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# The common bean growth habit gene *PvTFL1y* is a functional homolog of *Arabidopsis TFL1*

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**Abstract** In a common bean plant exhibiting determinate growth, the terminal shoot meristem switches from a vegetative to reproductive state, resulting in a terminal inflorescence. Contrary to this, indeterminate growth habit results in a terminal meristem that remains vegetative where it further regulates the production of lateral vegetative and reproductive growth. In the last century, breeders have selected determinate growth habit, in combination with photoperiod insensitivity, to obtain varieties with a shorter flowering period, earlier maturation and ease of mechanized harvest. Previous work has identified *TFL1* as a gene controlling determinate growth habit in *Arabidopsis thaliana.* In this work, we have validated that the *Phaseolus vulgaris* candidate gene, *PvTFL1y,* is the functional homolog of *TFL1* using three independent lines of evidence. First, in a population of ~1,500 plants, *PvTFL1y* was found to co-segregate with the phenotypic locus for determinate growth habit (*fin*) on chromosome 01. Second, using quantitative PCR, we found that two unique haplotypes associated with determinacy at the *PvTFL1y* locus, a 4.1-kb retrotransposon and a splice-site mutation, cause mRNA abundance to decrease 20–133 fold, consistent with the recessive nature of *fin*. Finally, using a functional complementation approach, through *Agrobacterium*-mediated transformation of determinate *Arabidopsis*, we rescued *tfl1-1* mutants with the wild-type *PvTFL1y* gene. Together, these three lines of evidence lead to the conclusion that *PvTFL1y* is the functional homolog of the *Arabidopsis* gene, *TFL1*, and is the gene responsible for naturally occurring variation for determinacy in common bean. Further work exploring the different haplotypes at the *PvTFL1y* locus may lead to improved plant architecture and phenology of common bean cultivars.

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## Introduction

A notable difference between wild and domesticated common bean is the increased variability of growth habit types that exist in domesticated classes (Smartt 1976). In wild-type common bean, the growth habit is indeterminate and the plant will flower under short-day conditions. A plant exhibiting indeterminate growth habit will have a terminal shoot meristem that remains in a vegetative state throughout the production of vegetative and reproductive structures (Ojehomon and Morgan 1969). In domesticated bean plants, both indeterminate and determinate growth habits are observed. In a common bean plant showing a determinate growth habit, the terminal meristem will switch from a vegetative to a reproductive state, thus, producing a terminal meristem (Ojehomon and Morgan 1969). A determinate growth habit selected along with photoperiod insensitivity produces common bean cultivars that mature more rapidly and also have a shortened flowering period, allowing for a shorter period until harvest and tolerance of mechanical harvesting (Cober and Tanner 1995; Koinange et al. 1996). The naturally occurring, determinate growth habit in common bean is controlled by a single recessive allele, *fin* (Norton 1915; Koinange et al. 1996). Conversely, indeterminacy is a dominant trait. Identification

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of the locus underlying determinate growth will allow for a better understanding of the domestication process and will allow for further, and faster, manipulation of growth habit and flowering time in future breeding efforts of common bean.

Apical meristem identity has been studied in many higher plants and great progress has been made in understanding the molecular foundations that control the phase transition from vegetative to reproductive growth (Weigel 1995; Yanofsky 1995; Bradley et al. 1997; Ma 1998; Pidkowich et al. 1999). In *Arabidopsis*, two opposing pathways have been identified that control the floral meristem fate. *FLOWERING LOCUS T* (*FT*) interacts with *FLOWERING LOCUS D* (*FD*) to form a heterodimer that binds to the promoter of *APETALA1* (*AP1*) to activate flowering initiation (Abe et al. 2005; Wigge et al. 2005). Antagonistically, *TERMINAL FLOWER 1* (*TFL1*) acts as a repressor for floral initiation and maintains the inflorescence meristem through suppression of the expression of *AP1* and *LEAFY* (*LFY*) (Bradley et al. 1997; Ohshima et al. 1997; Nilsson et al. 1998; Boss et al. 2004). Although *FT* and *TFL1* share a great degree of sequence identity, key amino acids have been identified that appear to be responsible for the functional divergence that exists between the two proteins. Specifically, Tyr85His in *FT* and His88Tyr in *TFL1* were found to be important residues for maintaining opposing functionality (Hanzawa et al. 2005) as well as a 14-amino acid segment in exon four that confers antagonistic activity between TFL1 and FT (Ahn et al. 2006).

