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# Phylogenetic analysis of phytochrome A gene from *Lablab purpureus* (L.) Sweet

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## Abstract

**Background:** Phytochromes are the best characterized photoreceptors that perceive Red (R)/Far-Red (FR) signals and mediate key developmental responses in plants. It is well established that photoperiodic control of flowering is regulated by *PHYA* (phytochrome A) gene. So far, the members of *PHYA* gene family remains unexplored in *Lablab purpureus*, and therefore, their functions are still not deciphered. *PHYA3* is the homologue of phytochrome A and known to be involved in dominant suppression of flowering under long day conditions by downregulating florigens in *Glycine max*. The present study is the first effort to identify and characterize any photoreceptor gene (*PHYA3*, in this study) in *Lablab purpureus* and decipher its phylogeny with related legumes.

**Results:** *PHYA3* was amplified in *Lablab purpureus* cv GNIB-21 (photo-insensitive and determinate) by utilizing primers designed from *GmPHYA3* locus of *Glycine max*. This study was successful in partially characterizing *PHYA3* in *Lablab purpureus* (*LprPHYA3*) which is 2 kb longer and belongs to exon 1 region of *PHYA3* gene. Phylogenetic analysis of the nucleotide and protein sequences of *PHYA* genes through MEGA X delineated the conservation and evolution of *Lablab purpureus PHYA3* (*LprPHYA3*) probably from *PHYA* genes of *Vigna unguiculata*, *Glycine max* and *Vigna angularis*. A conserved basic helix-loop-helix motif bHLH69 was predicted having DNA binding property. Domain analysis of *GmPHYA* protein and predicted partial protein sequence corresponding to exon-1 of *LprPHYA3* revealed the presence of conserved domains (GAF and PAS domains) in *Lablab purpureus* similar to *Glycine max*.

**Conclusion:** Partial characterization of *LprPHYA3* would facilitate the identification of complete gene in *Lablab purpureus* utilizing sequence information from phylogenetically related species of Fabaceae. This would allow screening of allelic variants for *LprPHYA3* locus and their role in photoperiod responsive flowering. The present study could aid in modulating photoperiod responsive flowering in *Lablab purpureus* and other related legumes in near future through genome editing.

**Keywords:** Phytochrome, *PHYA3*, GAF, PAS, Phylogenetic analysis

## Background

Photoperiod sensitivity is an important trait as it enables crops to adapt to different latitudes. Short-day (SD) crops like *Oryza sativa*, *Lablab purpureus*, *Phaseolus vulgaris* and *Glycine max* requires photoperiod insensitivity to adapt to a high latitudinal environment [1]. Photoperiod

is governed by phytochrome genes which play critical role in responding to the light quantity, quality and periodicity in plants and make communication between different biochemicals signaling pathway for growth and development of crops [2]. Phytochromes are Red (R)/Far-Red (FR) light receptors involving interconversion of inactive R (Pr) to active FR (Pfr) form by red light absorption which triggers its transfer to the nucleus and thus guides gene expression [3]. Phytochrome is the best characterized photoreceptor and its apoproteins are encoded by small multigene families. In recent decade, progress has been made in characterizing the number, molecular

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properties and biological activity of the photoreceptors that comprise a plant R/FR detecting framework [2].

Legumes have been documented with vast genetic variation for flowering and photoperiod has been quite involved in governing growth habit, flowering and maturity in these crops [4]. Legumes have been the focus of presently expanding genomics research and the availability of vast genomic resources and established synteny within the Fabaceae family has enabled the identification of candidate genes for flowering in other related species and exploring their molecular physiologies involved in downstream processes [5]. The fundamental genes governing flowering pathway in legumes are conserved from model plant *Arabidopsis* that contains five *PHY* (phytochrome) genes (*PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE*) and each of these gene have crucial role [6, 7]. Different maturity loci, i.e. E-series (*E1* to *E8*) represent a range of allelic composition and each *E* locus affects flowering time and maturity in *Glycine max* [8–14]. Photoperiod sensitivity under different light conditions was found to be related to *E1*, *E3*, *E4* and *E7* [15]. *E3* and *E4* encode *GmPhyA3* and *GmPhyA2*, respectively, which are homologues of photoreceptor phytochrome A [16]. *GmPHYA3* and *GmPHYA2* along with *GmPHYA1*, which is encoded by a homoeologous copy of *E4*, redundantly or complementarily function in floral induction and etiolation responses under various light conditions [17]. *E3* and *E4* coordinates flowering responses to long-day (LD) conditions with different Red-to-Far-Red (R:FR) quantum ratios. *E3* responds to light with high R:FR ratio; plants homozygous for the recessive *e3* allele can initiate flowering under the LD conditions generated by fluorescent lamps with a high R:FR ratio [18]. Photoperiod sensitivity and determinacy played important role in evolution of *Phaseolus vulgaris* [19]. *GmFT2a* and *GmFT5a*, two homologues of *FT* (Flowering locus T), are reported to induce photoperiodic flowering in *Glycine max* [20] and they are regulated by *E1*, *E3* (*PHYA3*) and *E4* genes under different photoperiod conditions to induce or repress flowering [15]. Photoperiodic response of *PHYA3* under short day and long day conditions affects growth habit by inducing other genes that activate florigens and guides floral initiation at shoot apex [3].

The research on photoperiod responsive flowering is mainly anchored to few major pulse legumes viz., *Glycine max*, *Pisum sativum*, *Phaseolus vulgaris*, *Cajanus cajan* and vegetable legumes endured despite being a promising nutritional source. Indian bean [*Lablab purpureus* (L.) Sweet] is one such underexploited legume with wide range of uses as vegetable, forage, cover crop, split pulse, fodder and medicinal [21]. The molecular information regarding this crop is scarce; nevertheless, it has the potential to become a model pulse crop in genomics era

owing to its immense genetic diversity and adaptability [22]. Most landraces of *Lablab purpureus* are photoperiod sensitive and indeterminate which flower only during short days, while few improved varieties with determinate growth and photoperiod insensitivity are available which flowers within 40 to 50 days across the year. Photoperiod responsive flowering along with growth habit might have played crucial role in domestication and evolution of this crop. Dominant nature of photoperiod sensitivity, indeterminate growth habit and purple flower was reported along with coupling phase of linkage between photoperiod insensitive flowering and determinate growth habit in *Lablab purpureus* [23]. Molecular tagging of photoperiod responsive flowering in *Lablab purpureus* discerned that photoperiod insensitive flowering and determinate growth habit is linked and they might be governed by recessive alleles of *GmPHYA3* and *Dt* homologs, respectively [24]. Most recently, allelic characterization of *TFL* (Terminal Flowering locus) governing growth habit has been reported along with involvement of splice site single nucleotide polymorphism (SNP) for growth habit differences in *Lablab purpureus* [25]. The role of *PHYA3* in photoperiod responsive flowering is already reported in *Glycine max* and *Phaseolus vulgaris*, and mutations in *E3/PHYA3* conferred photoperiod insensitive and early flowering [3, 26]. Lack of genome sequence data or any molecular information regarding *PHYA3* gene or any marker tightly linked to it in *Lablab purpureus* has limited the molecular characterization of photoperiod responsive flowering in this crop. Looking to possible role of *PHYA3* in photoperiod responsive flowering and lack of genome sequence information, the present work is focused on characterization of this gene in *Lablab purpureus* using candidate gene approach and phylogenetic analysis of legume phytochromes.

