD-L (0600 HR), LD-D (1400 HR), SD-L (0600 HR), and SD-D (1400 HR).

average insert size for the paired-end libraries was 300 base pairs (bp) (±50 bp). Subsequently, we carried out paired-end sequencing on a HiSeq. 4000, Illumina platform according to the vendor's recommended protocol.

NORMALIZATION OF GENE EXPRESSION LEVELS AND IDENTIFICATION OF DEGs. Sequencing reads were aligned to the reference sequences using SOAP aligner/soap 2 (Li et al., 2009). For each gene, the expression level was measured and expressed as reads per kilobase exon model per million map reads (RPKM) based on the number of uniquely mapped reads, to eliminate the influence of different gene lengths and sequencing discrepancies on the gene expression calculation. For genes with more than one alternative transcript, the longest transcript was selected for use in calculating the RPKM.

To infer the transcriptional changes under light conditions, DEGs in the light conditions were identified by comparing the expression levels in LD-L with those in SD-L, and the level in LD-D with those in SD-D (SD as control). To compensate for multiple testing errors, the false discovery rate (FDR) was calculated for use in adjusting the threshold of the probability value (Rajkumar et al., 2015). Transcripts with a minimal 2-fold difference in expression ($|\log_2 \text{Ratio}| \geq 1$) and an $\text{FDR} \leq 0.001$ were considered differentially expressed between the two time points. DEGs with higher expression levels in LD-D than in SD-L, as well as those higher in LD-D than in SD-D, were considered “upregulated,” whereas those in the exhibiting opposite trends were considered “downregulated.”

GENE EXPRESSION VALIDATION. Eight genes with varying expression patterns revealed through RNA-seq were randomly selected for validation by qRT-PCR. RNA extracted from the leaves of the three independent biological replicates of the different sampling times were used for qRT-PCR validation. First-strand cDNA was synthesized using a PrimeScriptRT reagent Kit (Takara Bio, Kusatsu, Japan). Gene copy specific primers for qRT-PCR were designed based on the corresponding sequences using Primer6 (Supplemental Table 2). The qRT-PCR was carried out using SYBR Premix Ex TaqII (Takara Bio) and determined in a PCR system (LightCycler 480; Roche, Basel, Switzerland) according to the manufacturer's instructions and the data were collected using method of relative quantification 2- $\Delta\Delta\text{Ct}$. Actin was used as the internal control to normalize small differences in template quantities. Three technical replicates were performed for each gene with the aim of obtaining reliable expression results from the RNA-seq. Similar expression patterns were observed in LD-D vs. SD-D and LD-L vs. SD-L when qRT-PCR and RNA-seq data were compared as shown in Supplemental Fig. 1.

FUNCTIONAL ANNOTATION AND GENE ONTOLOGY AND KYOTO ENCYCLOPEDIA OF GENES AND GENOMES CLASSIFICATION. BLAST (2.2.3) and BLAST2GO (2.2.5) were used for functional annotation of DEGs, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) for pathway enrichment analysis. Significantly enriched GO terms were determined through a hypergeometric test followed by Bonferroni correction to calculate the P value for each term. We established $P < 0.05$ as cutoff for a significantly enriched GO term. For the KEGG

pathway enrichment analysis, a hypergeometric test followed by an FDR analysis was carried out, and a cutoff of $q < 0.05$ was established for the determination of enriched KEGG pathways.

Results and Discussion

DIFFERENCE IN TIME-TO-FLOWER UNDER VARIOUS PHOTOPERIOD TREATMENTS. As a typical SD plant, common bean is sensitive to changes in daylength. Photoperiod conditions shorter than 12 h of light would promote flowering. By observing flowering times in the fields on the plant materials, we observed ‘Hong jin gou’ was one of the most photoperiod-sensitive cultivars. The flowering initiation times in ‘Hong jin gou’ were 36 and 69 d after sowing in Sanya and Harbin, respectively. The results also were verified by laboratory experiments: the SD condition promoted flowering in ‘Hong jin gou’ (Fig. 1). The time of flowering initiation was 33 d after sowing in the SD condition. In contrast, in the LD condition, the flowering initiation time was delayed by ≈ 37 d. One of the most visible effects of the plant clock is photoperiodism, the triggering of events such as flowering and senescence in response to the changing seasons (McWatters et al., 2000). In common bean, the major trigger is change in daylength. The results demonstrate that photoperiod had a considerable effect on flowering in ‘Hong jin gou’.

RNA-SEQ AND FUNCTIONAL ANNOTATION OF TRANSCRIPTOME. Twelve pools of mRNA samples from the SD and LD condition were used to build libraries for high-throughput sequencing. More than six G nucleotides were obtained from each library and all high-quality reads were selected and assembled. Approximately 38.14 to 46.59 million 125-bp pair-end reads were generated from the four samples through RNA-seq (Table 1). After sequence trimming, the retained high-quality reads from all the samples were merged into 27,012 genes, and 23,719 of the genes were functionally annotated with an e-value cutoff of 1×10^{-5} .