A QTL in common bean has been identified for growth habit on linkage group 1 (Koinange et al. 1996; Poncet et al. 2004). Within the growth habit QTL on linkage group 1, a candidate gene homologous to *TFL1*, *PvTFL1y* was recently identified and found to co-segregate (Kwak et al. 2008) with the phenotypic locus for determinate growth habit, *fin*. In this paper, we use three lines of evidence to support the hypothesis that *PvTFL1y* is the gene underlying the phenotypic determinate growth locus, *fin*, in common bean, *Phaseolus vulgaris*: (1) co-segregation of a large $F_2$ mapping population of the phenotypic locus and genotypic locus, (2) quantitative PCR studies showing a large decrease in mRNA transcripts (expression) of mutant *PvTFL1y* haplotypes as compared to wild-type, and (3) functional complementation, using *Agrobacterium*-mediated transformation, of the *Arabidopsis tfl1-1* mutant homolog with a wild-type *PvTFL1y* sequence.

## Materials and methods

### Sequences of PvTFL1y

#### *Plant materials*

The parental lines Midas and G12873 of a recombinant inbred population (MG) were used to identify the sequence and structure of *PvTFL1y*. The MG recombinant inbred population had been used to map domestication traits and homologous fragments for flowering time and determinacy genes (Koinange et al. 1996; Kwak et al. 2008). G12873 is a wild Mesoamerican accession (indeterminate growth habit) and Midas is a domesticated Andean cultivar (wax bean with a determinate growth habit).

#### *Primer walking for PvTFL1y*

Using the sequences obtained with degenerate primers (Kwak et al. 2008), the BD GenomeWalker™ Universal Kit (BD Biosciences Clontech, Mountain View, CA, USA) was used to extend sequences of *PvTFL1y* using the method recommended by the manufacturer. The genomic libraries of G12873 and Midas were constructed. The fragments obtained were cloned using the TOPO TA Cloning Kit for sequencing (Invitrogen, Carlsbad, CA, USA). Transformation and analysis followed manufacturer's instructions. Sequencing of the inserted fragments was performed with SP6 and T7 universal primers at the sequencing facility of the Division of Biological Sciences at UC Davis. To fill in unsequenced gaps of large inserts, sequencing primers were designed according to sequence availability. Primer walking was continued until the completed sequence of the cloned inserted was obtained (Kwak 2008). Using the primers developed as described, sequences of *PvTFL1y* were obtained for accessions G12873, Midas, BAT93, G00750 and California Dark Red Kidney (CDRK). The gene structure and putative translation product were determined using GENESCAN (http://genes.mit.edu/GENSCAN.html). The protein sequence similarity was determined using a Protein BLAST search at NCBI.

### Co-segregation

#### *Plant materials*

A large $F_2$ population ($n = 1{,}472$) resulting from the cross between G12873 and Midas was generated to confirm the co-segregation of the genotypic *PvTFL1y* and phenotypic *fin* loci. $F_2$ plants were grown in individual pots in the greenhouse; young leaf samples were collected after 2 weeks and frozen before genomic DNA extraction. The determinacy of $F_2$ plants was recorded periodically until plants reached full maturity at stage R8 (pod filling) (Gepts 1987; van Schoonhoven and Pastor-Corrales 1987).

#### *DNA extraction and genotyping of an $F_2$ population*

Genomic DNA from plant tissue samples was extracted using a modified high-throughput CTAB method with stainless steel beads (Chen and Ronald 1999). The 6-bp indel 3′ downstream of the wild-type *PvTFL1y* sequence in

G12873 was used to genotype $F_2$ plants (Fig. 1a). PCR mixtures contained approximately 30 ng of total genomic DNA, 200 mM of dNTP, 0.2 µM of PvTFL1y-F12 and PvTFL1y-R11 primers (Table 1), standard Taq buffer with 1.5 mM $MgCl_2$, and 1 unit of *Taq* polymerase (New England Biolabs, Ipswich, MA, USA) in a total reaction volume of 20 µl. The PCR cycle consisted of 2 min at 94°C and 35 cycles of 30 s at 94°C, 1 min at 57°C and then 30 s at 72°C followed by 3-min extension at 72°C with PTC-220 thermocycler (MJ research, Ramsey, MN, USA). To genotype the segregating diagnostic amplicon (172 bp in Midas and 166 bp in G12873) in the $F_2$, 8% non-denaturing polyacrylamide gels (8% 29:1 acrylamide: bis-acrylamide gels in 0.5× TBE buffer) were prepared using a Mega-Gel Dual High-Throughput Vertical equipment (C.B.S. Scientific, Del Mar, CA, USA). The fragments were then visualized using a Kodak Gel Logic 100 system.

