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Mapping Homologous Sequences for Determinacy and Photoperiod Sensitivity in Common Bean (*Phaseolus vulgaris*)

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Abstract

Determinacy and photoperiod insensitivity are agronomically important traits, selected during or after domestication in common bean. Determinacy reduces aboveground plant biomass and accelerates and synchronizes flowering. Photoperiod insensitivity allows common bean to be grown at higher latitudes under long days. In this study, we attempted to identify *Phaseolus vulgaris* homologues of 12 *Arabidopsis* genes that are involved in meristem identity determination and the photoperiod-dependent and autonomous flowering pathways. Amplification products with homology to the original Arabidopsis gene were obtained for 8 genes, 7 of which could be mapped onto the common bean-linkage map using the BAT93 × Jalo EEP 558 and Midas × G12873 recombinant inbred populations. Three *Terminal Flower 1* homologues (*PvTFL1x*, *PvTFL1y*, and *PvTFL1z*) were mapped to B4, B1, and B7, respectively. *PvTFL1y* cosegregated with the determinacy locus, *fin*. In addition, *PvTFL1z* mapped near or at a second determinacy locus on B7. A *Zeitlupe* homologue mapped near a quantitative trait locus (QTL) for flowering time on linkage group B9. *Constans*, *FCA*, *Flowering locus D*, *Gigantea*, and *Leafy* homologues did not cosegregate with currently mapped flowering time QTLs and photoperiod insensitivity loci in common bean. Further studies are needed to confirm the role of these homologues as potential candidate genes.

Wild common bean is indeterminate and flowers under short-day conditions. Plants are typically viny and grow on other plants that act as physical support. In addition, they require nights of at least 11–12 h for flowering induction. 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). Also, the determinate growth habit (GH) has been exploited in crop breeding to accelerate flowering and shorten the flowering period (Cober and Tanner 1995). In indeterminate cultivars, the terminal meristem remains in a vegetative state during which it controls the production of stems and leaves. In determinate cultivars, the terminal meristem switches from a vegetative to a reproductive state, that is, it produces a terminal inflorescence. Thus, photoperiod-insensitive, determinate common bean cultivars generally flower and mature early, allowing a shortening of the time to harvest (Koinange et al. 1996). Because common bean in the Andean region may have been domesticated without maize, which is used as a physical support for climbing bean in traditional agriculture, determinate genotypes would have been favored in that domestication region (Koinange et al. 1996).

In common bean, several major loci and quantitative trait loci (QTLs) have been reported for GH, photoperiod sensitivity, and flowering time (Norton 1915; Wallace et al. 1993; Jung et al. 1996; Bassett 1997; McClean et al. 2002; Tar'an et al. 2002; Kolkman and Kelly 2003). Among them, the recessive *fin* (determinacy) and the dominant *Ppd* (photoperiod sensitivity) loci are located on the B1 linkage group (Norton 1915; Koinange et al. 1996; Freyre et al. 1998). Isolation of these genes would allow us to better understand how selection for determinacy and photoperiod insensitivity has shaped, if at all, genetic diversity in the *fin*–*Ppd* region relative to other regions of the bean genome. It will also allow us to get a better understanding of their function for further utilization and improvement of these traits in bean breeding.

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Table 1. Flowering time and reproductive transition genes from *Arabidopsis* included in this study

Gene	Gene product	Function in *Arabidopsis*	Reference
Apetela1 (*AP1*)	Transcription factor	Floral promoter, promoting the floral transition	Gustafson-Brown et al. (1994)
Constans (*CO*)	Zinc finger–containing protein	Interaction between circadian rhythms and signaling pathway	Putterill et al. (1995)
Early Flowering 3 (*ELF3*)	Transcriptional regulator	Circadian clock input pathway	Hicks et al. (2001)
FCA	RNA-binding protein	Floral promoter, autonomous pathway	Macknight et al. (1997)
Flowering locus D (*FLD*)	Histone deacetylase	Regulation of *Flowering Locus C* expression, Autonomous pathway	He et al. (2003)
Flavin-Binding, Kelch repeat, F-Box1 (*FKF1*)	PAS protein	Photoreceptor, Controlling *CO*	Imaizumi et al. (2003)
Gigantea (*GI*)	Integral plasma membrane-localized protein	Phytochrome signaling, Light-dependent pathway	Huq et al. (2000); Park et al. (1999)
LEAFY (*LFY*)	Transcriptional activator	Floral promoter, promoting the floral transition	Schultz and Haughn (1991)
Phytochrome B (*PHYB*)	Photoreceptor	Light perception	Sharrock and Quail (1989)
Terminal Flower 1 (*TFL1*)	Phosphatidylethanolamine-binding protein	Floral repressor	Bradley et al. (1997)
Terminal Flower 2 (*TFL2*)	Heterochromatin protein	Maintaining of *Flowering Locus T* repression	Larsson et al. (1998)
Zeitlupe (*ZTL*)	PAS protein	Circadian clock input pathway	Somers et al. (2000)