Identified DEGs could be pooled into two major groups. Among the 1526 upregulated genes [Fig. 2A(a)], 1155 (61.6%) were transiently induced only in dark conditions, whereas 545 (29.1%) were only induced in light conditions, with 174 (9.3%) displaying increased expression in both conditions. In addition, we observed 1224 DEGs that exhibited downregulation [Fig. 2A(b)], 57 (4.3%) of which exhibited decreased expression in both light and dark conditions, 502 (37.5%) DEGs that were particularly downregulated under light conditions, and 779 (58.2%) that were downregulated in dark conditions [Fig. 2A(b)].

Fig. 2. Gene expression reprogramming under different photoperiod conditions in common bean. (A) Venn diagrams displaying the overlaps among differentially expressed genes identified in common bean plants exposed to light and dark conditions. Numbers in parentheses denote percentages with respect to the total upregulated (a) or downregulated (b) genes; long-day [LD (16/8 h light/dark)] and short-day [SD (8/16 h light/dark)] photoperiod × sampling time-point combination [LD-L (0600 HR), LD-D (1400 HR), SD-L (0600 HR), and SD-D (1400 HR)]. (B) Selected gene ontology (GO) terms enriched among differentially expressed genes identified in common bean. Color panels display the probability values of GO term enrichments.

After functional annotation of the DEGs, we performed GO classification and a GO term enrichment analysis as the first step in understanding the molecular changes that were induced by the different photoperiod conditions. The enrichment analysis revealed that one of the functions that dominated the photoperiod condition (light) was transcription factor activity (Fig. 2B). Other GO terms that were enriched were associated with the circadian rhythm. There was a significant enrichment of DEGs associated with flower development, circadian rhythm, and photosystem.

EXPRESSION PATTERN ANALYSIS OF FLOWERING TIME-RELATED GENES. Photoperiod is a critical factor regulating plant developmental processes, particularly flowering. As mentioned

previously, the results of the GO term enrichment analysis highlighted the relevance of transcription factor activity under different photoperiod conditions. Notably, among the 121 DEGs encoding putative transcription factors, whose expression were induced in both dark and light under LD and SD conditions, 15 circadian rhythm genes were observed to be significantly associated with flowering time genes in the current transcriptome data set (Table 2). The findings suggested that flowering time in common bean shares some molecular mechanisms with photoperiod responses in SD plants.

Circadian clocks are biological timing mechanisms used by pluricellular organisms to predicatively adjust physiological and molecular processes in anticipation of environmental changes that occur as a consequence of the day-night cycle, and they are the major factors that regulate flowering time. In the present transcriptomic analysis, to evaluate functions and expression patterns of circadian clock-related genes, we found eight DEGs associated with flowering time, based on an analysis using the KEGG database [*early flowering 3 (ELF3)*, *pseudo-response regulator 5 (PRR5)*, *pseudo-response regulator 7 (PRR7)*, *late elongated hypocotyl (LHY)*, *timing of cab expression 1 (TOC1)*, *gigantea (GI)*, *constans (CO)*, and *flowering locus T (FT)*] (Table 3). Only *LHY* and *TOC1* had different expression patterns under light and dark conditions.

Little is known about flowering time genes and flowering mechanisms in common bean. Although misregulation of several oscillator components results in altered flowering phenotypes, the precise molecular interactions between the circadian clock and photoperiodic flowering remain still poorly understood (Nagel and Kay, 2012). In the present study, we performed high-throughput transcriptome sequencing, revealing 15 flowering time-related genes in photoperiod pathways (Table 2). A total of eight putative flowering time genes were classified into the photoperiod pathways. *LHY* and *CCA1* repress floral transition under SD and LD conditions by inhibiting the expression of *TOC1* in the central loop of the circadian clock, and play partially redundant roles in the control of the circadian clock (Fujiwara et al., 2008; Lu et al., 2009; Zhuo et al., 2010). In this study, compared with the low expression levels of the homolog of *LHY*, the homolog of *TOC1* was highly expressed in all

Table 2. Fifteen genes associated with flowering time in common bean. Among the 121 differentially expressed genes encoding putative transcription factors, whose expressions were induced in dark and light conditions, 15 circadian rhythm genes were significantly associated with flowering time genes in the current transcriptome data set; *LHY* = late elongated hypocotyl, *PIF3* = phytochrome-interacting factor 3, *PRR7* = pseudo-response regulator 7, *PRR5* = pseudo-response regulator 5, *PHYB* = phytochrome, *CRY* = cryptochrome, *TOC1* = timing of cab expression 1, *HY5* = long hypocotyl 5, *FT* = flowering locus T, *GI* = gigantea, *CO* = CONSTANS, *CHS* = Chalcone and stilbene synthase, *SPA1* = suppressor of phyA-105, *ELF3* = early flowering 3.