## Real-time PCR

### *Plant material*

The *P. vulgaris* accessions BAT93, CDRK, and G00750 were used in this study. BAT93 was selected as an indeterminate control because it is one of the parents in the core mapping population of common bean and is a standard genotype for genomic studies (Freyre et al. 1998; Gepts et al. 2008). CDRK is a determinate accession of Andean origin and G00750 is a determinate accession of Mesoamerican origin. Tissue from the apical bud was taken at growth stages V1, V3, V4, R5, R7, and R8 (for the BAT93 and CDRK comparison) and V1, V4 and R7 (for the BAT93 and G00750 comparison) (V1 = Emergence, V3 = primary leaves, V4 = third trifoliolate leaf, R5 = preflowering, R7 = pod formation, and R8 = pod filling) (Gepts 1987; van Schoonhoven and Pastor-Corrales 1987).

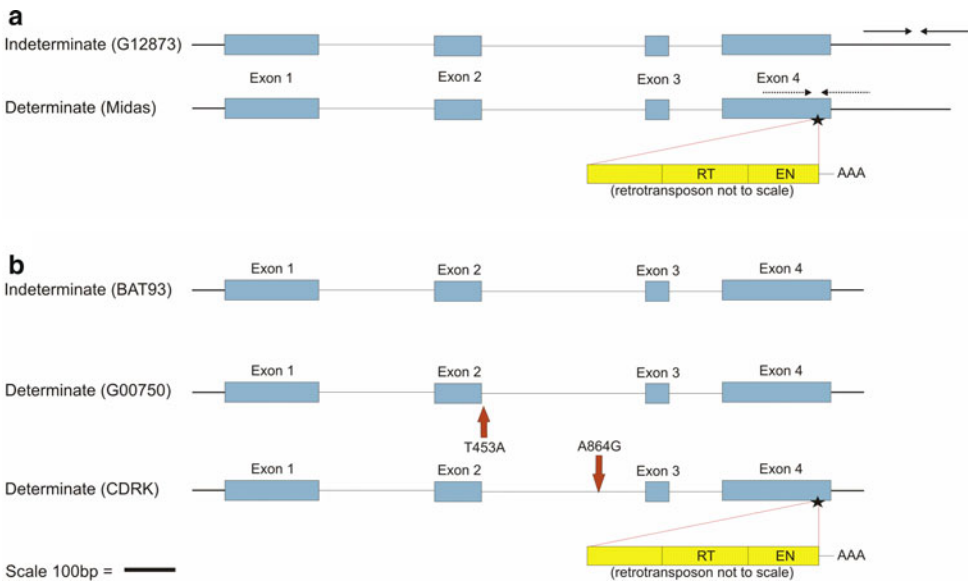


**Fig. 1** Coding sequence (CDS) of determinate and indeterminate *PvTFL1y* haplotypes in common bean. **a** Haplotypes used in the co-segregation study. *Solid line arrows* used for genotyping a 6-bp indel in the $F_2$ population. *Dashed line arrows* used for genotyping the 4.1 kb retrotransposon. **b** Haplotypes used in the quantitative PCR and transformation studies. *Arrows* indicate single base pair substitutions. *RT* reverse transcriptase domain and *EN* endonuclease domain

**Table 1** Primers used in this study

| Role | Primer | Sequence |
|---|---|---|
| $F_2$ genotyping | PvTFL1y-F12 Forward | 5′CAAACCAACAGTAAAAACCAG |
| | PvTFL1y-R11 Reverse | 5′TTGTAGGATCGTTGTGTCG |
| qPCR | PvTFL1y2a Forward | 5′GATGTTCCAGGCCCTAGTGA |
| | PvTFL1y4b Reverse | 5′CGAAATTGCGTGTGTTGAAG |
| | Actin2 Forward | 5′GAGGCACCGCTTAATCCTAA |
| | Actin2 Reverse | 5′TACGGCCACTAGCGTAAAGG |
| Cloning | PvTFL1y Forward | 5′GCGGAATTCATGGCAAGAATGCCTTTAGAA |
| | PvTFL1y Reverse | 5′CGCCTCGAGCTAGCGTCTTCTTGCAGCTGT |
| | TFL1 Forward | 5′GCCATTGATAATGGGGAGAG |
| | TFL1 Reverse | 5′CGGATTCAACTCATCCTTTGG |

*RNA extraction and expression analysis*

RNA was isolated from apical bud tissue at the V1, V3, V4, R5, R7, and R8 stages using the CARTAGEN Total RNA Isolation kit (Seattle, Washington, USA). cDNA was isolated using Invitrogen SuperScript® III First-Strand Synthesis SuperMix with random hexamers (Carlsbad, California, USA). Real-time PCR experiments were performed using intron spanning gene-specific primers, PvTFL1y 2a Forward and PvTFL1y 4b Reverse (Table 1), in a total volume of 20 µL with 5 µL of the RT reactions, 1 µM gene-specific forward and reverse primer, 4 µL distilled water and 10 µL SYBR Green Master mix (Applied Biosystems, Carlsbad, CA, USA) on an ABI 7900 real-time PCR machine (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. The common bean actin gene (GenBank no. EU369188.1) was used as an internal control (primers shown in Table 1). Gene expression data, from two biological replicates and three technical replicates, were processed and standardized according to the comparative Ct method where $\Delta\ C_t = C_t$ (target) $- C_t$ (control) and the comparative expression level is equal to $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001).