## Materials and methods

### Primer designing

The *GmPHYA3* sequence from *Glycine max* [3] was obtained in FASTA format from NCBI (National Centre for Biotechnology Information) GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>). *GmPHYA3* is 9.2kb longer, exon 1 was divided into three frames and primers for each frame were designed using web-based Primer BLAST (Basic Local Alignment Search Tool) from NCBI (Table 1). These primers were utilized for amplification of *PHYA3* in *Lablab purpureus* cv GNIB-21 which is determinate and photoperiod insensitive.

### Amplification and sequencing

Genomic DNA was isolated from young fresh trifoliolate leaves of GNIB-21 using CTAB method [27]. The DNA quality and quantity were assessed using gel

**Table 1** Primers used for partial amplification *LprPHYA3* locus

Frame	Primer	Sequence	Amplicon length (bp)
Frame-1	93F	5'TGCATCAGATAACAGTGGGAAGA3'	951
	94R	5'TTGTAGGATTGCAGGGCTCC3'	
Frame-2	115F	5'ATTTTGAGCCGGTCAAGCCT3'	987
	116R	5'CAGCTGCCATTCCACATGC3'	
Frame-3	133F	5'TTGTCTGATGCAGGCTTCCC3'	964
	134R	5'CCAAGCTGATGGGACCAGAA3'	

F and R represent forward and reverse primers, respectively

electrophoresis and spectrophotometer (Nanodrop 2000, Thermo Fisher, USA). Both the stock and diluted DNA preparations were stored at  $-50^{\circ}\text{C}$  until use. The target frames were amplified in GNIB-21 through Polymerase Chain Reaction (PCR) with *Taq* DNA polymerase (TaKaRa, Clontech, Japan). PCR mixture prepared in 200  $\mu\text{l}$  contained approximately 100 ng genomic DNA, 200  $\mu\text{M}$  of dNTPs, 10 pmol of forward and reverse primers, standard *Taq* buffer ( $\text{Mg}^{2+}$  plus) and 1 unit of *Taq* DNA polymerase in total volume of 25  $\mu\text{l}$  reaction. The PCR cycle involved initial denaturation of  $95^{\circ}\text{C}$  for 7 min followed by 35 cycles of  $94^{\circ}\text{C}$  (30 s),  $51\text{--}55^{\circ}\text{C}$  (45 s) and  $72^{\circ}\text{C}$  (1 min) and a final extension of 10 min at  $72^{\circ}\text{C}$ . Amplicons giving the single discrete band when resolved on 1.5% agarose gel electrophoresis with the expected product size were purified using column purification with SLS PCR Clean-up Kit (Saffron Life science, Surat, India). Sanger sequencing reaction of purified PCR amplicon was carried out with specific primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer (Applied Biosystems). Bidirectional sequence data obtained from each amplicon were processed using BioEdit [28]. The bidirectional sequence information obtained were processed by merging the sequences from three frames and overlapping sequences were identified in both directions to construct consensus sequence.

#### Sequence retrieval, alignment and phylogenetic analysis

The processed *LprPHYA3* sequence was used as query for BLASTn at <https://blast.ncbi.nlm.nih.gov/Blast.cgi> for finding homologous sequences with reference to NCBI (National Center for Biotechnology Information) nucleotide database [29]. Sequences showing matches with *LprPHYA3* from Fabaceae family were retrieved from NCBI nucleotide database. MEGA X (Molecular Evolution Genetics Analysis) software [30] was used to align *PHYA* nucleotide sequences of 16 species from Fabaceae family in addition to *Arabidopsis thaliana* using the CLUSTAL W alignment algorithm [31]. All the

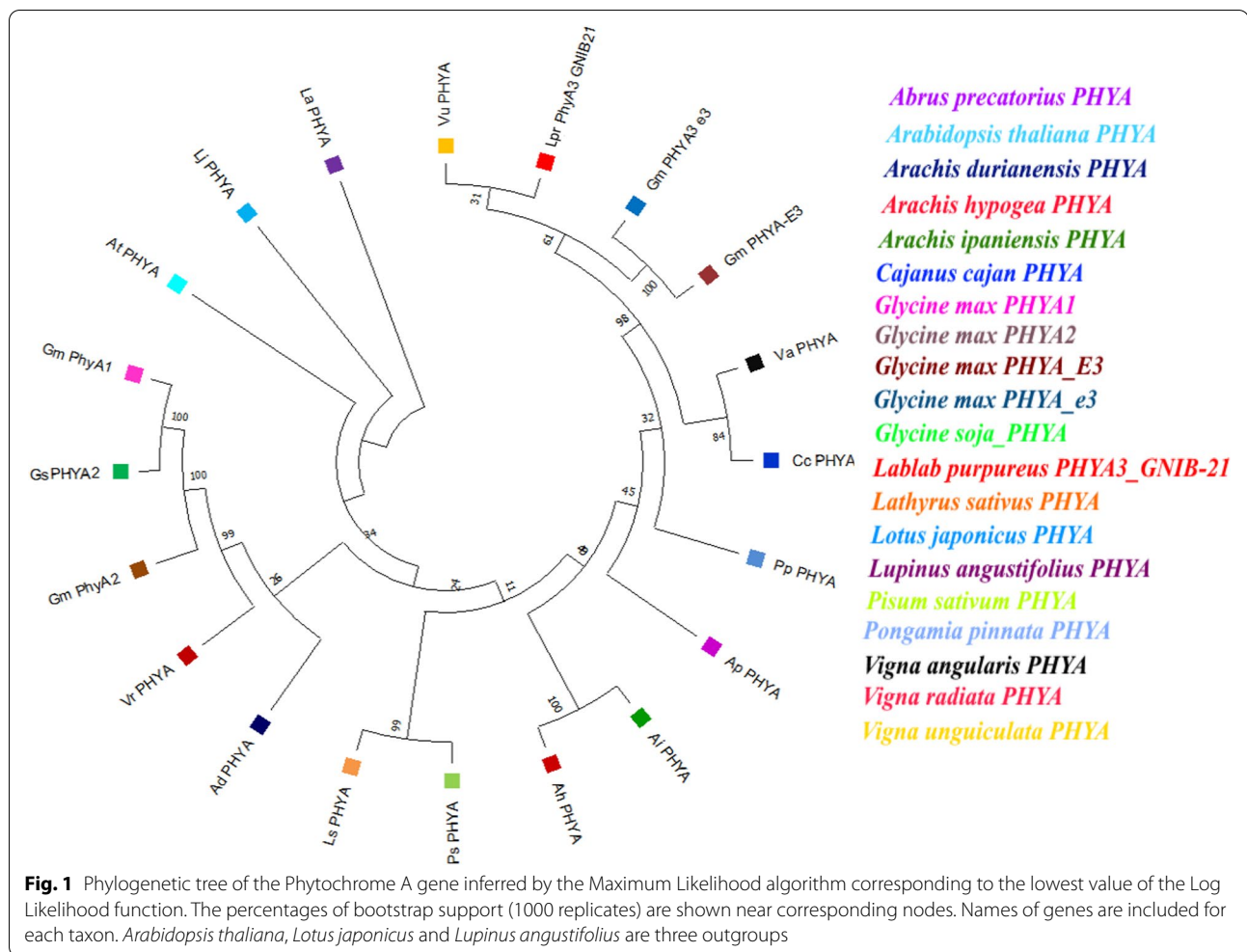
alignment settings were employed at default values; 2160 and 2051 positions with and without gaps were obtained, respectively. The nucleotide substitutions selected with complete deletion of gaps or missing data were used to analyse sequences. The phylogenetic tree was inferred using Maximum Likelihood method based on Tamura-Nei Model [32]. The initial tree was inferred with default setting using Neighbour Joining method and Nearest-Neighbour Interchange was used as ML heuristic search method. The reconstructions of phylogenetic trees were conducted using Maximum Likelihood Method. Bootstraps with 1000 replicates for Poisson correction model were performed to assess node support [33]. The best-scoring ML tree was searched simultaneously to represent the evolutionary history of the 20 specimens tested.