Code	Identity	Gene name	Biological function	P value
1	Phvul.006G086700	<i>LHY</i>	Homeodomain-like superfamily protein	5.24E-11
2	Phvul.008G133600	<i>PIF3</i>	Basic helix-loop-helix (bHLH) DNA-binding superfamily protein	1.81E-04
3	Phvul.008G147400	<i>PRR7</i>	CCT motif family protein	1.02E-03
4	Phvul.009G213400	<i>PHYB</i>	Phytochrome E	3.88E-03
5	Phvul.010G153200	<i>CRY</i>	DNA photolyase family protein	4.65E-02
6	Phvul.002G039100	<i>CHS</i>	Chalcone and stilbene synthase family protein	3.45E-03
7	Phvul.003G188400	<i>TOC1</i>	CCT motif-containing response regulator protein	4.60E-09
8	Phvul.006G029200	<i>HY5</i>	Basic-leucine zipper (bZIP) transcription factor family protein	4.87E-03
9	Phvul.007G083500	<i>GI</i>	Gigantea protein (GI)	5.63E-33
10	Phvul.008G257300	<i>PRR5</i>	CCT motif family protein	1.96E-02
11	Phvul.010G111200	<i>SPA1</i>	SPA (suppressor of phyA-105) protein family	1.77E-05
12	Phvul.004G088300	<i>GI</i>	Gigantea protein (GI)	7.62E-04
13	Phvul.001G097200	<i>FT</i>	PEBP (phosphatidylethanolamine-binding protein) family protein	6.12E-04
14	Phvul.006G005200	<i>CO</i>	CONSTANS-like	2.18E-04
15	Phvul.010G142900	<i>ELF3</i>	Protein early flowering 3	6.50E-06

Table 3. Expression patterns of eight differentially expressed genes related to flowering time in common bean. To evaluate functions and expression patterns of circadian clock-related genes, eight differentially expressed genes were found to be relate to flowering time, based on analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database; *ELF3* = early flowering 3, *PRR7* = pseudo-response regulator 7, *PRR5* = pseudo-response regulator 5, *LHY* = late elongated hypocotyl, *TOC1* = timing of cab expression 1, *GI* = gigantea, *CO* = constans, *FT* = flowering locus T.

Code	Gene	Gene name	Regulation	
			Light	Dark
1	Phvul.010G142900	<i>ELF3</i>	Down	Down
2	Phvul.008G257300	<i>PRR5</i>	Down	Down
3	Phvul.008G147400	<i>PRR7</i>	Down	Down
4	Phvul.006G086700	<i>LHY</i>	Down	Up
5	Phvul.003G188400	<i>TOC1</i>	Down	Up
6	Phvul.004G088300, Phvul.007G083500	<i>GI</i>	Down	Down
7	Phvul.006G005200	<i>CO</i>	Down	Up
8	Phvul.001G097200	<i>FT</i>	Down	Down

libraries. Its expression in dark conditions was 2-fold than in light conditions, whereas it was negatively regulated by the expression of the *GI* (Supplemental Fig. 2). The results suggest that *TOC1* plays a more important role than *LHY* in regulating the circadian clock rhythm in common bean. Notably, other genes involved in the molecular clock also were detected as being differentially regulated. *TOC1* played the key role of transmitting signals to regulate the flowering process. *TOC1* transmitted signals to downstream genes, and dark and light conditions could induce the upregulation and downregulation of *TOC1* expression, respectively, thereby activating the flowering process in common bean (Supplemental Fig. 2). The circadian-regulated gene, *CO*, is a critical regulator of the pathway, and coordinates light and clock inputs in leaves to trigger the expression of florigen gene *FT* (Suárez-López et al., 2001). In *A. thaliana*, an LD plant, the transcript peak of *CO* mRNA occurs late in the day, but occurs after dusk in an SD

plant (Suárez-López et al., 2001). In the present study, common bean is an SD plant and the transcript peak of the *CO* mRNA is the exact opposite of the one in *A. thaliana*. We can infer that the expression pattern of *CO* was influenced by photoperiod.