As a first step toward isolating these genes in common bean, we sought to use information obtained in model species such as *Arabidopsis* through a candidate gene approach. Genetic regulation of flowering induced by light has been studied most intensively in model species, especially in *Arabidopsis* (Hayama and Coupland 2003; Komeda 2004; Corbesier and Coupland 2005). Photoperiodism involves regulation of flowering time through a circadian clock (Ratcliffe and Riechmann 2002; Komeda 2004). Thus, identification of candidate genes for photoperiod sensitivity in target species can be initiated with testing genes of the light-dependent flowering pathway from *Arabidopsis*. For example, in rice, heading date is controlled by photoperiod. One of the major QTL for heading date, *Hd1*, is a homologue of the *Arabidopsis Constans* (*CO*) gene. This gene codes for a zinc finger–containing protein, which interacts between circadian rhythms and the flowering signaling pathway (Yano et al. 2000; Hayama and Coupland 2004).

Determinacy and photoperiod sensitivity traits play an important role in common bean evolution and breeding, but the actual genes have not yet been isolated in this species. In this study, several common bean homologues related to floral transition and photoperiod sensitivity mainly from *Arabidopsis* were isolated and mapped onto the consensus common bean-linkage map. This work will provide genetic background information to manipulate GH and photoperiod sensitivity for future common bean breeding as well as identifying candidate genes for those traits in other legume species.

Materials and Methods

Polymerase Chain Reaction Primers

Table 1 shows the *Arabidopsis* genes included in this study. Amplifications of *LFY* and *TFL1* homologues were performed with degenerate primers developed prior to this study by Frohlich and Meyerositz (1997) and Foucher et al. (2003), respectively. To identify other homologous genes in common bean, degenerate primers were developed in this study (Table 2). Using *Arabidopsis* sequences, the corresponding homologues of soybean, peanut, Medicago, or/and lotus sequences were obtained by BLAST searches in GenBank. The sequences were aligned with ClustalX (Thompson et al. 1997). The degenerate primers were developed based on conserved motifs among the sequences from those species.

Amplification of Homologous Sequences

Genomic DNA was extracted from young leaves using the Cetyl TrimethylAmmonium Bromide method (Doyle and Doyle 1987). The target fragments in BAT93, G12873, Jalo EEP558, and Midas (parents of the mapping populations used in this study) were amplified using polymerase chain reaction (PCR) with *Taq* DNA polymerase (New England Biolabs, Ipswich, MA). PCR reaction mixtures contained approximately 100 ng of total DNA, 0.2 mM of dNTP, 0.2 μM of forward and reverse primers, standard *Taq* buffer with 1.5 mM $MgCl_2$, and 1 unit of *Taq* polymerase in a total volume 50 μl reaction. The PCR cycle consisted of 2 min at 95 °C and 35 cycles of 1 min at 95 °C, 1 min at 48 °C (*PvLFY*), 54°C (*PvFKF1* and *PvZTL*), 55 °C (*PvTFL1x*, *PvTFL1y*, and *PvTFL1z*), 56 °C (*PvFCA*), 57 °C (*ELF3*), 58 °C (*PvGI*), or 60 °C (*PvCO* and *PvFLD*), and lastly 1–3 min at 72 °C followed by a 7 min extension at 72 °C. For amplification of *AP1*, *AG*, and *TFL2* fragments, various annealing temperatures from 50 °C to 62 °C were tested. For amplification of a *PHYB* fragment, the PCR cycle consisted of 35 cycles of 1 min at 92 °C, 1.5 min at 53 °C, and 4.5 min at 68 °C. The fragments obtained were

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Table 2. Primer sequences. The nested primers used for genotyping are shown in bold