#### Prediction of conserved motifs

The detection of conserved motifs in DNA sequence of phytochrome gene was performed using online tool MEME (Multiple Em for Motif Elicitation) (<https://meme-suite.org/meme/>) [34]. This online web-based analysis was performed with minimum and maximum motif width of 6 and 12 residues, respectively, which were used to identify probable motifs, keeping the rest of the parameters at default. The MEME output in HTML showed the motifs as local multiple alignments of the input sequences, as well as in several other formats. MEME HTML output were allowed one or all of the motifs to be forwarded for additional investigation. The results of the MEME analysis were applied to TOMTOM (<http://memesuite.org/tools/tomtom>), which is the online software performing comparison of given motifs with available databases. The output generated from TOMTOM include sequence-logo graphics on behalf of the alignment of two motifs with p and q value (a measure of false discovery rate) of the match [35].

#### Exon prediction, protein prediction and functional analysis

The identified partial sequence of *LprPHYA3* was subjected to exon prediction using Eukaryotic GeneMark. hmm version 3.54 [36]. The nucleotide sequence of exon-1 obtained in such a way was translated to protein sequence using standard codon table. This amino acid sequence obtained was further compared with *GmPHYA3* sequence. The protein sequence of exon 1 from *Lablab purpureus* GNIB-21 was used to perform BLASTp in NCBI GenBank database and the sequences showing homology were further used to create multiple alignment using the CLUSTAL W algorithm [29, 31]. Phylogenetic analysis with amino acid sequence of *PHYA* in 21 different plant species was also performed using JTT model in MEGA X [30, 37]. The online web-based functional analysis tool SMART-EMBL (Simple Modular



Architecture Research Tool) (<http://smart.embl-heidelberg.de/>) Version 9 was used for predicting domains in both *GmPHYA3* and *LprPHYA3* protein sequences [38].

## Results

### *LprPHYA3* characterization and phylogenetic analysis

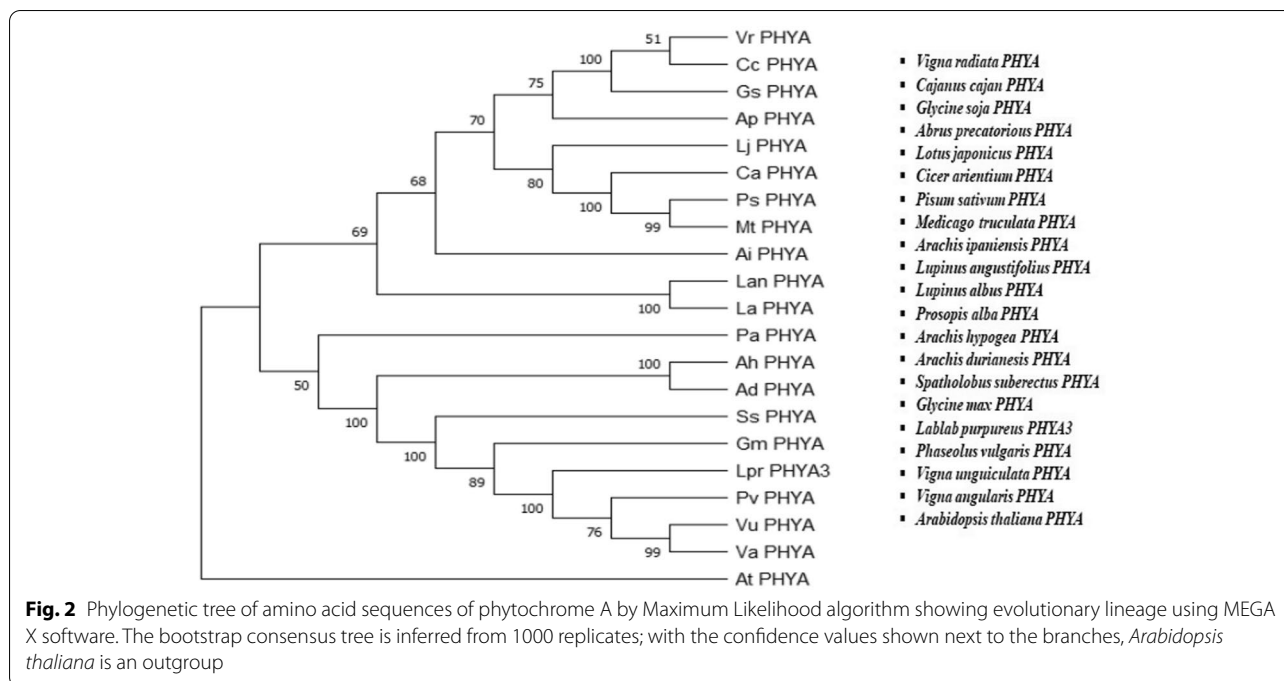
In the present study, *Lablab purpureus* cv GNIB-21, which is determinate and photo-insensitive, was used for characterizing *PHYA3* gene. The sequencing data after processing and analysis revealed the successful characterization of the exon-1 of *PHYA3* gene in Indian bean (*LprPHYA3*- *Lablab purpureus* *PHYA3*) for the first time in the world. BLASTn analysis of nucleotide sequence *LprPHYA3* indicated highest identity with *Vigna unguiculata* (95.76%) followed by *Vigna angularis* (95.67%) and *Glycine max* (90.90%), all with *E*-value close to zero.

Phylogenetic tree depicts formation of different clades on the basis of evolutionary changes between the sequences. *LprPHYA3* nucleotide sequence was compared with the nucleotide sequences of phytochrome

genes of other plant species, most of which, belonged to the Fabaceae family. Phylogenetic analysis showed that this phytochrome A gene evolved from common ancestry root but diverged into different clades during the course of evolution (Fig. 1). The *PHYA* sequences from *Lablab purpureus*, *Vigna unguiculata* and *Glycine max* form independent clade and are closest to *Vigna angularis* and *Cajanus cajan*. This indicates that *LprPHYA3* has maximum closeness to *Vigna unguiculata*, *Glycine max*, *Cajanus cajan* and *Vigna angularis* *PHYAs* suggesting the evolutionary closeness of the gene in these species. The three outgroups depicted in the tree are *Lupinus angustifolius*, *Lotus japonicus* and *Arabidopsis thaliana*.

Realizing the importance of both DNA and protein sequence alignment in phylogenies, the protein sequence predicted from the exon 1 of *LprPHYA3* gene was also studied along with the other sequences to delineate the amino acid changes in the sequence during the course of evolution. Alignment of the amino acid sequences from *PHYA* homologs was done by CLUSTAL W alignment





algorithm and phylogenetic tree for protein sequences was constructed (Fig. 2). The protein alignment also depicts the most probable evolution of *LprPHYA3* from common ancestral PHYA gene of *Glycine max*, *Phaseolus vulgaris*, *Vigna unguiculata* and *Vigna angularis*. This tree depicts *Arabidopsis thaliana* as an outgroup.

#### Determination of common motifs in PHYA sequence

Identification of conserved motifs in the PHYA was performed by comparing DNA sequences from these 20 PHYA sequences from different species. The analysis resulted in three conserved sites present in pool of studied sequence. The best result was a 12-nt-long motif GTGCAAAGCATG which was found in all 20 analysed sequence (Table 2). The *E*-value for best match discussed here was  $3.4 \times 10^{-45}$ . Subsequently, the motif was compared using TOMTOM with a database of *Arabidopsis thaliana* DAP motifs which resulted in identification of bHLH69 basic helix-loop-helix motif containing transcription factor (*p* value  $1.16 \times 10^{-4}$  and *E* value  $1.01 \times 10^{-1}$ ) (Fig. 3).