The number of reports for economically relevant traits in the common bean is still very limited. Kamfwa et al. (2015), using 237 genotypes of common beans genotyped for 5398 single-nucleotide polymorphisms (SNPs), found two significant SNPs for phenological traits days to flowering and days to maturity. These authors suggested the positional gene *Phvul.001G221100*, located on Pv01, as a candidate gene for controlling photoperiod sensitivity and flowering in common bean. In this study, *Phvul.001G221100* was particularly upregulated under light conditions, and downregulated in dark conditions. But *Phvul.001G221100* was not detected in the results of the GO term enrichment analysis. It was not observed to be significantly associated with flowering time genes in the current transcriptome data set. Moyses et al. (2018) investigated potential candidate genes in the region around seven significant SNPs. Three genes involved in the flowering pathways were identified, including *Phvul.001G214500*, *Phvul.007G229300*, and *Phvul.010G142900.1* on Pv01, Pv07, and Pv10, respectively. In this study, *Phvul.010G142900.1* observed *ELF3* that exhibited downregulation in both light and dark conditions. It is proposed that, in the absence of *ELF3* to gate light input, the clock is continually reset until the plants are returned to darkness. Thus, *ELF3* might be predicted to be very important in maintaining the rhythm as days are lengthening toward summer and, indeed, *elf3* mutants show an inability to adapt properly to LDs, becoming responsive rather than predictive in *A. thaliana* (Covington et al., 2001; Liu et al., 2001; McWatters et al., 2000). In the network, the circadian-regulated gene, *ELF3*, is a key regulator of the pathway that coordinates light and circadian clock inputs in leaves to trigger the expression of downstream genes.

In summary, our analysis revealed that signaling molecules under LD or SD conditions induce changes in the expression of plant genes that participate in circadian rhythms, and facilitate the observation of a delayed flowering phenotype. In addition, our

results illustrate how flowering time can be modulated by alternative transcript processing under different photoperiods. Our results highlight, to the best of our knowledge, novel biological and molecular aspects that could facilitate the understanding and identification of downstream responses in photoperiod regulation, and provide critical information on flowering time genes, which would facilitate further investigations on mechanisms of flower development. However, further studies of these flowering time-related genes are needed to better understand the network for regulating flowering time in common bean.

Literature Cited

- Aidyn, M., C. Frédéric, and G. Coupland. 2002. Control of flowering time: Interacting pathways as a basis for diversity. *Plant Cell* 14:111–130.
- Bhakta, M.S., S.A. Gezan, J.A. Clavijo Michelangeli, M. Carvalho, L. Zhang, and J.W. Jones. 2017. A predictive model for time-to-flowering in the common bean based on QTL and environmental variables. *Genes Genomes Genet.* 7:3901–3912.
- Cober, E.R. and J.W. Tanner. 1995. Performance of related indeterminate and tall determinate soybean lines in short-season areas. *Crop Sci.* 35:361–364.
- Covington, M.F., S. Panda, X.L. Liu, C.A. Strayer, D.R. Wagner, and S.A. Kay. 2001. ELF3 modulates resetting of the circadian clock in *Arabidopsis*. *Plant Cell* 13:1305–1315.
- Doust, A.N., M. Mauro-Herrera, J.G. Hodge, and J. Stromski. 2017. The C4 model grass *Setaria* is a short day plant with secondary long day genetic regulation. *Front. Plant Sci.* 8:1062.
- Fujiwara, S., A. Oda, and R. Yoshida. 2008. Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. *Plant Cell* 20:2960–2971.
- Gepts, P. and D.G. Debouck. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.), p. 7–53. In: A. van Schoonhoven and O. Voysest (eds.). *Common beans: Research for crop improvement*. Commonwealth Agricultural Bureaux Intl., Wallingford, UK.
- Gu, W., J. Zhu, D. Wallace, S. Singh, and N. Weeden. 1998. Analysis of genes controlling photoperiod sensitivity in common bean using DNA markers. *Euphytica* 102:125–132.
- Itoh, H., Y. Nonoue, M. Yano, and T. Izawa. 2010. A pair of floral regulators sets critical day length for Hd3a florigen expression in rice. *Nat. Genet.* 42:635–638.
- Kojima, S., Y. Takahashi, Y. Kobayashi, L. Monna, and T. Sasaki. 2002. Hd3a, a rice ortholog of the *Arabidopsis* FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant Cell Physiol.* 43:1096–1105.
- Kornegay, J., J.W. White, J.R. Dominguez, G. Tejada, and C. Cajiao. 1993. Inheritance of photoperiod response in Andean and Mesoamerican common bean. *Crop Sci.* 33:977–984.
- Kwak, M., D. Velasco, and P. Gepts. 2008. Mapping homologous sequences for determinacy and photoperiod sensitivity in common bean (*Phaseolus vulgaris*). *J. Hered.* 99:283–291.
- Kamfwa, K., K.A. Cichy, and J.D. Kelly. 2015. Genome-wide association study of agronomic traits in common bean. *Plant Genome* 8:1–12.
- Kitae, S., K.H. Chul, S. Seungho, K. Kyung-Hee, M. Jun-Cheol, and K.J. Yoo. 2017. Transcriptome analysis of flowering time genes under drought stress in maize leaves. *Front. Plant Sci.* 8:267.
- Liu, X.L., M.F. Covington, C. Fankhauser, J. Chory, and D.R. Wagner. 2001. ELF3 encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. *Plant Cell* 13:1293–1304.
- Lee, S. and G. An. 2007. Diversified mechanisms for regulating flowering time in a short-day plant rice. *J. Plant Biol.* 50:241–248.
- Li, R., C. Yu, Y. Li, T.W. Lam, S.M. Yiu, K. Kristiansen, and J. Wang. 2009. SOAP2: An improved ultrafast tool for short read alignment. *Bioinformatics* 25:1966–1967.
- Lu, S.X., S.M. Knowles, C. Andronis, M.S. Ong, and E.M. Tobin. 2009. Circadian clock associated1 and late elongated hypocotyl function synergistically in the circadian clock of *Arabidopsis*. *Plant Physiol.* 150:834–843.
- McWatters, H.G., R.M. Bastow, A. Hall, and A.J. Millar. 2000. The ELF3 zeitnehmer regulates light signalling to the circadian clock. *Nature* 408:716–720.
- Moyses, N., A.C.C. Nascimento, and F.F.E. Silva. 2018. Quantile regression for genome-wide association study of flowering time-related traits in common bean. *PLoS One* 13:0190303.
- Nagel, D.H. and S.A. Kay. 2012. Complexity in the wiring and regulation of plant circadian networks. *Curr. Biol.* 22:648–657.
- Putterill, J., R. Laurie, and R. Macknight. 2004. It's time to flower: The genetic control of flowering time. *BioEssays* 26:363–373.
- Rajkumar, A.P., P. Qvist, R. Lazarus, F. Lescai, J. Ju, M. Nyegaard, O. Mors, A.D. Børglum, Q. Li, and J.H. Christensen. 2015. Experimental validation of methods for differential gene expression analysis and sample pooling in RNA-seq. *BMC Genomics* 16:548.
- Salome, P.A., K. Bomblies, R.A.E. Laitinen, L. Yant, and R. Mott. 2011. Genetic architecture of flowering-time variation in *Arabidopsis thaliana*. *Genetics* 188:421–433.
- Simpson, G.G. and C. Dean. 2002. *Arabidopsis*, the Rosetta Stone of flowering time. *Science* 296:285–289.
- Suárez-López, P., K. Wheatley, F. Robson, H. Onouchi, F. Valverde, and G. Coupland. 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410:1116–1120.
- Tamaki, S., S. Matsuo, H. Wong, S. Yokoi, and K. Shimamoto. 2007. Hd3a protein is a mobile flowering signal in rice. *Science* 316:1033–1037.
- Wallace, D.H., P.A. Gniffke, P.N. Masaya, and R.W. Zobel. 1991. Photoperiod, temperature, and interaction effects on days and nodes required for flowering of bean. *J. Amer. Soc. Hort. Sci.* 116:534–543.
- White, J. and D. Laing. 1989. Photoperiod response of flowering in diverse genotypes of common bean (*Phaseolus vulgaris*). *Field Crops Res.* 22:113–128.
- White, J., J. Kornegay, and C. Cajiao. 1996. Inheritance of temperature sensitivity of the photoperiod response in common bean (*Phaseolus vulgaris* L.). *Euphytica* 91:5–8.
- Yanovsky, M. and S. Kay. 2002. Molecular basis of seasonal time measurement in *Arabidopsis*. *Nature* 419:308–312.
- Zhuo, J.W., P. Bao-Ping, and F. Yong-Fu. 2010. Bioinformatics analysis of the TOC1 homologs in soybean genome. *J. Henan Agr. Sci.* 56:14–19.

