

Circadian clock and photoperiodic flowering genes in adzuki bean (*Vigna angularis* [Willd.] Ohwi & H. Ohashi)

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Abstract

Adzuki bean (*Vigna angularis* [Willd.] Ohwi & H. Ohashi) is one of the most important legume crops cultivated in East Asia and northern South Asia. Despite its agronomic importance, the lack of available sequence information has made it difficult to improve important agronomic traits. In the present study, we performed *de novo* assembly of transcript sequences produced by short-read sequencing to construct 59,860 full-length protein-coding sequences in adzuki bean. These genes were subjected to a BLASTP search to identify putative homologues of the 84 *Arabidopsis* genes involved in the circadian clock and photoperiodic flowering pathway. A large proportion of these *Arabidopsis* genes were found to be conserved in adzuki bean. However, there were no homologues of six genes including *FLOWER LOCUS D* (*FD*) and *LEAFY* (*LFY*). Furthermore, the phylogenetic relationships of 25 highly homologous matches to *CONSTANS* (*CO*) or *CONSTANS-LIKE* (*COL*) of *Arabidopsis* indicated the lack of a *CO* orthologue in adzuki bean. *FLOWER LOCUS T* (*FT*) and its homologues were found to have two homologous counterparts in adzuki bean. This study provides primary genetic resources that may be useful for producing adzuki bean with improved flowering and fruiting performance in response to environmental changes.

Keywords: adzuki bean; circadian clock; *de novo* assembly; full-length cDNA; photoperiodic flowering pathway

Introduction

Adzuki bean (*Vigna angularis* [Willd.] Ohwi & H. Ohashi) is a summer legume crop that has traditionally been cultivated in East Asia and northern South Asia (Isemura *et al.*, 2007). Adzuki bean is a quantitative short-day (SD) plant with a critical photoperiod of 12–13 h. Great progress has been made in understanding the flowering pathway in *Arabidopsis thaliana* and more than 180 *Arabidopsis* genes that regulate flowering time have been identified (Fornara *et al.*, 2010). In this study, we performed *de novo* assembly of RNA sequences expressed in the leaves and flowers of adzuki bean

and identified putative homologues of the *Arabidopsis* genes involved in circadian clock and photoperiodic flowering.

Materials and methods

Leaf triplets and flowers at the V4 stage were harvested from *V. angularis* accession IT213134 in daytime under SD (12 h light–12 h dark) conditions for RNA isolation. The purified mRNA was fragmented, and the RNA fragments were used to construct template-sequencing libraries. Each library was sequenced using the Illumina HiSeq™ 2000 platform to produce paired-end sequences of approximately 5 Gb. Transcript *de novo* assembly was performed using the program Trinity (Grabherr *et al.*, 2011), and the Perl script available in Trinity

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(transcripts_to_best_scoring_ORFs.pl) was used to identify the coding sequences. Complete coding sequences were translated into protein sequences using the Transeq software in the EMBOSS package (Rice *et al.*, 2000).

Results and discussion

Transcriptome sequencing and de novo assembly in adzuki bean

Illumina sequencing of two libraries from the leaves and flowers of adzuki bean generated 7,668,523,172 total bases, constituting 75,925,972 short reads with an average length of 101 bp (Table S1, available online). Due to the absence of adzuki bean reference genome, *de novo*

assembly was performed to construct transcripts. A total of 86,353 and 51,811 contigs were constructed from the leaf and flower complementary DNA (cDNA) libraries, respectively. Among these, 59,860 contigs were full-length cDNA sequences, with an average N50 of 1627 bp. The number of complete cDNAs in flowers was five times less than that in leaves owing to the low sequencing depth of the leaf cDNA library.

Conservation of Arabidopsis genes involved in circadian clock and photoperiodic flowering in adzuki bean

The photoperiodic flowering pathway is mediated by a complex interaction between an internal rhythm, the