#### Domain analysis of GmPHYA3 and LprPHYA3

Domain analysis of *GmPHYA3* sequence retrieved from NCBI GenBank database after performing BLASTp search with *LprPHYA3* that encodes a protein with 1130 amino acids was carried out using EMBL-SMART platform. This protein displayed normal features of Phytochrome A with five domains viz., GAF (cGMP-specific phosphodiesterase-adenylyl cyclase-FhA), two PAS

(period-ARNT-single-minded) domains and two Histidine kinase-related domains (HKRD) viz., HisKA and HATPase\_c as depicted in Fig 4a. The amino acid sequence of *LprPHYA3* showed two domains, i.e. GAF and PAS (Fig 4b). This finding is in congruity with the domain analysis from *GmPHYA3*.

#### Discussion

The deciphering of putative molecular pathways in legumes involving different phytochrome genes and their involvement in governing flowering time will pave way for future research. Various genetic models for this regulatory framework of photoperiod-based flowering with different known loci is available for *Glycine max*. It is studied in depth for photoperiod control of flowering and different *E* loci have been reported, out of which, *E3* (*GmPHYA3*) is considered a strong candidate for *FT3* and mutations in *GmPHYA3* results in early flowering [3]. Different mutations of *E3* have been reported in *Glycine max* and *Phaseolus vulgaris* that confer significantly early flowering and photoperiod insensitivity [3, 26]. Additionally, naturally occurring *E3* mutants depicted a large insertion in fourth intron and one SNP for non-synonymous amino acid substitution in third exon. Naturally occurring *e3* allele carries a large deletion spanning exon 4, whereas an induced mutant *e3* allele has sustained a 40-bp deletion and frameshift in the middle of exon 1 [3]. Flowering time is a very important trait governing several other correlated functions and molecular identification of flowering networks with these *E* loci can

**Table 2** Predicted conserved motifs in 20 sequences of Phytochrome A gene

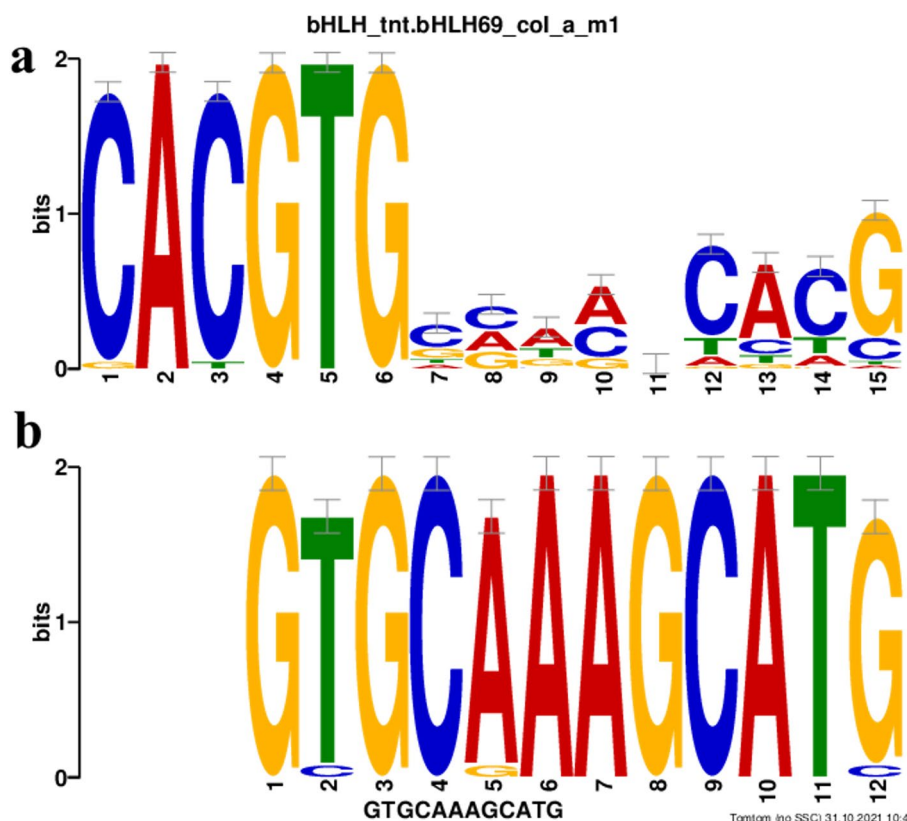
Sequence	Length	Start	P-value	Sites		
<i>Ad_PHYA</i>	2054	1598	$5.50 \times 10^{-8}$	AGATGGGGTG	GTGCAAAGCATG	ACCCTGGTGA
<i>Ah_PHYA</i>	2057	884	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAGGTGCT
<i>Ai_PHYA</i>	2057	884	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAGGTGCT
<i>Ap_PHYA</i>	2073	896	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAAGTCTT
<i>At_PHYA</i>	2057	1601	$1.42 \times 10^{-7}$	AGATGGGGAG	GTGCAAAGCATG	ATCCAGATGA
<i>Cc_PHYA</i>	2076	896	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TTAAGGTGCT
<i>Gm_PHYA1</i>	2077	1589	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AGGCTGGAGA
<i>Gm_PHYA2</i>	2072	1589	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AGGCTGGAGA
<i>Gm_PHYA3_E3</i>	2077	899	$5.50 \times 10^{-8}$	GTTGATTGTT	GTGCAAAGCATG	TGAATGTGCT
<i>Gm_PHYA3_e3</i>	2077	899	$5.50 \times 10^{-8}$	GTTGATTGTT	GTGCAAAGCATG	TGAATGTGCT
<i>Gs_PHYA2</i>	2056	1595	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AAGCTGGAGA
<i>La_PHYA</i>	2063	454	$5.50 \times 10^{-8}$	CGCTGGGGTG	GTGCAAAGCATG	AACCTGGTGA
<i>Lj_PHYA</i>	2059	1598	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AACCTGGAGA
<i>Lpr_PHYA3</i>	2077	902	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAGGTGCT
<i>Ls_PHYA</i>	2059	1598	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AACCGGGCGA
<i>Pp_PHYA</i>	474	26	$3.18 \times 10^{-7}$	GTTGATTGTC	GCGCAAAGCATC	TGAAGTTTCT
<i>Ps_PHYA</i>	2056	1598	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AACCGGGCGA
<i>Va_PHYA</i>	2079	902	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAGGTGCT
<i>Vr_PHYA</i>	2051	1595	$5.50 \times 10^{-8}$	CGATGGGGTG	GTGCAAAGCATG	AAGCTGGAGA
<i>Vu_PHYA</i>	2079	902	$5.50 \times 10^{-8}$	GTTGATTGTC	GTGCAAAGCATG	TGAAGTTTCT