Supplemental Fig. 1. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) validation of the relative expression levels of genes selected from the RNA-sequencing (RNA-seq) analysis of common bean–photoperiod {long-day [LD (16/8 h light/dark)] and short-day [SD (8/16 h light/dark)]} interaction. Expression profiles of eight genes (color coded) as determined by qRT-PCR and RNA-seq. The x-axis indicates two groups of comparisons: LD-D vs. SD-D. and LD-L vs. SD-L [LD-L (0600 HR), LD-D (1400 HR), SD-L (0600 HR), and SD-D (1400 HR)]. The y-axis shows the fold change increases/decreases in expression levels of genes; *LRZFP1* = *LR zinc finger protein*, *GI* = *gigantea*, *HST* = *homogentisate solanesyl transferase*.

Supplemental Fig. 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Regulation of circadian rhythm genes in response to photoperiod in common bean. Schematic representation of key circadian clock components identified in common bean and their transcriptional interactions (red: upregulation, blue: downregulation). *LHY* = late elongated hypocotyl, *PIF3* = phytochrome-interacting factor 3, *PRR9* = pseudo-response regulator 9, *PRR7* = pseudo-response regulator 7, *PRR5* = pseudo-response regulator 5, *PHYB* = phytochrome, *CRY* = cryptochrome, *TOC1* = timing of cab expression 1, *HY5* = long hypocotyl 5, *FT* = flowering locus T, *GI* = gigantea, *ELF3* = early flowering 3, *CO* = constans, *PHYA* = phytochrome A, *COP1* = E3 ubiquitin-protein ligase RWD2, *FKF1* = flavin-binding kelch repeat F-box protein 1, *ZTL* = clock-associated PAS protein, *CHE* = transcription factor TCP21, *CCA1* = circadian clock associated 1, *CK2* = casein kinase II, *CHS* = chalcone synthase, *PAP1* = transcription factor MYB75, *CDF1* = Dof zinc finger protein DOF5.5.