Transformation

*Plant material*

The *P. vulgaris* accession BAT93 (indeterminate) and the *A. thaliana* accessions CS39005 (indeterminate; *TFL1*), and CS6167 (determinate; *tfl1-1*) were used in this study. *A. thaliana* accessions were obtained from the Arabidopsis Biological Resource Center (ABRC). *P. vulgaris* was grown in a greenhouse with natural day length and an average temperature of 24°C. *Arabidopsis* was grown in a growth chamber at 24°C under the condition of 16 h of 120 µE $m^{-2}$ $s^{-1}$ light and 8 h of darkness. *Arabidopsis* was chosen for the transformation species because the *PvTFL1y* gene was initially identified due to its high sequence homology to *TFL1*, the gene found to control determinate growth in *Arabidopsis* (Kwak et al. 2008). Additionally, many studies have shown that *P. vulgaris* has lower transformation efficiency than *Arabidopsis* (Zambre et al. 2005; Bonfim et al. 2007) and a similar study in *Glycine max* had success using an *Arabidopsis* transformation system (Tian et al. 2010).

*RNA isolation and PCR amplification*

RNA was isolated from BAT93 using the CARTAGEN Total RNA Isolation kit. cDNA was isolated using Invitrogen SuperScript® III First-Strand Synthesis SuperMix with gene-specific primers for *PvTFL1y* (PvTFL1y Forward and PvTFL1y Reverse) (Table 1).

*Plant phenotyping*

Days until flowering (DUF) in *Arabidopsis* was measured as the number of days between germination and the opening of the first flower, also referred to as Stage 6 in *Arabidopsis* development (Boyes et al. 2001). Plant height was recorded after the plant had reached its maximum height and fruits began to shatter (Stage 8). Plants were phenotyped for indeterminate or determinate growth habit after the onset of flowering (Stage 6.9).

*Plasmid construction and transformation*

The full length coding sequence, amplified from indeterminate common bean cultivar BAT93, was inserted into cloning vector pBSSK (Stratagene, Santa Clara, CA, USA). The PCR cycle consisted of 3 min at 95°C and 35 cycles of 30 s at 95°C, 1 min at 55°C and then 1 min at 72°C followed by 5-min extension at 72°C with C1000 thermocycler (Bio-Rad, Hercules, CA, USA). Next, the construct was sub-cloned into a plant expression binary, pB5, which is a modified pBIN19 (Clontech, Mountain View, CA, USA) derivative with a double CaMV 35S promoter and TMV omega translational enhancer for strong constitutive expression in plants. Subsequently, the construct was sub-cloned into *Agrobacterium* strain GV3101, Rif (chromosomal), Gent and Kan (Ti plasmid), and then introduced into *Arabidopsis tfl1-1* and *TFL1* plants by the floral dip procedure (Zhang et al. 2006). The presence of the *PvTFL1y* construct was confirmed by sequencing the PCR fragment obtained after amplification with primers PvTFL1y Forward and PvTFL1y Reverse (Table 1). Additionally, presence or absence of the *Arabidopsis tfl1-1* allele, characterized by a single nucleotide substitution G to A at the 5′ end of the fourth exon leading to a missense mutation Gly to Asp at residue 105, was confirmed by PCR (primers in Table 1) and sequencing. The PCR cycle consisted of 3 min at 95°C and 35 cycles of 30 s at 95°C, 1 min at 55°C and then 1 min at 72°C followed by 5-min extension at 72°C with C1000 thermocycler (Bio-Rad, Hercules, CA, USA).