The column representing sequences are phytochrome A (PHYA) nucleotide sequences from 20 species. *Ad\_PHYA* *Arachis durianensis*, *Ah\_PHYA* *Arachis hypogea*, *Ai\_PHYA* *Arachis ipaniensis*, *Ap\_PHYA* *Abrus precatorious*, *At\_PHYA* *Arabidopsis thaliana*, *Cc\_PHYA* *Cajanus cajan*, *Gm\_PHYA1*, *Gm\_PHYA2*, *Gm\_PHYA3\_E3*, *Gm\_PHYA3\_e3* *Glycine max* PHYA A homologs, *Gs\_PHYA2* *Glycine soja*, *La\_PHYA* *Lupinus angustifolius*, *Lj\_PHYA* *Lotus japonicus*, *Lpr\_PHYA3* *Lablab purpureus*, *Ls\_PHYA* *Lathyrus sativus*, *Pp\_PHYA* *Pongamia pinnata*, *Ps\_PHYA* *Pisum sativum*, *Va\_PHYA* *Vigna angularis*, *Vr\_PHYA* *Vigna radiata*, *Vu\_PHYA* *Vigna unguiculata*. The following columns present length of DNA sequence, start position, random letters probability and the sequence of the conserved motif

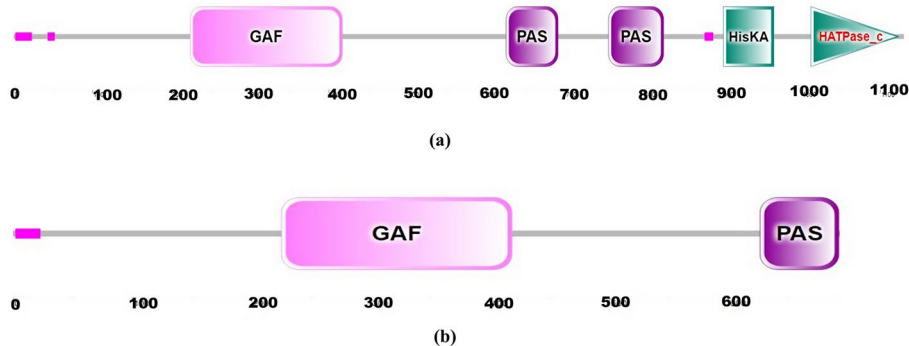
be used for more efficient breeding strategies [39]. These pathways have been more or less conserved in related legume species involving numerous loci with known or unknown functions. On the basis of available information on related genes and pathway undermining photoperiodic flowering in legumes, a theoretical model for photoperiod dependent flowering pathway is proposed (Fig. 5). In *Lablab purpureus*, photoperiodic response of flowering may also be governed by circadian clock as it is reported in *Glycine max*. CONSTANS (CO) is a circadian-regulated gene and acts as prime regulator of this pathway. It activates the expression of florigen gene *FT* by coordinating light and clock input to leaves. The different genes, their responses to photoperiod and their putative roles mediating this process have been proposed. It is speculated that under SD condition (low R:FR), *E3* and *E4* are repressed due to lack of exorbitant light condition and thus *E1* is also suppressed which thus has no effect on *FT2a* and *FT5a* genes leading to early flowering phenotype. Under enriched light condition, i.e. LD condition *PHYA3* (*E3*) and *PHYA2* (*E4*) are expressed activating the expression of *E1* which eventually leads to *FT2a/FT5a* downregulation, flowering repression, indeterminate growth (if *TFL1* is present) and delayed maturity. The

presence of *TFL1* and/or *tfl1* conveys indeterminate and determinate growth habit, respectively [1, 15]. Dominant *TFL* allele suppresses development of floral architecture at shoot apex in indeterminate types; racemes emerge from axillary bud only upon short day conditions where *FTs*, expressed due to favourable photoperiod condition, might be able to nullify the effect of *TFL* in competitive manner. Determinate growth habit results from non-functional allele (*tfl*) of *TFL* which is unable to suppress flowering in shoot apex and results into determinate growth habit and photoperiod insensitive flowering. This is unveiled by the presence of splice site SNP at third exon in *Lpr tfl* which renders non-functional protein and is responsible for determinate growth habit and photoperiod insensitive flowering in *Lablab purpureus* cv GNIB-21 [25]. A non-synonymous SNP in exon 4 of *TFL1* locus in cowpea also resulted in determinate growth habit [40]. However, photoperiod responsive expression of growth habit cannot be ignored [41].

Legumes have always been of persistent interest due to vast genetic variation for photoperiod responses mediating crucial traits like growth habit, flowering and maturity. A summary of different phytochrome A genes



**Fig. 3** The sequence-Logo comparison of conserved motifs between **a** *Arabidopsis thaliana* bHLH69 and **b** the motif found in putative phytochrome A genes

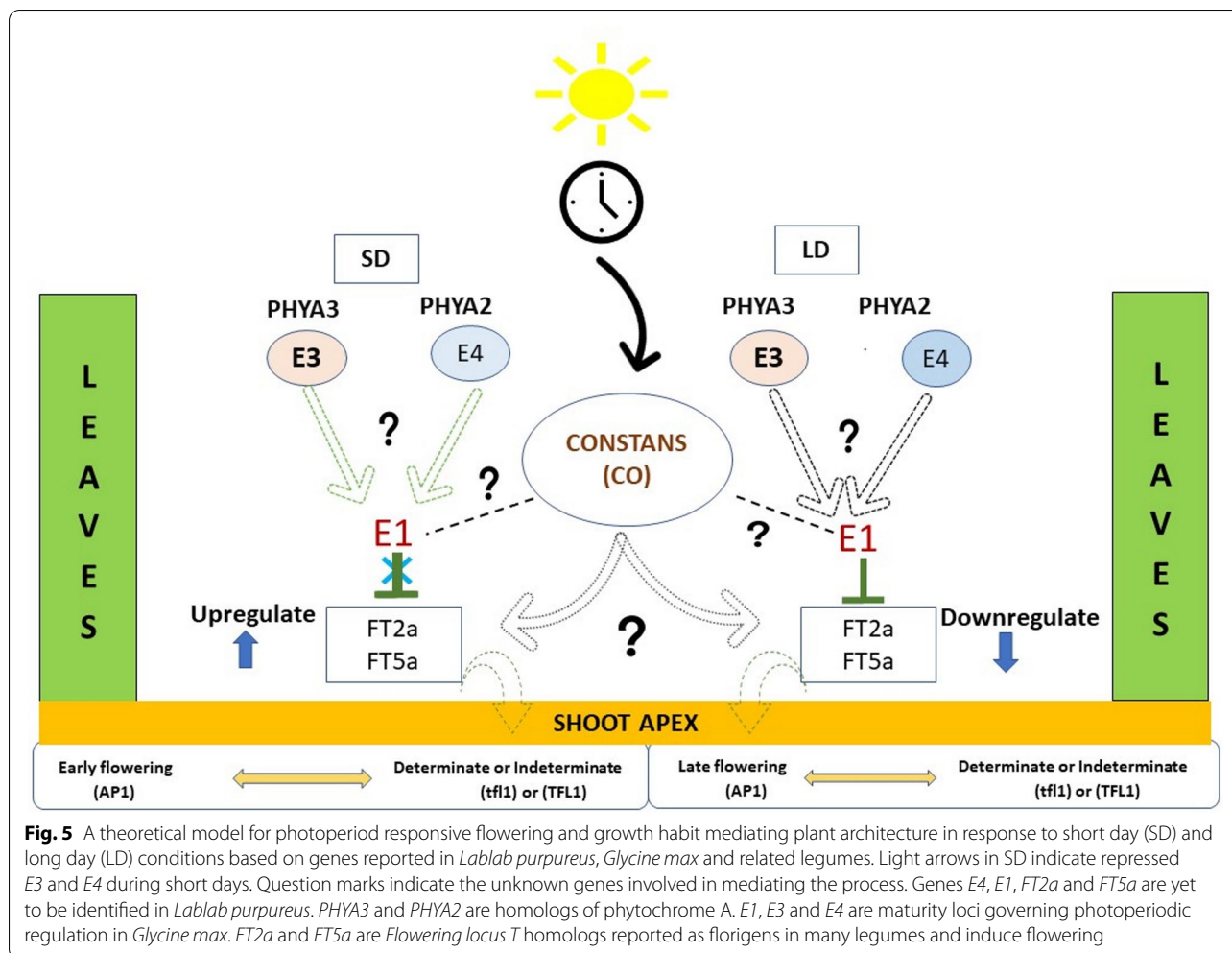


**Fig. 4** **a** Domain structure analysis of amino-acid sequence for *GmPHYA3*. **b** Domain structure analysis of exon-1 of *LprPHYA3*. The numbers represent position of amino acid. The domains for *PHYA3* protein included GAF (cGMP-specific phosphodiesterase-adenylyl cyclase-FhIA), PAS (period-ARNT-single-minded) and Histidine kinase-related domains (HKRD) viz., HisKA and HATPase\_c

in some legumes along with their putative role is represented (Table 3).