Gene	Primer	Sequences
AP1	AP1-l1-f	AGCTCATGAGATCTCTGTTC
	AP1-l1-r	AGCGYTCIAGHATCTTCTCC
PvCO	Constans5	AACAAGCTCGCMTCSCGCCAC
	Constans3	TGCACCGGAACGACKCCGTC
ELF3	ELF3-F1	CGCAATCTTCGYTCTCAC
	ELF3-R2	GCAGRGCCAGGAGG
PvFCA	FCA-F1	AAGCAAGCTTTCATTCATCTC
	FCA-R4	GTAACTCCATATGCCTGG
	FCA-F3	CAGATGATGCAGCCTTC
	FCA-R6	CAGTACAGCTATTTAGAACC
PvFLD	FLD-F1	TTGGAATATGCAAATGCTGGG
	FLD-R2	CAGCTTCACCAGCCAC
	FLD-F3	GCTGGGTGCCTTTCAAATC
	FLD-R4	CAGCCACCAGCGCAAC
PvFKF1	FKF1-F1	GTTGTGKCTGAGATTAG
	FKF1-R2	GCTATGWCCCCAAG
PvGI	GI-F1	GTGATGATGAAGTTGCTCG
	GI-R4	CATTTGAGCTGTAACTCCAAG
	GI-F3	GAGAATTTGCACCATTTGGG
PvLFY	LFY1[a]	CACCCACGACCITTYATIGTIA CIGARCCIGGIG
	LFY2[a]	CCTGCCIACRTARTGICKCATY TTIGGYTT
PHYB	PHYB-F1	TCCACGCCGTYTTCG
	PHYB-R2	CCCKCTTRTTTGCAGTCA
	PHYB-F3	GGCTCCTCAYGGTTG
	PHYB-R4	GAGATGGTGCATASCG
PvTFL1	TFL1-1[b]	ATGGGGAGAGTGATHGGRGAWG
	TFL1-2[b]	TCACTAGGRCCWGGAACATCWGG
	TFLC_ph-f	TCTGCAAAGAGAAAAGGAGACA
	TFLC_ph-r	GGTTGAGATTGGTGGAGGAG
	TFL1-3[b]	GATGTTCCWGGWCCTAGTGAYCC
	TFL1-5[b]	CTTGCAGCRGTYYTCYCTYTG
	TFLABlong_ph	CTTCTTGTGATGTAAGTGTTTG
	TFLa-f	TGGTTAGTCACTCTCTTACC
	TFLa-r	TCTGTGGATTCCTATCACTG
TFL2	TFL2-F	TTCTGTCAAGAGGTTCAAGAG
	TFL2-R	TCCACCATCACTTCTGTTCC
PvZTL	ZTL-F1	GTTAATCGTGGAGTTTGTGG
	ZTL-R2	ACAGGAGCTATGCCAAG

[a] Frohlich and Meyerowitz (1997)

[b] Foucher et al. (2003)

sequenced at the sequencing facility in the Division of Biological Sciences at University of California, Davis. For *TFL1* homologues, fragments of approximately 360 bp, 520 bp, and 800 bp were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). Transformation and analysis were done following manufacturer's instructions. Sequencing of inserted fragments was performed with SP6 and T7 universal primers. The identity of the sequences was confirmed with a tBLASTx search. Sequence polymorphisms between parental lines were searched for restriction enzyme cleavage sites. For *PvFCA*, *PvFLD*, *PvGI*, *PvTFL1x*, *PvTFL1y*, and *PvTFL1z*, nested primers to improve amplification quality were designed and used for the genotyping and mapping experiment (Table 2).

Mapping Populations

The BAT93 × Jalo EEP 558 (BJ, $n = 80$) and Midas × G12873 F_8 (MG, $n = 58$) recombinant inbred (RI) populations were used to integrate the homologous sequences into the common bean molecular linkage map. The BJ RI population is the common bean core mapping population (Freyre et al. 1998; Blair et al. 2003). BAT93 is a multiple disease–resistant breeding line belonging to the Mesoamerican gene pool, whereas Jalo EEP558 is a Brazilian cultivar from the Andean gene pool. The MG RI population was used to investigate the inheritance of domestication syndrome traits in common bean (Koinange et al. 1996). Midas is a domesticated Andean accession, and G12873 is a wild Mesoamerican accession. This MG population shows several segregating traits related to domestication, including determinacy (*fin*) and photoperiod sensitivity (*Ppd*), which do not segregate in the BJ population. However, because the map of MG is a low-density map, homologous sequences were mapped in both the BJ and MG populations. Huron and Newport (HN), the parental lines of the HN population used for mapping an additional determinacy locus on B7 (Kolkman and Kelly 2003), were used to sequence *PvTFL1z*, an additional homologous sequence.