## Results

Gene structure of PvTFLly

Full length *PvTFL1y* genomic sequences were obtained for G12873 (GenBank JN418231) and Midas (GenBank JN418250). The G12873 coding sequence contained four exons and translated into 173 amino acids (Fig. 1a). Within the Phaseoleae tribe, *G. max* and *P. vulgaris* shared 79% nucleotide similarity. The derived *PvTFL1y* protein

sequence had 95% similarity with *Dt1* (*Glyma19g37890*-http://phytozome.net/soybean.php) in *G. max* (Tian et al. 2010), 87% with *Det* in *P. sativum* (Foucher et al. 2003), and 75% with *TFL1* of *Arabidopsis*. In contrast, the *PvTFL1y* coding sequence from the accession Midas contained a 4,170-bp fragment inserted in the fourth exon (Fig. 1a). Using a BLAST search, the inserted sequence in Midas was identified as a non-LTR retrotransposon (rvt1) in the endonuclease/exonuclease/phosphatase family (*e* value $1.7e^{-53}$). Full length coding sequence was also obtained for BAT93 (GenBank JN418230), G00750 (GenBank JN418223), and CDRK (GenBank JN418248). BAT93 translated into the same 173 amino acid sequence as G12873. Similar to Midas, CDRK also had the 4.1 kb retrotransposon insertion in exon four (Fig. 1b). The accession G00750 had a T453A mutation at the end of exon two that is located in a putative splice site (Fig. 1b).

### Co-segregation

The *PvTFL1y* fragment containing the 6-bp indel 3′ downstream of the *PvTFL1y* sequence co-segregated with the *fin* locus (coding for the determinacy phenotype) in the MG recombinant inbred population ($n = 58$), suggesting that *PvTFL1y* is a candidate gene for determinacy (Kwak et al. 2008). Because the size of this population was small, we verified this finding with a larger $F_2$ population from the same cross. Among 1,472 $F_2$ plants, 1,049 plants were indeterminate and 423 plants were determinate. A goodness-of-fit test for a 3 indeterminate: 1 determinate segregation ration resulted in a $\chi^2$ value of 10.96 (1 *df*; $P = 0.001$). All determinate plants were homozygous for the $PvTFL1y^{Midas}$ haplotype. Among indeterminate $F_2$ plants, 657 were heterozygous for the $PvTFL1y^{Midas}$ and $PvTFL1y^{G12873}$ haplotypes and 392 were homozygous for the $PvTFL1y^{G12873}$ haplotype. A goodness-of-fit test for a 2 indeterminate (heterozygous for $PvTFL1y^{G12873/Midas}$):1 indeterminate (homozygous for the $PvTFL1y^{G12873}$) gave a $\chi^2$ value of 7.68 (1 *df*; $P = 0.01$). Overall, the goodness-of-fit test for a 1 ($PvTFL1y^{Midas}$):1 ($PvTFL1y^{G12873}$) ratio in this generation gave a $\chi^2$ value of 0.70 (1 *df*; $P = 0.6$). Although the two *PvTFL1y* alleles appear to have been transmitted as expected (1:1 ratio), there was either an excess of homozygous determinate or heterozygotes in the region marked by the *fin* locus. The original recombinant inbred population of the Midas × G12873 cross also exhibited an excess of homozygous determinate types (Koinange et al. 1996).

### Real-time PCR

Transcription level of three *PvTFL1y* haplotypes [$PvTFL1y^{BAT93}$ (indeterminate), $PvTFL1y^{G00750}$ (determinate) and $PvTFL1y^{CDRK}$ (determinate)] was monitored by quantitative real-time PCR. A 32–133 fold decrease in expression was observed between the $PvTFL1y^{BAT93}$ (indeterminate, wild-type) haplotype and the determinate, mutant $PvTFL1y^{CDRK}$ haplotype (Fig. 2a) and a 20–91 fold reduction in expression was found between the $PvTFL1y^{BAT93}$ indeterminate haplotype and the determinate $PvTFL1y^{G00750}$ haplotype (Fig. 2b). The wild-type $PvTFL1y^{BAT93}$ haplotype had the highest mRNA levels (lowest $C_t$ levels) between stages V4 and R5, which corresponds with the onset of flowering (Gepts 1987). The $PvTFL1y^{CDRK}$ and $PvTFL1y^{G00750}$ haplotypes had extremely low mRNA levels throughout development with $C_t$ values ranging from 32 to 39 (Fig. 2c, d).