Seeking the importance of *GmPHYA3* (or *E3*) in photoperiod adaptation of short-day legumes like *Glycine max* and *Phaseolus vulgaris*, it becomes quite important to understand its role in *Lablab purpureus* which is also

a short-day crop. Due to the lack of genome sequence database, there is very scanty information available about *Lablab purpureus* at molecular level. The present study was successful in characterizing exon-1 in *LprPHYA3* by utilizing *GmPHYA3* sequence as reference. Comparative gene mapping has been quite useful in deducing genomic



**Fig. 5** A theoretical model for photoperiod responsive flowering and growth habit mediating plant architecture in response to short day (SD) and long day (LD) conditions based on genes reported in *Lablab purpureus*, *Glycine max* and related legumes. Light arrows in SD indicate repressed E3 and E4 during short days. Question marks indicate the unknown genes involved in mediating the process. Genes E4, E1, FT2a and FT5a are yet to be identified in *Lablab purpureus*. PHYA3 and PHYA2 are homologs of phytochrome A. E1, E3 and E4 are maturity loci governing photoperiodic regulation in *Glycine max*. FT2a and FT5a are Flowering locus T homologs reported as florigens in many legumes and induce flowering

structure from related plant species due to conservation of genetic content, gene order and function [25, 46]. This marks the significance of phylogenetic analysis to depict evolutionary closeness of different plant species. DNA-based phylogenies have been dominated in recent years mainly because it yields more phylogenetic information than protein. Since non-synonymous mutations do not affect amino acid sequence but do alter nucleotide sequences of a pair of homologous genes. Both DNA- and amino acid sequence-based phylogenies have been conducted in present study for validation of results obtained by phylogenetic analysis. DNA sequence phylogenies provide opportunity to the researcher for examination of both coding and non-coding regions of gene. However, the use of protein sequences in establishing evolutionary relationships cannot be ignored as amino acid sequences are more conserved than DNA sequences. The degeneracy in genetic code and difference in codon usage in different species makes it less accepted. Protein sequence gives more sensitive sequence alignment as DNA has

only 4 characters, while protein has 20. The translation of DNA to protein gives a higher signal-to-noise ratio and thus sharpens up the analysis making it better for phylogenetic studies [47]. PHYA3 gene has most probably been evolved from common ancestral PHYA gene of these species, as depicted from the tree. Previous studies have also shown closeness of *Lablab purpureus* to *Vigna unguiculata* and their predictable evolution from *Glycine max* in Phaseoleae clade [48]. This study has delineated the conservation of PHYA3 among the phaseoleae clade legumes indicating evolutionary closeness with *Vigna unguiculata*, *Vigna angularis* and *Glycine max* employing both DNA and protein sequences for phylogenetic analysis (Figs. 1 and 2). The evolutionary relationship of *Lablab purpureus* to *Glycine max*, *Vigna unguiculata* and *Phaseolus vulgaris* among the Phaseoleae clade has been reported earlier [48–50]. This will aid in isolating allelic variants of PHYA3 from *Lablab purpureus* by utilizing model plants like *Glycine max* and *Phaseolus vulgaris*. Characterization of the full gene could pioneer the



**Table 3** Summary of identified *Phytochrome A* genes in different crop legumes

Gene	Legume species	Function/role	Reference
<i>PHYA3 E3</i>	<i>Glycine max</i>	R/FR photoreceptor; represses FT; delays flowering in LD	[3]
<i>PHYA3 e3</i>	<i>Glycine max</i>	Promotes flowering in LD	[42]
<i>PHYA2 E4</i>	<i>Glycine max</i>	R/FR photoreceptor; represses FT; delays flowering in LD	[17]
<i>PHYA1</i>	<i>Glycine max</i>	Unknown	[17]
<i>PHYA2 E4</i>	<i>Glycine soja</i>	Inhibits flowering	[42]
<i>PHYA</i>	<i>Pisum sativum</i>	R/FR photoreceptor	[43]
<i>PHYA3</i>	<i>Phaseolus vulgaris</i>	R/FR photoreceptor; represses FT; delays flowering in LD	[26]
<i>PHYA and PHYB</i>	<i>Arachis hypogea</i>	Gynophore enlargement	[44]
<i>PHYA</i>	<i>Vigna radiata</i>	Control FT	[45]

extensive photoperiodic flowering control mechanism in *Lablab purpureus*.

Phytochromes mediate light responses by interacting with multiple partners to modulate transcription of downstream target genes. The transcription factor (TF) containing basic helix-loop-helix (bHLH) motif interacts physically with red and far-red photoreceptor, phytochrome, called Phytochrome Interacting Factors (PIF) [51]. The present study was also successful in identifying bHLH69 as conserved motif for *PHYA* gene (Fig. 3 and Table 2). The TF with bHLH motif has already been known to be involved in regulating circadian rhythm in *Arabidopsis* [52, 53]. Presence of DNA binding motif (bHLH) in *PHYA* indicates that it might compete with PIFs for DNA binding to repress flowering [51]. The present study also deciphered the domains encoded by exon 1 of *LprPHYA3* (Fig. 4) which are in congruity with the *Glycine max PHYA3* [3]. This implies to the fact that exon 1 in *LprPHYA3* codes for GAF and PAS domains with chances of conserved functions. The plant phytochromes detect light *via* their amino-terminal photosensory module (PSM) comprising N-terminal extension (NTE), period-ARNT-single-minded (PAS), cGMP-specific phosphodiesterase-adenylyl cyclase-FhlA (GAF) and phytochrome-specific (PHY) domains with the help of a bilin chromophore. C-terminal output module (OPM) is shared by two PAS on the N-terminal side and a histidine kinase-related domain (HKRD) [54]. NTE is related to the stability of light-activated phytochromes and interacts with the part of GAF which binds to P $\phi$ B for lyase activity and reversible Pr/Pfr photo-transformation. PAS domain represents transducer domain that mediates light signal from input photosensory domain to output module. HKRD domain plays major roles in dimerization, nuclear import and localization [55]. *LprPHYA3*-Exon 1 encodes GAF and PAS domain of phytochrome genes which belongs to the photo-sensory module and is responsible for convertible Pr/Pfr transformation as well

as light-signal transduction from this module to output module, respectively [56]. Characterization of full gene sequence of *PHYA3* in *Lablab purpureus* would unravel the different domains involved in downstreaming light-mediated response to the signaling pathway along with their putative roles.

### Conclusion

Partial characterization of *LprPHYA3* would facilitate allelic characterization in relation to photoperiod responsive flowering in *Lablab purpureus*. Phylogenetic analysis indicated that complete characterization of *LprPHYA3* would be possible utilizing sequence information from related legumes. The presence of conserved DNA binding motif (bHLH69) in *PHYA* gene indicated that it might repress flowering by competing for DNA binding with bHLH containing TFs. Domain analysis of protein-encoding *LprPHYA3* would unfold the signaling pathways and their interaction with different proteins from PEBP (Phosphatidyl ethanolamine-binding protein) family genes that would guide flowering response.