Genotyping Amplified Fragments

To genotype fragments in the 2 RI populations, *PvCO*, *PvFLD*, *PvFKF1*, *PvLFY*, and *PvTFL1y* sequences were amplified and digested with *Taq*I, *Bsm*AI, *Bsss*SI, *Cvi*JI, and *Mse*I, respectively (Table 3). For *PvZTL* sequences, amplified fragments in the BJ population were digested with Taq^{α}I and the fragments of the MG population were digested with *Hpy*188I. Digestions followed the manufacturer's instructions. Digested fragments were separated by 1.5% agarose gel or 6% polyacrylamide gel electrophoresis depending on their size and size differences. To genotype *PvFCA*, *PvGI*, and *PvTFL1x*, the fragments of the RI populations were amplified and genotyped directly in a 6% polyacrylamide gel according to their size difference. *PvTFL1z* was genotyped by sequencing.

Genetic Mapping of Homologous Sequences

Homologous sequences were mapped with MAPMAKEWR/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992; Freyre et al. 1998) using a minimum LOD score of 3.0 and a maximum distance of 50 cM. The markers were ordered using the "Sequence" command and the new homologous sequences in this study were placed using the "Try" and "Compare" commands into their respective linkage group. The likelihood of their placement was tested using the "Ripple" command to find the most likely order. The linkage maps obtained are represented using the MapChart program (Voorrips 2002).

Results

The PCR amplifications using degenerate primers designed in this study were successful in identifying homologous

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Table 3. Primers and polymorphisms for genotyping homologues sequences and genotyping method

		Amplification						
		Mesoamerican		Andean				
Gene tested	Primers	G12873	BAT93	Midas	Jalo EEP 558	Polymorphism	Restriction enzyme/genotyping method	
AP1[a]	No amplification obtained							
PvCO	Constans5/Constans3		+	+	+	+	SNP	*Taq*I
ELF3[a]	ELF3-F1/ELF3-R2		+	+	+	+	Sequencing unsuccessful: mixed sequences	
PvFCA	FCA-F3/FCA-R6		−	+	+	+	9 bp indel in BJ	Gel electrophoresis
PvFLD	FLD-F3/FLD-R4		+	+	+	+	SNP	*Bsm*AI
PvFKF1	FKF1-F1/FKF-R2		+	+	+	+	SNP	*Bsss*SI
PvGI	GI-F3/GI-R4		+	+	+	+	24 tandem repeat in MG	Gel electrophoresis
PvLFY	LFY1/LFY2		+	+	+	+	SNP	*Cvi*JI
PHYB[a]	PHYB-F1/PHYB-R2	2 kb	+	+	+	+	See text	
		1 kb	+	+	−	−	See text	
		750 bp	+	+	+	+	See text	
		400 bp	+	+	+	+	See text	
	PHYB-F3/PHYB-R4	1.4 kb	+	+	+	+	See text	
		700 bp	+	+	−	−	See text	
		600 bp	+	+	+	+	See text	
		300 bp	+	+	+	+	See text	
PvTFL1x	TFLa-f/TFLa-r		+	+	+	+	2-bp indel in MG; 2-bp and 1-bp indels in BJ	Gel electrophoresis
PvTFL1y[b]	TFLABlong_ph/TFL1-3		+	+	+	+	SNP	*Mse*I
PvTFL1z	TFLC_ph-f/TFLC_ph-r		+	+	+	+	SNP	Sequencing
TFL2[a]	No amplification obtained							
PvZTL	ZTL-F1/ZTL-R2		+	+	+	+	SNP	*Taq*$^{\alpha}$I in BJ *Hpy*188I in MG

[a] Excluded from genotyping and mapping analysis due to failure of amplifying or sequencing a homologous target fragment.

[b] *PvTFL1y* (ca. 800-bp) fragment was not amplified with TFL1-3 and TFL1-5 from Midas. However, 251-bp *PvTFL1y* sequence of Midas was obtained by amplification with TFLABlong_ph nested primers and TFL1-3.

sequences potentially coding for determinacy and photoperiod sensitivity in common bean, except for homologues of *AP1* and *TFL2* (Table 3). BLAST searches of GenBank revealed that the sequences obtained in this study are very similar to reproductive transition and photoperiod sensitivity genes, mostly of *Medicago truncatula*, considered a legume model plant (Table 4). For amplification of *ELF3*, a fragment of approximately 1200 bp was obtained from the 4 parental lines. However, the sequencing of this fragment was not successful and *ELF3* was excluded from further analyses. For amplification of homologous sequences of *PHYB*, fragments of approximately 2 kb, 1 kb, 750 bp, and 400 bp were obtained with PHYB-F1 and PHYB-R2 primers and of approximately 1.4 kb, 700 bp, 600 bp, and 300 bp were obtained with PHYB-F3 and PHYB-R4 primers. However, tBlastx searches did not identify any sequences homologous to flowering time or GH genes. Thus, *PHYB* was excluded from further analysis as well. Using the primers developed by Foucher et al. (2003), an approximately 800-bp *PvTFL1y* fragment was not amplified from Midas as expected. However, because a 251-bp *PvTFL1y* fragment could be amplified using nested primers for *PvTFL1y*, genotyping in the MG population was successful (Table 3).