### Transformation

In order to determine if the function of wild-type *PvTFL1y* complements the function of *TFL1* in *Arabidopsis*, we transformed the BAT93 (indeterminate) *PvTFL1y* full length cDNA, excluding UTRs, into the determinate *Arabidopsis* mutant *tfl1-1*. Additionally, the *PvTFL1y* full length cDNA was transformed into wild-type *Arabidopsis* (*TFL1*), as a negative control, to verify that the transgene does not alter the wild-type phenotype. Transformed *tfl1-1* and *TFL1* plants are shown alongside wild-type *tfl1-1* and *TFL1* plants at 20 days after planting (DAP) and 40 DAP in Fig. 3a and b. Nineteen transgenic *tfl1-1* and 34 transgenic *TFL1* lines were produced. Seventeen of the 19 transformed *tfl1-1* mutants had fully restored indeterminate growth and all 34 *TFL1* wild-type plants remained indeterminate. The conversion of the mutant to wild-type phenotype corroborates that the *PvTFL1y* transgene fully complements the function of *TFL1* in *Arabidopsis*. The presence of the *PvTFL1y* transgene was confirmed by PCR amplification and sequencing. All transformed plants were correspondingly genotyped for the presence of the *Arabidopsis tfl1-1* (A) or *TFL1* (G) allele. Nontransformed *tfl1-1* plants were much shorter in stature (average = 11 cm) and flowered earlier (average = 21 days) compared to nontransformed *TFL1* (average 45 cm and 28 days), transformed *tfl1-1*(average 52 cm and 46 days), and transformed *TFL1* (average 55 cm and 38 days) plants. Furthermore, transformed plants had much larger ranges of DUF and plant height (Fig. 4).

## Discussion

In this paper, we present three compelling arguments that *PvTFL1y* is indeed the gene underlying the determinacy phenotype observed among non-artificially mutagenized domesticated beans. A second locus, *PvTFL1z,* was mapped on LG 7 by Kolkman and Kelly (2003) and Kwak et al.