Supplemental Table 1. List of common bean cultivars, growth habits, and phenotypes used in this study. A total of 215 common bean cultivars were sown in Harbin [northeast China (lat. 44°30'24"N, long. 125°42'41"E)] and Sanya [south China (lat. 18°09'34"N, long. 108°56'30"E)] on 21 May and 1 Nov. 2017, respectively.

Code	Cultivar	Growth habit	Time to flowering (d)	
			Harbin	Sanya
1	Hong jin gou	Indeterminate viny	69	36
2	Xiang jiao you dou	Indeterminate viny	51	38
3	Sheng feir	Indeterminate viny	42	40
4	Qi ji	Indeterminate viny	41	42
5	Zao feng	Indeterminate viny	39	38
6	Kuai jian	Indeterminate viny	40	37
7	Da zi pao	Indeterminate viny	46	38
8	Di dou	Determinate brush	39	38
9	Jiu yue	Determinate brush	41	39
10	Jia you dou	Determinate brush	39	37
11	Yi ke song	Determinate brush	40	38
12	Dan se qiu	Determinate brush	40	39
13	73-8	Determinate brush	40	34
14	97-5	Indeterminate viny	40	36
15	923	Indeterminate viny	41	38
16	Te nen	Indeterminate viny	46	41
17	Tai man	Indeterminate viny	38	37
18	Wu jin 6	Indeterminate viny	44	42
19	F0119	Indeterminate viny	47	45
20	F0678	Determinate brush	42	39
21	F0705	Determinate brush	42	40
22	F1736	Determinate brush	47	45
23	F2320	Determinate brush	42	38
24	F3420	Indeterminate viny	54	37
25	F4150	Indeterminate viny	49	50
26	F4226	Indeterminate viny	50	48
27	BE221-1	Determinate brush	41	38
28	Shuang feng NO.3	Indeterminate viny	41	39
29	D6	Determinate brush	41	37
30	D8	Determinate brush	40	36
31	D10	Determinate brush	42	39
32	D11	Determinate brush	39	39
33	D13	Determinate brush	40	39
34	Zao wu jin	Indeterminate viny	43	41
35	Chao shang si ji dou	Indeterminate viny	55	39
36	Shi li chang	Indeterminate viny	49	45
37	Da ma zhang	Indeterminate viny	49	47
38	851-923-9	Indeterminate viny	42	39
39	97-5	Indeterminate viny	42	40
40	Yin bai tiao	Indeterminate viny	42	41
41	Zao man jia	Indeterminate viny	43	38
42	Qing feng di dou	Determinate brush	40	40
43	Qing dao dou	Determinate brush	40	40
44	81-6	Determinate brush	41	39
45	Shuang sheng jia dou	Determinate brush	40	40
46	Tian ma di dou	Determinate brush	40	38
47	Shuang sheng di dou	Determinate brush	41	39
48	5991(81-6)	Determinate brush	41	37
49	No. 16	Indeterminate viny	51	49
50	4-10	Indeterminate viny	49	49
51	Tu zi fan bai yan	Indeterminate viny	50	38
52	96-9-17	Indeterminate viny	41	41
53	96-8-50	Indeterminate viny	44	40
54	96-8-14	Indeterminate viny	49	47
55	96-8-7	Indeterminate viny	41	40

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Supplemental Table 1. Continued.