The polymorphisms in the *PvCO*, *PvFCA*, *PvFLD*, *PvLFY*, *PvTFL1x*, *PvTFL1y*, and *PvTFL1z* sequences among parents of the 2 RI populations reflected generally the origin of bean accessions. Midas and Jalo EEP558 represent accessions of Andean origin, whereas G12873 and BAT93 originated in the Mesoamerica gene pool. Four SNPs were found in *PvZTL* sequences. Whereas 1 SNP distinguished G12873 from the 3 other genotypes in *PvZTL* sequences, 3 SNPs distinguish BAT93 from the other 3 sequences. Three SNPs and a 24-bp tandem repeat were found in *PvGI* sequences. These SNPs in *PvGI* sequences distinguished the G12873 sequence from the others. Because we could not find any polymorphism between BAT93 and Jalo EEP 558 for the *PvGI* sequence, the BJ population was not genotyped for *PvGI* sequences. Likewise, because a *PvFCA* sequence could not be amplified in G12873, *PvFCA* was not genotyped in the MG population. The enzymes and genotyping methods are summarized in Table 3. The segregations of these alleles in the RI populations are summarized in Tables 5 and 6.

The distribution of flowering and GH homologues in the common bean-linkage map is shown in Figure 1. *TFL1* sequences mapped to 3 different linkage groups: B1, B4, and B7. The *PvTFL1y*, *PvTFL1z*, and *PvFLD* loci had similar map positions in the MG and BJ maps. *PvTFL1y* cosegregated with the *fin* locus in the MG RI population. *PvTFL1z* mapped to linkage group B7. Although there was no segregating locus for GH trait located on B7 in the MG and BJ populations, a determinate morphology in the HN population had been mapped to the B7 linkage group in a similar location (Kolkman and Kelly 2003). In addition, Tar'an et al. (2002) mapped a plant height (PH) QTL on B7 in the OAC Seaforth × OAC 95-4 cross. However, the absence of polymorphism between the Huron and Newport

Table 4. Common bean homologues identified in this study. All queries were made using tBLASTx against the nonredundant database with default parameters (BLUSUM62 MATRIX)

Common bean homologues	GenBank accessions number	Sequences similar to		e value
PvCO	EF643220, EF643221, EF643222, EF643223	AAC99310	*Malus × domestica CONSTANS*-like protein2	7×10^{-40}
PvFCA	EF643224, EF643225, EF643226	AC135100	*Medicago truncatula* clone mth2-17j9, complete sequence	2×10^{-32}
		AY805329	*Pisum sativum FCA* gamma	4×10^{-32}
PvFLD	EF643227, EF643228, EF643229, EF64323	CT573339	*M. truncatula* DNA sequence from clone MTH2-82H11	1×10^{-98}
		NM_112218	*Arabidopsis thaliana* amine oxidase/oxidoreductase	6×10^{-89}
		AY830930	*P. sativum FLOWERING LOCUS D* (*FLD*)	4×10^{-76}
PvFKF1	EF643231, EF643232, EF643233, EF643234	AC140104	*M. truncatula* clone mth2-10g22	0.0
		AP004911	*Lotus japonicus* genomic DNA, chromosome 4	0.0
		NM_105475	*A. thaliana FKF1*	0.0
PvGI	EF643235, EF643236, EF643237, EF643238	AC148397	*M. truncatula* clone mth2-22h4, complete sequence	0.0
		AY682088	*A. thaliana GIGANTEA* gene, complete cds	0.0
PvLFY	EF643239, EF643240, EF643241, EF643241	DQ448809	*Glycine max LEAFY*-like Protein	2×10^{-40}
PvTFL1x	EF643243, EF643244, EF643245, EF643246	AB183451	*Populus nigra PnFTL1a* gene for flowering locus T like protein	6×10^{-50}
PvTFL1y	EF643247, EF643248, EF643249, EF643250	AY423715	*L. japonicus CEN/TFL1*-like GTP-associated binding protein	6×10^{-47}
PvTFLz	EF643251, EF643252, EF643253, EF643254, EF643255, EF643256	AY186738	*Lycopersicon esculentum SP9D* gene	1×10^{-36}
PvZTL	EF643257, EF643258, EF643259, EF643260	AC148970	*M. truncatula* chromosome 2 BAC clone mth2-74j13	7×10^{-56}
		DQ309278	*Ipomoea nil ZTL* mRNA, complete cds	6×10^{-54}