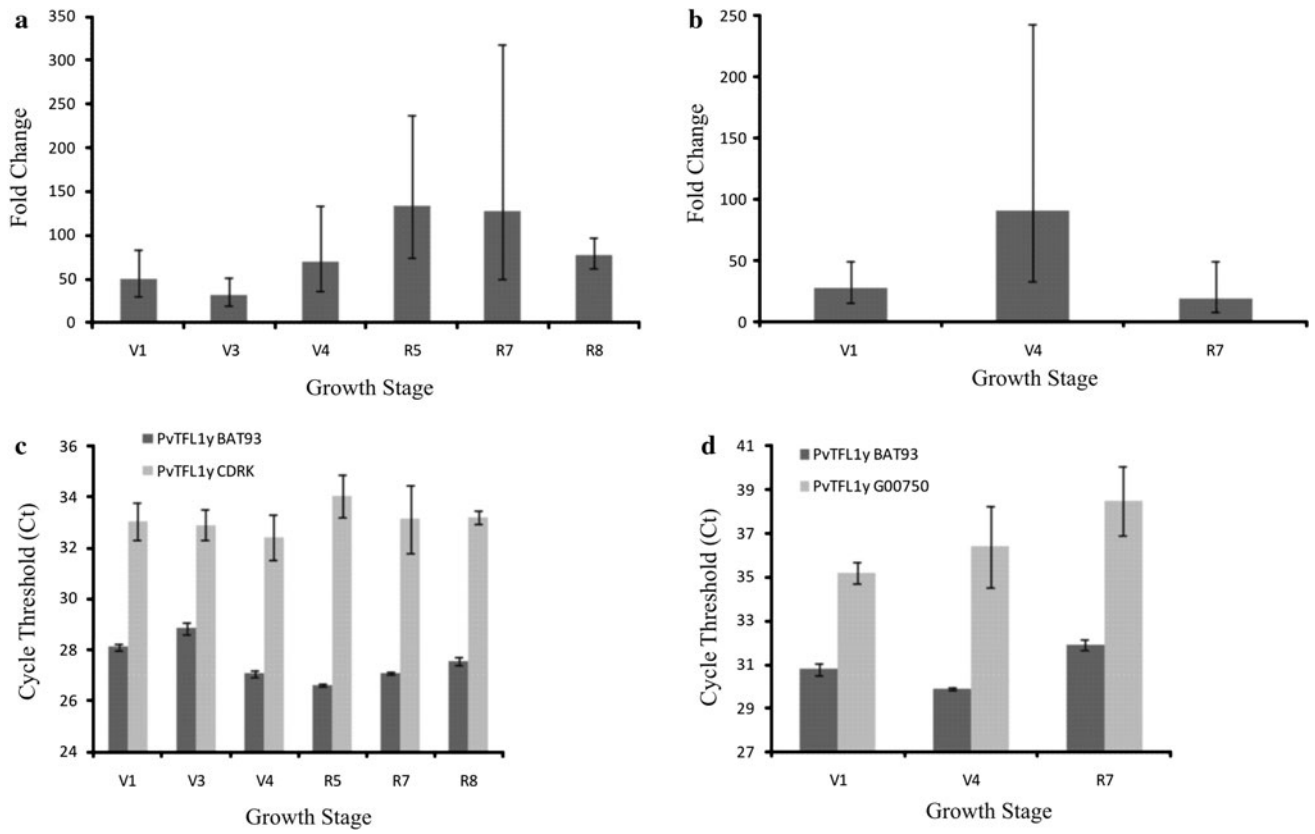


**Fig. 2** **a** Fold change in expression ($2^{-\Delta\Delta C_t}$ of the CDRK haplotype as compared to the BAT93 haplotype at six different developmental stages. **b** Fold change in expression ($2^{-\Delta\Delta C_t}$) of the G00750 haplotype as compared to the BAT93 haplotype at three different developmental stages. *Error bars* represent $1\sigma$ confidence intervals derived from experimental standard deviations. **c** Direct comparison of $C_t$ values between CDRK and BAT93 at six developmental stages. **d** Direct comparison of $C_t$ values between G00750 and BAT93 at three developmental stages. *Error bars* represent experimental standard deviations

(2008) and is likely responsible for determinacy observed in Michigan navy bean cultivars, which arose from an artificial mutagenesis program in the 1950s (Kelly 2001). However, this artificially mutagenized locus is not being addressed in the research presented here. Our evidence includes co-segregation in a large $F_2$ population between *PvTFL1y* and *fin* (the phenotypic locus for determinacy), demonstration of the sharp reduction in expression of the *PvTFL1y* locus in selected determinate haplotypes, and rescue of the *tfl1-1* phenotype in *Arabidopsis* with the *PvTFL1y* dominant allele associated with indeterminacy.

## Co-segregation between *PvTFL1y* and *fin*

Genetic mapping with large populations is one of the primary ways of identifying genomic regions underlying phenotypes of interest. We found a 6-bp indel linked to the *PvTFL1y* locus which co-segregated in a large $F_2$ mapping population with the phenotypic locus *fin*. While co-segregation does not definitively identify a causal gene, it is very useful in narrowing down a large number of genomic regions, or genes, to several high confidence regions or candidate genes. This restriction can be beneficial when allocating future resources toward discovery of genes underlying important economic traits.

## Expression level variation at the *PvTFL1y* locus

Two unique and contrasting mutant *PvTFL1y* haplotypes, in CDRK and G00750, respectively, were assessed to see if they caused a reduction of overall mRNA expression, as expected from a recessive mutation. The CDRK haplotype, of Andean origin, has a 4.1-kb retrotransposon inserted in the 4th exon of *PvTFL1y* and represents the most common mutant haplotype associated with determinacy, accounting for 72% of all determinate accessions analyzed (M. Kwak and P. Gepts, in preparation). The G00750 haplotype, of Mesoamerican origin, is a rare, putative splice-site-failure mutation that is located at the end of the second exon. These haplotypes were chosen to represent both common bean gene pools and investigate if rather different mutant *PvTFL1y* haplotypes lead to various expression patterns. In the study, both mutant haplotypes were found to cause a drastic reduction, 20–133 fold, of mRNA expression at the