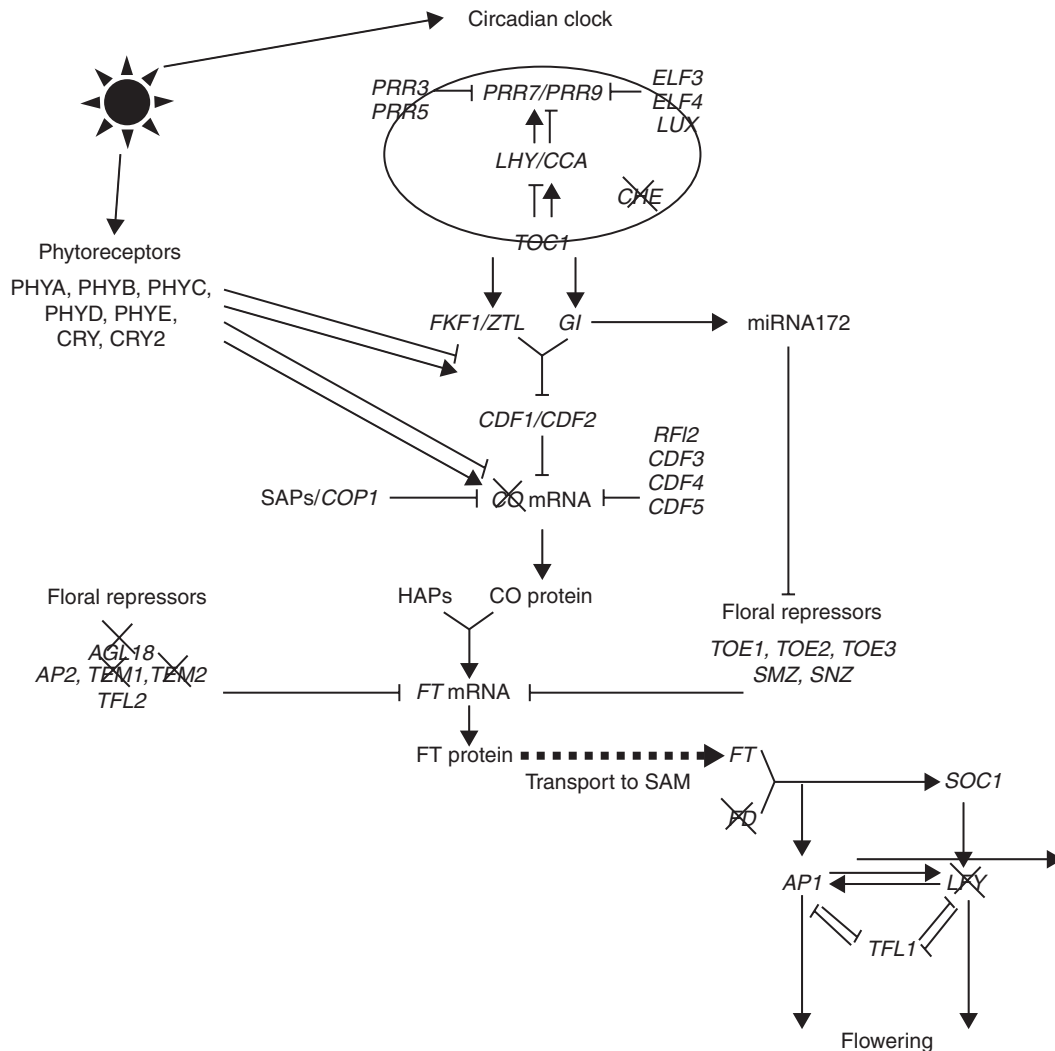


Fig. 1. Diagram of the circadian clock and photoperiodic flowering pathway in *Arabidopsis*. Genes lacking full-length complementary DNA homologues in adzuki bean are indicated by 'X'. These genes include *CHE*, *CO*, *FD*, *LFY*, *AGL18*, *TEM1* and *TEM2*. Standard arrows represent positive regulatory relationships and T-arrows represent negative regulatory relationships. See Table S1 (available online) for a list of gene names and abbreviations.

circadian clock, and external light signals (Piñeiro and Jarillo, 2013). A list of genes with demonstrated involvement in the circadian clock-based photoperiodic flowering pathway was obtained from the literature, including 84 *Arabidopsis* genes (Fig. 1; Tables S2 and S3, available online). The 59,860 genes of adzuki bean were subjected to a BLASTP search to identify putative homologues involved in the photoperiodic flowering pathway, with an *e*-value cut-off of 1×10^{-5} . Our analysis

revealed that a large proportion of the *Arabidopsis* photoperiodic flowering genes are probably conserved in adzuki bean and that most of the genes have more than two homologues (Fig. 1; Table S3, available online). However, we could not detect the homologues of six *Arabidopsis* genes in adzuki bean, including *TEMPRANILLO 1 (TEM1)* and *TEMPRANILLO 2 (TEM2)*, *AGAMOUS-LIKE 18 (AGL18)*, *FLOWER LOCUS D (FD)*, *CCA1 HIKING EXPEDITION (CHE)* and *LEAFY (LFY)*