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Table 5. Chi-square test of segregation ratios for homologues sequences and linkage map position in the BJ population

Common bean homolog	Segregation ratio			Map location
	Observed			
	BAT93 allele	Jalo EEP 558 allele	χ^2 (1:1)	
PvCO	36	37	0.01	B9
PvFCA	31	36	0.37	B3
PvFLD	26	26	0	B11
PvFKF1	32	27	0.42	Not assigned
PvLFY	34	33	0.01	B9
PvTFL1x	37	14	10.37*	B4
PvTFl1y	22	49	10.26*	B1
PvTFL1z	53	14	22.7*	B7
PvZTL	35	22	2.96	B9

* Significantly different from 1:1 at $P = 0.05$.

PvTFL1z sequences precluded comparative mapping of the *PvTFL1z* and determinacy loci on B7 in the HN population. We could not find any morphological marker cosegregating with *PvTFL1x* on linkage group 4.

A *PvZTL* fragment mapped to the B9 linkage group in population BJ (Figure 1). The position of *PvZTL* was similar to that of a GH gene identified by Tar'an et al. (2002). This GH locus is correlated with the number of days to flowering and maturity (Tar'an et al. 2002). The *PvCO*, *PvLFY*, *PvZTL*, and *PvTFL1x* could not be assigned in the MG population with a LOD score of at least 3.0. The *PvFKF1* sequence could not be assigned in either population.

Discussion

Gene discovery using public sequence databases of model plants is useful especially for specialty crops that do not have this type of information yet (Mahalakshmi and Ortiz 2001). The strategy to identify certain homologous genes with degenerate primers designed from sequence information in model plants databases was partially useful in this study. The sequences obtained in this study had a high BLAST match with corresponding sequences in *M. truncatula* or other species (Table 5). Considerable progress has been made in legume genomics with large genomic or EST sequencing efforts in soybean (*Glycine max*), *M. truncatula*, and *Lotus japonicus* (Gepts et al. 2005). The availability of these resources made the design of degenerate primers considerably easier than it was 5 years ago when degenerate primers for *Phaseolus vulgaris* had to be established based on homologies with nonlegume sequences (Sambatti J. and Gepts P., unpublished data: e.g., TFL1 homologues: AY028710 and AY028711). However, of the 12 *Arabidopsis* genes included in this study, homologous *P. vulgaris* sequences could be amplified for only 8 of them. No amplified product could be obtained for 2 genes (*AP1* and *TFL2*). Predicted sequences could not be obtained for 2 other genes (*ELF3* and *PHYB*). The lack of success can be attributed to at least 2 causes. First, and most likely, sequence divergence within the legume family may make the design of primers more difficult. Further attempts at designing primers could ultimately yield sequenceable amplicons. Second, the specific homologues may not exist in common bean. However, given the ubiquitousness of these sequences, this possibility seems unlikely although not impossible in individual cases. Sequences homologous to *AP1* have been identified in other legumes (Berbel et al. 2001; Hecht et al. 2005). Through a BLAST search using an *Arabidopsis TFL2* sequence (AB073490), a similar sequence was identified in soybean (TC230509; *e* value $2.1 \times 10{-}^{23}$).

An indeterminate common bean plant produces stem nodes until maturity and senescence take over the growth processes. After floral initiation, each node consists of an internode with above it, 1 compound leaf and a compound raceme inflorescence in its axil. However, a determinate common bean plants ceases producing stem nodes well before the onset of maturity and senescence because the terminal meristem of stems produces a terminal inflorescence. The growth pattern through successive series of modular units is similar to that of tomato (Sage and Webster 1987; Schmitz and Theres 1999). In tomato, *Self-Pruning* (*SP*) and *Falsiflora* (*FA*) control meristem identity. *SP* gene suppresses the transition of vegetative to reproductive state, keeping a plant indeterminate (Pnueli et al. 1998). *FA* is responsible for floral meristem identity and promotes flowering (Molinero-Rosales et al. 1999). *SP* is a member of the *CETS* family (*Centroradialis* [*CEN*] in *Antirrhinum*, *TFL1* in *Arabidopsis*, and *FT* in *Arabidopsis* [Pnueli et al. 2001]). *FA* is an orthologue of *Arabidopsis LFY* and *Antirrhinum Floricaula* (*FLO*). *LFY* in *Arabidopsis* activates directly *AP1*, causing flowering (Komeda 2004; Saddic et al. 2006). Recently, a pea homologue of *TFL1*, *PsTFL1a*, has been found to correspond to the determinacy locus (*Determinate*; *DET*) in that species (Foucher et al. 2003). Another pea *TFL1* homologue, *PsTFL1c*, is a homologue of Late Flowering, a repressor of flowering. In common bean, cosegregation between the *PvTFL1y* and *fin* loci in the MG population suggests that *PvTFL1y* may be a candidate gene for the *fin* locus. Further data are needed to confirm this finding. Furthermore, because amplification using Foucher et al. (2003) was not successful in Midas, it implies that Midas might possess a mutation in primer site on the putative forth exon of *PvTFL1y*. Thus, further research to investigate relationship of this mutation in Midas *PvTFLly* and determinacy should be pursued.