The continued progress in this direction would entice further questions to address in future like characterization and identification of allelic variants for *LprPHYA3* and their role in modulating photoperiod responsive flowering. Additionally, qPCR studies could also be undertaken for relative expression studies of *PHYA3* in LD and SD conditions. The role of *LprPHYA3* may be confirmed through genome editing by utilizing partial sequence reported in the present study. These efforts would accelerate the understanding of flowering time and growth habit regulation in *Lablab purpureus* in response to changed photoperiod.

### Abbreviations

R/FR: Red/Far-Red; *PHY*: Phytochrome; FT: Flowering locus T; LD: Long day; SD: Short day; DT: Determinate; IDT: Indeterminate; PS: Photosensitive; PIS: Photo-insensitive; SNP: Single nucleotide polymorphism; NCBI: National Centre

for Biotechnology Information; BLAST: Basic Local Alignment Search Tool; MEGA: Molecular Evolution Genetics Analysis; MEME: (Multiple Em for Motif Elicitation); CTAB: Cetyl trimethyl ammonium bromide; PCR: Polymerase chain reaction; SMART: Simple Modular Architecture Research Tool; DNA: Deoxyribonucleic acid; PSM: Photosensory module; NTE: N-Phosphodiesterase-adenylyl cyclase-FhIA (GAF); PHY: Phytochrome-specific; OPM: Output module; HKRD: Histidine kinase-related domain; PIF: Phytochrome-interacting factors; CO: Constans; AP1: Apetela 1; TF: Transcription factors; bHLH: Basic helix-loop-helix; PEBP: Phosphatidyl ethanolamine-binding protein.

## Supplementary Information

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**Additional file 1.** Sequencing data.

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### Authors' contributions

SK recorded observations, did laboratory work and wrote the manuscript. KM conceptualized the research and analysed the data. VP guided laboratory works and interpreted the results. RP ensured funding for the laboratory work, helped in field evaluation and guided the research work. DC provided the experimental material, assisted in phenotyping and guided the research work. Further, all the authors have read and approved the manuscript.

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### Availability of data and materials

The dataset supporting the conclusions of this article are included within the article. The sequencing data have been submitted as [supplementary material](#).

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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### References

- Xu M, Xu Z, Liu B, Kong F, Tsubokura Y, Watanabe S, Xia Z et al (2013) Genetic variation in four maturity genes affects photoperiod insensitivity and PHYA-regulated post-flowering responses of soybean. *BMC Plant Biol* 13:91–105. <https://doi.org/10.1186/1471-2229-13-91>
- Sharrock RA, Mathews S (2006) Phytochrome genes in higher plants: structure, expression, and evolution. In: *Photomorphogenesis in plants and bacteria*. Kluwer Academic Publishers, Dordrecht. [https://doi.org/10.1007/1-4020-3811-9\\_7](https://doi.org/10.1007/1-4020-3811-9_7)
- Watanabe S, Hideshima R, Zhengjun X, Tsubokura Y, Sato S, Nakamoto Y, Yamanaka N et al (2009) Map-based cloning of the gene associated with the soybean maturity locus E3. *Genetics* 182:1251–1262. <https://doi.org/10.1534/Genetics.108.098772>
- Weller JL, Ortega R (2015) Genetic control of flowering time in legumes. *Front Plant Sci*. <https://doi.org/10.3389/Fpls.2015.00207>
- Cronk Q, Ojeda I, Pennington RT (2006) Legume comparative genomics: progress in phylogenetics and phylogenomics. *Curr Opin Plant Biol* 9:99–103. <https://doi.org/10.1016/j.Pbi.2006.01.01>
- Clack T, Mathews S, Sharrock RA (1994) The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequences and expression of PHYD and PHYE. *Plant Mol Biol* 25:413–427. <https://doi.org/10.1007/BF00043870>
- Sharrock RA, Quail PH (1989) Novel phytochrome sequences in Arabidopsis thaliana: structure, evolution, and differential expression of a plant regulatory photoreceptor family. *Genes Dev* 3:1745–1757. <https://doi.org/10.1101/Gad.3.11.1745>
- Bernard RL (1971) Two major genes for time of flowering and maturity in soybeans 1. *Crop Sci* 11:242–244. <https://doi.org/10.2135/Cropsci1971.0011183X001100020022x>
- Bonato ER, Vello NA (1999) E6, a dominant gene conditioning early flowering and maturity in soybeans. *Genet Mol Biol* 22:229–232. <https://doi.org/10.1590/S1415-47571999000200016>
- Buzzell RI (1971) Inheritance of a soybean flowering response to fluorescent daylength conditions. *Can J Genet Cytol* 13:703–707. <https://doi.org/10.1139/G71-100>
- Buzzell RI, Voldeng HD (1980) Research notes: inheritance of insensitivity to long daylength. *Soybean Genet Newsl* 7:26–28
- Cober ER, Voldeng HD (2001) A new soybean maturity and photoperiod-sensitivity locus linked to E1 and T. *Crop Sci* 41:698–701. <https://doi.org/10.2135/Cropsci2001.413698x>
- Cober ER, Molnar SJ, Charette M, Voldeng HD (2010) A new locus for early maturity in soybean. *Crop Sci* 50:524–527. <https://doi.org/10.2135/Cropsci2009.04.0174>
- McBlain BA, Bernard RL, Cremeens CR, Korczak JF (1987) A procedure to identify genes affecting maturity using soybean isoline testers 1. *Crop Sci* 27:1127–1132. <https://doi.org/10.2135/Cropsci1987.0011183X002700060008x>
- Xia Z, Watanabe S, Yamada T, Tsubokura Y, Nakashima H, Zhai H, Anai T et al (2012) Positional cloning and characterization reveal the molecular basis for soybean maturity locus E1 that regulates photoperiodic flowering. *Proc Natl Acad Sci* 109:E2155–E2164. <https://doi.org/10.1073/Pnas.1117982109>
- Franklin KA, Quail PH (2010) Phytochrome functions in Arabidopsis development. *J Exp Bot* 61:11–24. <https://doi.org/10.1093/Jxb/Erp304>
- Liu B, Kanazawa A, Matsumura H, Takahashi R, Harada K, Abe J (2008) Genetic redundancy in soybean photoresponses associated with duplication of the phytochrome a gene. *Genetics* 180:995–1007. <https://doi.org/10.1534/Genetics.108.092742>
- Cober ER, Tanner JW, Voldeng HD (1996) Soybean photoperiod-sensitivity loci respond differentially to light quality. *Crop Sci* 36:606–610. <https://doi.org/10.2135/Cropsci1996.0011183X003600030014x>
- Kwak M, Velasco D, Gepts P (2008) Mapping homologous sequences for determinacy and photoperiod sensitivity in common bean (*Phaseolus Vulgaris*). *J Hered* 99:283–291. <https://doi.org/10.1093/Jhered/Es9005>
- Kong F, Liu B, Xia Z, Sato S, Kim BM, Watanabe S, Yamada T et al (2010) Two coordinately regulated homologs of Flowering Locus T are involved in the control of photoperiodic flowering in soybean. *Plant Physiol* 154:1220–1231. <https://doi.org/10.1104/Pp.110.160796>
- Maass BL, Knox MR, Venkatesha SC, Angessa TT, Rammes S, Pengelly BC (2010) Lablab purpureus—a crop lost for Africa? *Trop Plant Biol* 3:123–135. <https://doi.org/10.1007/S12042-010-9046-1>
- Dhaliwal SK, Talukdar A, Gautam A, Sharma P, Sharma V, Kaushik P (2020) Developments and prospects in imperative underexploited vegetable