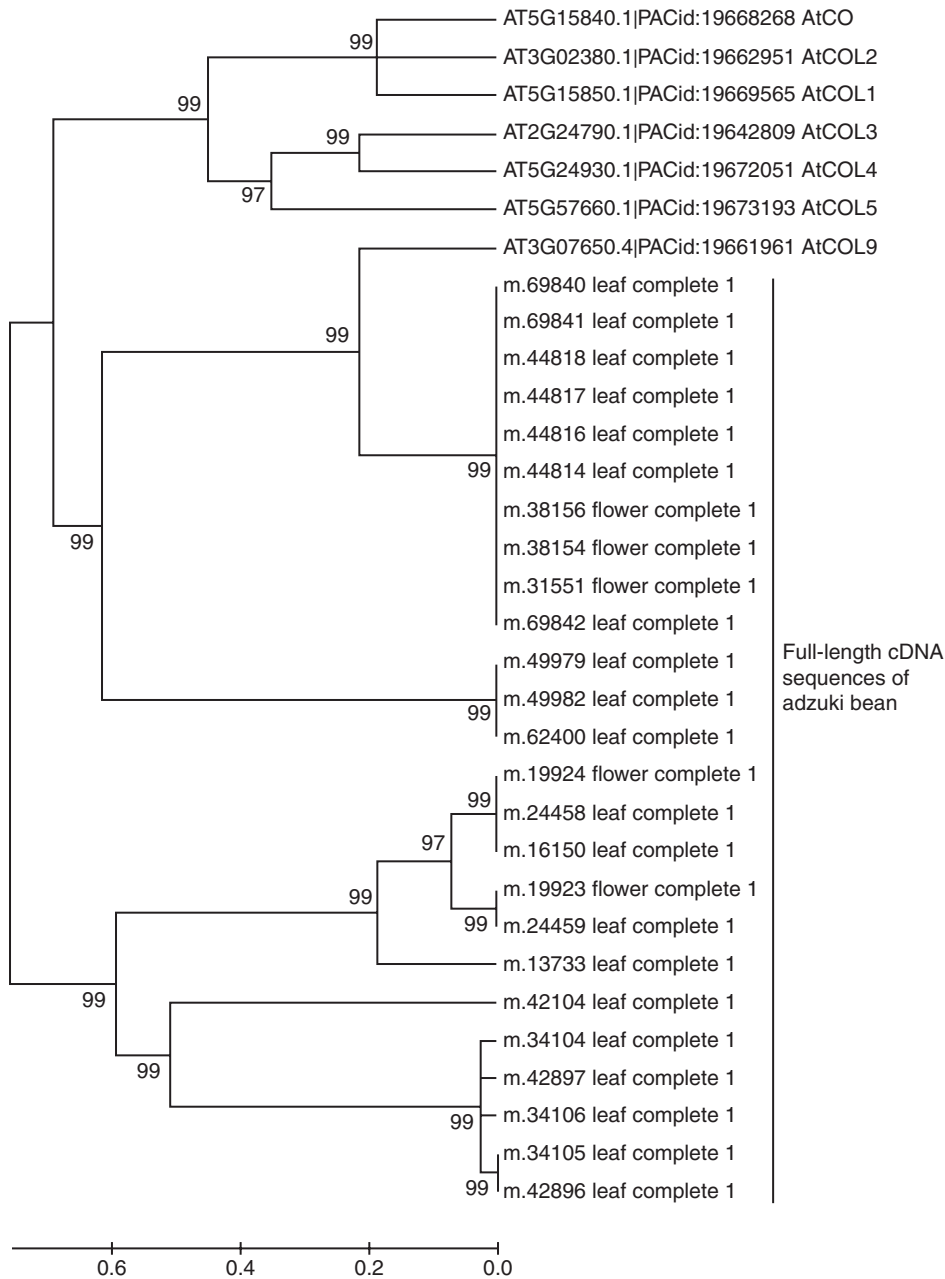


Fig. 2. Phylogenetic analysis of *CONSTANS-LIKE (COL)* genes in adzuki bean. A neighbour-joining tree of concatenated B-box and CCT domain nucleotides is shown. Bootstrap values from 1000 replicates are indicated as a percentage above each branch. cDNA, complementary DNA.

(Fig. 1; Table S3, available online). One of reasons for the absence of homologues of these genes in adzuki bean may be gaps in the gene coverage of cDNA libraries. Considering the expression oscillation of photoperiodic flowering genes, incomplete sampling for particular cell types such as a shoot apical meristem (SAM), developmental stages and photoperiodic conditions may also have affected these results.