PvLFY mapped to the B9 linkage group in this study, but we could not find any morphological trait associated with it. However, because a large single leaf mutation (*Unifoliata-2*), similar to pea *uni* has been reported in common bean (Garrido et al. 1991), the role of *PvLFY* as a potential candidate gene for *Uni-2* should be analyzed further. *LFY* homologues have been isolated from many species and their function is well conserved in angiosperms. *Unifoliata* (*UNI*) in pea is a *LFY* homologue and regulates indeterminacy of leaf and flower morphogenesis (Hofer et al. 1997).

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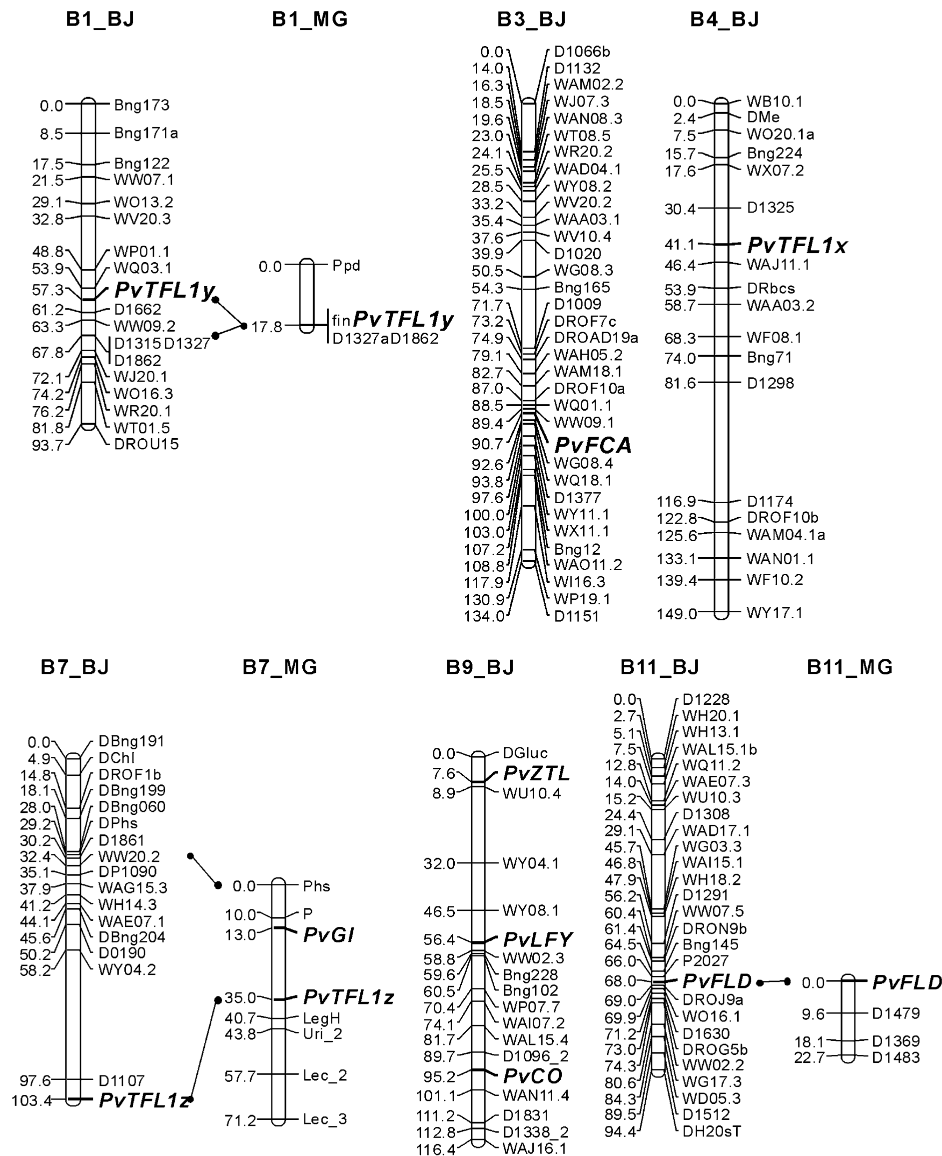

Figure 1. Positions of *Phaseolus vulgaris* homologues of *Arabidopsis* flowering and photoperiod sensitivity genes are shown with larger fonts on the core linkage map (Freyre et al. 1998) of common bean. BJ: BAT × Jalo EEP 558; MG: Midas × G12873. Genetic distances in Kosambi units.