- legumes breeding: a review. *Int J Mol Sci* 21:9615. <https://doi.org/10.3390/ijms21249615>
23. Modha K, Kale B, Borwal D, Ramtekey V, Arpit B (2019) Inheritance pattern of photoperiod responsive flowering, growth habit and flower colour in indian bean [Lablab Purpureus (L.) Sweet.]. *Electron J Plant Breed* 10:297. <https://doi.org/10.5958/0975-928X.2019.00037.1>
  24. Ramtekey V, Bhuriya A, Ayer D, Parekh V, Modha K, Kale B, Vadodariya G et al (2019) Molecular tagging of photoperiod responsive flowering in indian bean [Lablab Purpureus (L.) Sweet.]. *Indian J Genet Plant Breed* 79:264–269. <https://doi.org/10.31742/IJGPB.79S.1.17>
  25. Kaldate S, Patel A, Modha K, Parekh V, Kale B, Vadodariya G, Patel R (2021) Allelic characterization and protein structure analysis reveals the involvement of splice site mutation for growth habit differences in Lablab purpureus (L.) sweet. *J Genet Eng Biotechnol* 19:34. <https://doi.org/10.1186/S43141-021-00136-Z>
  26. Weller JL, Vander Schoor JK, Perez-Wright EC, Hecht V, González AM, Capel C, Yuste-Lisbona FJ et al (2019) Parallel origins of photoperiod adaptation following dual domestications of common bean. *J Exp Bot* 70:1209–1219. <https://doi.org/10.1093/jxb/ery455>
  27. Doyle JJ, Doyle JLA (1987) Rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
  28. Hall TA (1999) A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
  29. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25(17):3389–3402. <https://doi.org/10.1093/nar/25.17.3389>
  30. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
  31. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Higgins DG et al (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21):2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
  32. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10:512–526. <https://doi.org/10.1093/oxfordjournals.molbev.a040023>
  33. Felsenstein (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39(4):783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
  34. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* 37:W202–W208. <https://doi.org/10.1093/nar/gkp335>
  35. Aman Beshir J, Kebede M (2021) In silico analysis of promoter regions and regulatory elements (motifs and CpG islands) of the genes encoding for alcohol production in *Saccharomyces cerevisiae S288C* and *Schizosaccharomyces pombe 972h*. *J Genet Eng Biotechnol* 19:8. <https://doi.org/10.1186/s43141-020-00097-9>
  36. Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, Borodovsky M (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. *Nucleic Acids Res* 33:6494–6506. <https://doi.org/10.1093/Nar/Gki937>
  37. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* 8:275–282. <https://doi.org/10.1093/bioinformatics/8.3.275>
  38. Letunic I, Doerks T, Bork P (2009) SMART 6: recent updates and new developments. *Nucleic Acids Res* 37:D229–D232. <https://doi.org/10.1093/nar/gkn808>
  39. Harada K, Watanabe S, Xia Z, Tsubokura Y, Yamanaka N, Anai T, Krezhova D (2011) Positional cloning of the responsible genes for maturity loci E1, E2 and E3 in Soybean. In: *Soybean—Genetics Nov. Tech. Yield Enhanc*
  40. Dhanasekar P, Reddy KS (2015) A novel mutation in TFL1 homolog affecting determinacy in cowpea (*Vigna unguiculata*). *Mol Genet Genomics* 290:55–65. <https://doi.org/10.1007/S00438-014-0899-0>
  41. Kim SE, Okubo H (1995) Control of growth habit in determinate lablab bean (Lablab purpureus) by temperature and photoperiod. *Sci Hortic* 61(3–4):147–155
  42. Tsubokura Y, Matsumura H, Xu M, Liu B, Nakashima H, Anai T, Kong F et al (2013) Genetic variation in soybean at the maturity locus E4 is involved in adaptation to long days at high latitudes. *Agronomy* 3:117–134. <https://doi.org/10.3390/Agronomy3010117>
  43. Weller JL, Batge SL, Smith JJ, Kerckhoffs LHJ, Sineshchekov VA, Murfet IC, Reid JB (2004) A dominant mutation in the pea PHVA gene confers enhanced responses to light and impairs the light-dependent degradation of Phytochrome A. *Plant Physiol* 135:2186–2195. <https://doi.org/10.1104/Pp.103.036103>
  44. Zhang Y, Sun J, Xia H, Zhao C, Hou L, Wang B, Li A et al (2018) Characterization of peanut phytochromes and their possible regulating roles in early peanut pod development. *PLoS One* 13:5. <https://doi.org/10.1371/Journal.Pone.0198041>
  45. Hwang WJ, Ha J, Lee T, Jeong H, Kim MY, Kim SK, Lee Y-H et al (2017) A candidate flowering gene in mungbean is homologous to a soybean phytochrome a gene. *Euphytica* 213:79. <https://doi.org/10.1007/S10681-017-1866-8>
  46. Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu S-C et al (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269:1714–1718. <https://doi.org/10.1126/Science.269.5231.1714>
  47. Opperdoes (2003) Phylogenetic analysis using protein sequences. In: *Phylogenetics Handb. A Pract. Approach to DNA Protein Phylogeny*
  48. McClean PE, Mamidi S, McConnell M, Chikara S, Lee R (2010) Synteny mapping between common bean and soybean reveals extensive blocks of shared loci. *BMC Genomics* 11:184. <https://doi.org/10.1186/1471-2164-11-184>
  49. Chang Y, Liu H, Liu M, Liao X, Sahu SK, Fu Y, Song B et al (2019) The draft genomes of five agriculturally important African orphan crops. *Gigascience* 8:3. <https://doi.org/10.1093/Gigascience/Giy152>
  50. Wang Z, Yang R, Devisetty UK, Maloof JN, Zuo Y, Li J, Shen Y et al (2017) The divergence of flowering time modulated by FT/TFL1 is independent to their interaction and binding activities. *Front Plant Sci* 8:697. <https://doi.org/10.3389/Fpls.2017.00697>
  51. Pham VN, Kathare PK, Huq E (2017) Phytochromes and phytochrome interacting factors. *Plant Physiol* 176:1025–1038. <https://doi.org/10.1104/pp.17.01384>
  52. Hanano S, Stracke R, Jakoby M, Merkle T, Domagalska MA, Weisshaar B, Davis SJ (2008) A systematic survey in *Arabidopsis thaliana* of transcription factors that modulate circadian parameters. *BMC Genomics* 9:182. <https://doi.org/10.1186/1471-2164-9-182>
  53. Nakamichi N (2011) Molecular mechanisms underlying the Arabidopsis circadian clock. *Plant Cell Physiol* 52(10):1709–1718. <https://doi.org/10.1093/pccp/pcr118>
  54. Oide M, Nakasako M (2021) Red light-induced structure changes in phytochrome A from *Pisum sativum*. *Sci Rep* 11:2827. <https://doi.org/10.1038/S41598-021-82544-2>
  55. Rockwell NC, Su Y-S, Lagarias JC (2006) Phytochrome structure and signaling mechanisms. *Annu Rev Plant Biol* 57:837–858. <https://doi.org/10.1146/Annurev.Arplant.56.032604.144208>
  56. Cheng M-C, Kathare PK, Paik I, Huq E (2021) Phytochrome signaling networks. *Annu Rev Plant Biol* 72:217–244. <https://doi.org/10.1146/Annurev-Arplant-080620-024221>

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