Code	Cultivar	Growth habit	Time to flowering (d)	
			Harbin	Sanya
56	96-8-4	Indeterminate viny	47	46
57	96-8-2	Indeterminate viny	43	40
58	Chao chang si ji dou	Indeterminate viny	44	43
59	San dao mei	Indeterminate viny	40	39
60	Da you dou	Indeterminate viny	41	41
61	Hei huang dou	Indeterminate viny	52	39
62	Ya ta jia	Indeterminate viny	46	47
63	Ji dou jiao	Indeterminate viny	53	53
64	Ha cai dou NO. 8	Indeterminate viny	40	38
65	Di you dou	Determinate brush	39	39
66	You dou	Indeterminate viny	44	38
67	Chao chang jia dou	Indeterminate viny	44	37
68	Ju feng	Indeterminate viny	45	47
69	Bo li cui	Indeterminate viny	44	40
70	2504Jia dou	Indeterminate viny	43	42
71	Lv long dou	Indeterminate viny	44	38
72	Ha cai dou NO.1	Indeterminate viny	42	37
73	Jin long	Indeterminate viny	41	38
74	Yi chuan feng	Indeterminate viny	40	38
75	Fan bai yan	Indeterminate viny	46	49
76	Zi hua you dou	Indeterminate viny	40	37
77	Yu dou	Indeterminate viny	49	40
78	78-3	Indeterminate viny	43	48
79	Mao yan	Indeterminate viny	53	49
80	Lian pu	Indeterminate viny	51	38
81	Shuang qing NO. 1	Indeterminate viny	44	41
82	Wu jin NO. 1	Indeterminate viny	46	47
83	Hong qi you dou	Indeterminate viny	43	47
84	Jiu li bai	Indeterminate viny	42	40
85	Wei fang bai li	Indeterminate viny	45	46
86	Hong fu	Indeterminate viny	43	40
87	Tai guo dou	Indeterminate viny	44	43
88	9Z-3-15	Indeterminate viny	44	39
89	Da hui dou	Indeterminate viny	43	41
90	You dou	Indeterminate viny	41	36
91	Lao lai lv	Indeterminate viny	47	39
92	Ji dou	Indeterminate viny	54	56
93	Lao lai shao	Indeterminate viny	56	39
94	You dou	Indeterminate viny	55	48
95	Ma que dan	Indeterminate viny	47	45
96	Cheng gou zi	Indeterminate viny	49	49
97	You dou	Indeterminate viny	54	55
98	Jia qiao dan	Indeterminate viny	44	40
99	Cheng feng	Indeterminate viny	50	48
100	Zi hua	Indeterminate viny	45	44
101	Jia que dan	Indeterminate viny	51	51
102	96-2-36	Indeterminate viny	42	40
103	Zao you dou	Indeterminate viny	42	43
104	96-2-1	Indeterminate viny	42	40
105	Long you dou	Indeterminate viny	46	49
106	Hu lan	Indeterminate viny	45	44
107	Sui hua di dou	Indeterminate viny	47	48
108	Wu ying	Indeterminate viny	60	40
109	Qing miao	Indeterminate viny	42	40
110	Se mei	Indeterminate viny	49	47
111	Re na ya	Determinate brush	40	39

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Supplemental Table 1. Continued.

Code	Cultivar	Growth habit	Time to flowering (d)	
			Harbin	Sanya
112	Ai zi dou	Determinate brush	41	40
113	Ai huang dou	Determinate brush	38	39
114	Ji zao sheng	Determinate brush	40	41
115	Gong ji zhe	Determinate brush	40	40
116	P-15-61 Borlotlo U	Determinate brush	39	39
117	D-15-02 Contender M	Determinate brush	40	39
118	Rocquercoldot H	Determinate brush	43	42
119	Hua pi dou	Indeterminate viny	61	59
120	Yu long	Indeterminate viny	50	51
121	Chi yu	Indeterminate viny	44	43
122	Jia huang	Indeterminate viny	43	40
123	Hong guang	Indeterminate viny	43	39
124	Feng lan	Indeterminate viny	43	40
125	Ai guo zhe	Indeterminate viny	43	41
126	Fang ming	Determinate brush	39	38
127	Sheng ri	Determinate brush	40	40
128	Xu shen	Determinate brush	41	40
129	Qi jiang	Determinate brush	41	39
130	Guang hui	Determinate brush	41	34
131	Yin bao	Determinate brush	42	36
132	A95-24	Determinate brush	41	38
133	A18-13	Indeterminate viny	41	41
134	B96-1	Determinate brush	40	37
135	Qing lang	Determinate brush	41	42
136	Wu shi	Determinate brush	42	39
137	Li ze	Determinate brush	43	40
138	Sheng qi	Determinate brush	42	41
139	Fang zheng	Determinate brush	40	39
140	A99-4	Determinate brush	40	40
141	A2000-3	Determinate brush	40	40
142	Zao sheng	Determinate brush	45	41
143	ABA-18	Indeterminate viny	45	42
144	BZLDAFRRL	Indeterminate viny	42	41
145	CIPT	Determinate brush	42	43
146	VPC	Indeterminate viny	42	44
147	CIAT-107	Determinate brush	41	41
148	DDF-309	Indeterminate viny	46	50
149	E-MAR	Indeterminate viny	46	43
150	GER	Indeterminate viny	46	45
151	JAX	Indeterminate viny	45	48
152	VAX	Indeterminate viny	47	47
153	VAXL	Indeterminate viny	46	48
154	VAT	Indeterminate viny	46	45
155	XAV309	Indeterminate viny	46	44
156	Z-MAR	Indeterminate viny	51	50
157	G0446	Determinate brush	40	43
158	G173441	Indeterminate viny	51	50
159	Qi san ba	Indeterminate viny	40	40
160	Jiu li hong	Indeterminate viny	40	38
161	Yi li wang	Indeterminate viny	40	41
162	Hei cha ma zhang	Indeterminate viny	41	40
163	Xin zi cha	Indeterminate viny	40	42
164	Zi cha you dou	Indeterminate viny	41	42
165	Chao zao jia dou	Indeterminate viny	42	41
166	Jia NO.57	Indeterminate viny	42	42
167	Xiao cha ba	Indeterminate viny	43	43

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Supplemental Table 1. Continued.