The map position of *PvTFL1z* on B7 is similar to that of another possible determinacy locus (Tar'an et al. 2002; Kolkman and Kelly 2003). This determinacy may correspond to the determinate mutant generated by artificial mutagenesis in navy bean (Kelly 2000; Kolkman and Kelly 2003). Because we obtained a partial sequence of the putative first and second exons and first intron of Huron and Newport, it may be possible to find polymorphisms outside of the sequences obtained. Thus, a further linkage experiment between *PvTFL1z* and determinacy locus on B7 as well as morphological characterization of determinate phenotypes and possible epistatic interactions between *PvTFL1y* and *PvTFL1z* should be performed. This work may be helpful in manipulating plant architecture to improve common bean.

Among economic plants in the legume family, soybean, pea, and common bean have several genotypes or mutant lines with a determinate GH. The phenotype of 2 determinate soybean mutants (*dt-1* and *Dt-2*) is identical to the determinacy phenotype of common bean. Although genetic and physical maps for soybean have been constructed, the actual determinacy genes have not yet been identified in that species (Tasma and Shoemaker 2003; Wu et al. 2004). *TFL1* homologues in soybean should be tested as candidate genes to elucidate the nature of soybean determinacy genes. In pea, it has been shown recently that

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Table 6. Chi-square test of segregation ratios for homologues sequences and linkage map position in the MG population

Common bean homologs	Segregation ratio: Observed: Midas allele	G12873 allele	χ^2 (1:1)	Map location
PvCO	23	36	2.86	Not assigned
PvFLD	21	34	3.07	B11
PvFKF1	29	25	0.30	Not assigned
PvGI	18	30	3.00	B7
PvLFY	23	29	0.69	Not assigned
PvTFL1x	30	28	0.07	Not assigned
PvTFl1y	36	20	4.57*	B1
PvTFL1z	29	29	0	B7
PvZTL	23	33	1.79	Not assigned

* Significantly different from 1:1 at $P = 0.05$.

the determinate mutant (*det*) is caused by mutations in a homologue of the *Arabidopsis TFL1* gene. These mutations are synonymous or nonsynonymous substitutions at the junction between an exon and an intron resulting in splicing failure (Foucher et al. 2003).

In this study, we found that the map position of the *PvZTL* on B9 is similar to that of a GH locus in common bean. Because determinacy causes an early flowering, there is a positive correlation between earliness and PH. Tar'an et al. (2002) reported correlation of days to flowering and days to maturity to this locus. This raises the question whether the *PvZTL* locus could be a candidate gene for this locus.

With the exception of the *PvTFL1y*, *PvTFL1z*, and *PvZTL* sequences, we could not find any further sequences associated with a morphological trait associated in this study. In particular, we have not been able so far to identify a potential candidate gene for the *Ppd* locus. Therefore, of the 8 *Arabidopsis* sequences mapped, only 2 are currently associated with a mapped flowering-related gene in common bean. However, this study is based on segregation of photoperiod and GH traits that are present in only 3 study populations. It is possible that additional morphological traits will be identified that segregate in other populations and will cosegregate with homologous sequences mapped in this study. Also, the genetic mapping of potential candidate genes will provide a database for their potential role in photoperiod and reproductive transition pathways. Further isolation of mapping of flowering and GH genes is warranted to obtain a more complete picture of the genes involved in this important developmental stage of the bean plant.

In summary, our study was able to identify homologous sequences that either cosegregate with or map in the vicinity of known major genes controlling flowering. The case of the *PvTFL1y* homologue, which cosegregates with the *fin* gene is tantalizing given the similarity in phenotype action (if not the phenotype). However, the segregating population is small (n ~ 65). Thus, this cosegregation will have to be confirmed in a larger population if *PvTFL1y* is to be considered as a candidate gene for the *fin* locus. In addition, polymorphisms at this locus should correlate with the determinacy/indeterminacy phenotype. Ultimately, transformation and complementation tests will determine if *fin* is coded by *PvTFL1y*.

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