In plants, there is an array of photoreceptors including red/far-red PHYTOCHROME (PHY) photoreceptors and CRYPTOCHROMES (CRYs) that perceive blue and ultraviolet light. Adzuki bean contains putative homologues of these photoreceptors. The *Arabidopsis* circadian clock consists of central regulatory loops, where two myeloblastosis (MYB) transcriptional regulators of *CIRCADIAN CLOCK-ASSOCIATED 1 (CCA)* and *LATE ELONGATED HYPOCOTYL (LHY)* repress the expression of the pseudo-response regulator (PRR) *TIMING OF CAB EXPRESSION 1 (TOC1)*, also known as *PRR1* in the morning (Imaizumi, 2010). Additional essential genes have been proposed to function in interconnected morning and evening feedback loops, including the other members of the TOC1 family, i.e. *PRR3*, *PRR5*, *PRR7* and *PRR9*, *CHE*, *EARLY FLOWERING 3 (ELF3)*, *EARLY FLOWERING 4 (ELF4)*, *LUX ARRHYTHMO (LUX)* or *PHYTOCLOCK (PCL)*, and *GIGANTEA (GI)* (Imaizumi, 2010). All molecular frames of the circadian clock, with the exception of *CHE*, are likely to be conserved in adzuki bean (Fig. 1). *CHE* is a TEOSINTE-LIKE1, CYCLOIDEA, and PROLIFERATING CELL FACTOR1 (TCP) transcription factor (Imaizumi, 2010). Adzuki bean was predicted to lack a homologue of *CHE*.

In *Arabidopsis*, the *CONSTANS (CO)* gene, which encodes a zinc finger and CONSTANS, CO-like, and TOC1 (CCT) domain-containing transcription factor, is a key player in the photoperiodic pathway (Lagercrantz, 2009). We utilized *CO* and six *CO-LIKE (COL)* genes to identify the adzuki bean homologues of *CO* (Fig. 2). There are 25 highly homologous matches to *CO* or *COL* in adzuki bean (Fig. 2; Table S3, available online). The cladogram in Fig. 2 shows that *CO* and *COL1* to *COL5* only form one clade, which does not contain any adzuki bean genes. Half of the adzuki bean homologues fall within the *COL9* clade and the others are more divergent from the *Arabidopsis COL* genes. These results indicate the absence of *CO* gene in adzuki bean, consistent with the previous reports in other legumes (Hecht *et al.*, 2005; Ono *et al.*, 2010; Kim *et al.*, 2013).

SD and long-day (LD)-responding plants have evolved different mechanisms to regulate the activation of a floral pathway integrator (FPI) gene *FLOWER LOCUS T (FT)* (Jarillo and Piñeiro, 2011), but increased *FT* expression is required in both types of plants, indicating that *FT* is a highly universal promoter of flowering (Lagercrantz, 2009). *FT* and its homologues encode a phosphatidylethanolamine-binding protein (PEBP) (Wigge, 2011). The *FT* homologues contain

TWIN SISTER OF FT (TSF), *TERMINAL FLOWER 1 (TFL1)*, *MOTHER OF FT AND TFL1 (MFT1)*, *BROTHER OF FT AND TFL1 (BFT)* and *TERMINAL FLOWER 2 (TFL2)*. Adzuki bean was found to have two putative homologues of the *Arabidopsis* members of the *PEBP* gene family. The *FT* protein in SAM forms a complex with *FD* encoding a basic-leucine zipper (bZIP) transcription factor. In adzuki bean, a *FD* homologue was not detected in either of the cDNA libraries. Another FPI, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*, also known as *AGAMOUS-LIKE 20*, is up-regulated by the complex *FT/FD* (Lagercrantz, 2009). *SOC1* is a MCM1-AGAMOUS-DEFICIENS-SRF (MADS)-box transcription factor. Eleven adzuki bean homologues of *SOC1* were detected, but these genes also exhibited high homologies with other *AGAMOUS-LIKE* genes. *LFY* plays a dual role in flower development as both an FPI and a meristem identity gene in *Arabidopsis* (Parcy, 2005). *LFY* encodes a plant-specific transcription factor and functions downstream of *SOC1*. We could not identify a homologue of *LFY* in the adzuki bean gene sets.

In addition, four photoperiodic flowering genes, *E1–E4*, have been isolated in soybean. *E2 (GmGI)* is orthologous to *Arabidopsis GI* (Watanabe *et al.*, 2011), and *E3 (GmPHYA3)* and *E4 (GmPHYA2)* are the homologues of phytochrome A (Liu *et al.*, 2008; Watanabe *et al.*, 2009). *E1* encodes a low-copy protein with a nuclear localization signal and a B3 domain-related region, which is expressed under LD conditions (Xia *et al.*, 2012). Among these genes, only *E1* did not have a homologue in the adzuki bean grown under SD conditions.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1479262114000252>

Acknowledgements

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: PJ008060, Project No. PJ00806003)”, Rural Development Administration, Republic of Korea.

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