Code	Cultivar	Growth habit	Time to flowering (d)	
			Harbin	Sanya
168	Fan bai yan	Determinate brush	41	40
169	Hong yu	Determinate brush	42	42
170	Da cha ba	Determinate brush	39	38
171	Qing lv	Indeterminate viny	42	43
172	D312G-4-3	Indeterminate viny	41	42
173	Da qi	Indeterminate viny	42	42
174	TG-4-7-2-7	Indeterminate viny	43	41
175	Yu shu you dou	Indeterminate viny	43	43
176	Da que dan	Indeterminate viny	43	43
177	Lv jiao hei zhen zhu	Indeterminate viny	43	45
178	D312G-9-4-2	Determinate brush	42	41
179	D312G-3	Indeterminate viny	41	42
180	D312G-111	Indeterminate viny	43	43
181	D312G-1-1-7	Indeterminate viny	44	45
182	RGGH-8-6-4-1	Indeterminate viny	43	48
183	61GH-3-17-2-1	Indeterminate viny	40	46
184	21RG-1-5-1-2	Indeterminate viny	42	42
185	35M-9-1	Indeterminate viny	39	43
186	GH15-3-2-1	Indeterminate viny	40	40
187	HG-5-1	Indeterminate viny	40	41
188	16-9-1-3	Indeterminate viny	41	38
189	61-11-2	Indeterminate viny	41	39
190	GZG	Indeterminate viny	39	40
191	Jiao long338	Indeterminate viny	41	44
192	Hong bian bian	Indeterminate viny	42	42
193	Hong ni	Indeterminate viny	39	39
194	Lu wang No.2	Indeterminate viny	41	38
195	Huang se wu jia	Indeterminate viny	41	42
196	Qing yu di dou	Indeterminate viny	40	40
197	Tai guo wu jin	Indeterminate viny	39	39
198	Wu jin di dou	Indeterminate viny	43	43
199	Tian ma	Indeterminate viny	44	45
200	81-8	Indeterminate viny	41	42
201	Hou wan yao	Indeterminate viny	41	45
202	Jia qiao dan	Indeterminate viny	41	44
203	Xin peng shi wang	Indeterminate viny	41	43
204	Ji feng	Indeterminate viny	41	48
205	Huang jiao zhen zhu	Indeterminate viny	41	40
206	Qing long wu jin	Indeterminate viny	42	43
207	Xi nuo jia dou	Indeterminate viny	41	41
208	Bai li qing jia	Indeterminate viny	40	43
209	D312G-4-16	Determinate brush	41	42
210	Yi ke song	Indeterminate viny	41	41
211	Jiu yue qing	Indeterminate viny	46	42
212	Long yan	Indeterminate viny	44	41
213	HG911	Indeterminate viny	46	45
214	Ha you san	Indeterminate viny	40	39
215	Wan xia	Indeterminate viny	42	41

Supplemental Table 2. List of primers used to amplify the selected genes for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses. Eight genes with varying expression patterns revealed through RNA-sequencing were randomly selected for validation by qRT-PCR. RNA extracted from the leaves of the three independent biological replicates of the different sampling times were used for qRT-PCR validation. Gene copy specific primers for qRT-PCR were designed based on the corresponding sequences using Primer6; *LRZFP1* = *LR zinc finger protein*, *GI* = *gigantea*, *HST* = *homogentisate solanesyl transferase*.

Code	Gene name	Identity	Forward primer (5'-3')	Reverse primer (5'-3')
1	<i>LRZFP1</i>	Phvul.011G168500	TCGTCCTCTGTCAAGTCA	GCAGTATAACCACCACCAT
2	<i>GI</i>	Phvul.007G083500	GCCAAGAGGTGAAGATAACT	CAGCAGCCAGAACATAAGA
3	<i>Photoperiod</i>	Phvul.009G225500	AACTGCTAAGTGCCTGTT	GTAGTGTTACCTAATCCAAGAG
4	<i>Circadian rhythm</i>	Phvul.009G045000	CAACCTCACACTCTCAACA	GAACTTGGACTTGGCATTG
5	<i>HST</i>	Phvul.001G265300	CTGAGAGTGCTGGTGTTT	ATTGCGGTAGTATGCTGTAA
6	<i>ABC transporters</i>	Phvul.004G027800	TGGCACATCGTCTATCAAC	GTCTTGGCAATGAGTTCTTC
7	<i>Chl metabolism</i>	Phvul.008G185100	TGAATTAAGAACCAGGAT	AGCCGTGTGGATTATTGAG
8	<i>Antenna proteins</i>	Phvul.004G071500	CTGCCAACACATTGATGAG	GCATCCAGTCAGAAGTCAT