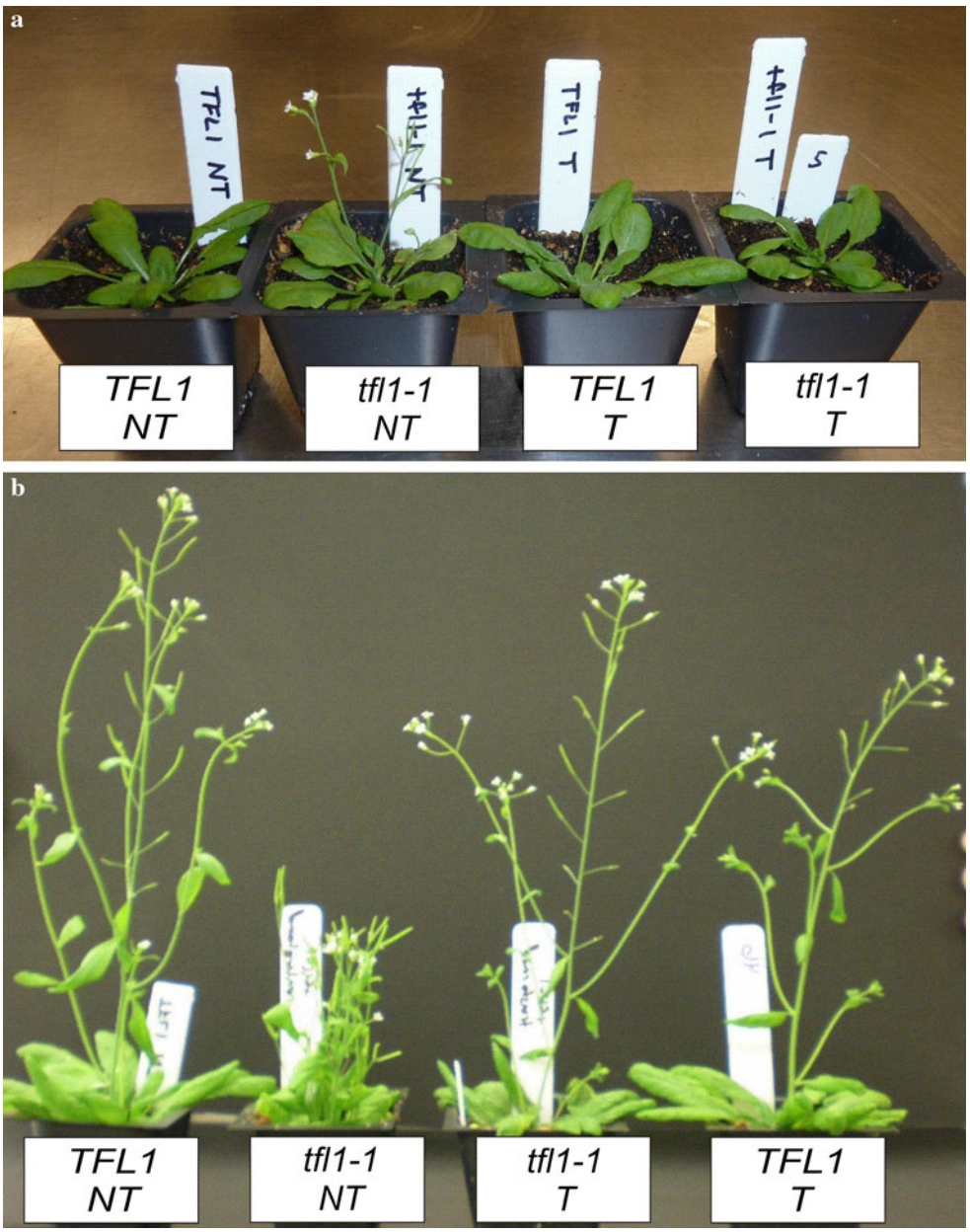


**Fig. 3** **a** Transformed (*T*) and nontransformed (*NT*) *TFL1* (indeterminate) and *tfl1-1* (determinate) plants 20 days after planting. **b** Transformed (*T*) and nontransformed (*NT*) *TFL1* (indeterminate) and *tfl1-1* (determinate) plants 40 days after planting

*PvTFL1y* locus. This finding aligns well with the recessive inheritance of determinate growth habit in that it likely is caused by mutations leading to a drastic reduction or complete loss-of-function.

The qPCR findings also suggest that expression of *PvTFL1y* in wild-type plants is heightened at the V4 and R5 stages, which corresponds with the onset and continuation of flowering. Elevated expression levels at the onset of flowering have also been observed in pea, lotus, and soybean *TFL1* homologs (Foucher et al. 2003; Guo et al. 2006; Tian et al. 2010). This is not surprising since *Arabidopsis TFL1* has been identified to play a large role in the inflorescence meristem development pathway by antagonizing the activity of the floral promoter *FT* (Shannon and Meeks-Wagner 1991; Alvarez et al. 1992; Bradley et al. 1997; Hanzawa et al. 2005; Ahn et al. 2006).

## Transgene complementation

Lastly, it has been shown in this study that the common bean *PvTFL1y* gene is a functional homolog of the *Arabidopsis TFL1* gene through complementation. When *PvTFL1y* was introduced into *tfl1-1 Arabidopsis* mutants, it restored the wild-type indeterminate phenotype in 17 of the

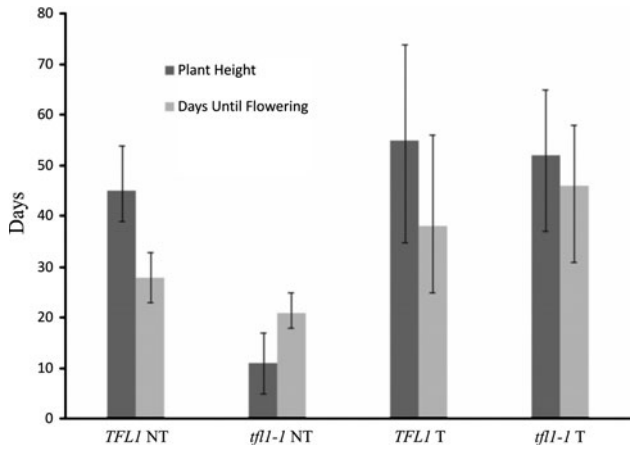


**Fig. 4** Average number of days until flowering and average plant height observations for transformed (*T*) and nontransformed (*NT*), *TFL1* (indeterminate) and *tfl1-1* (determinate) plants. *Error bars* represent range of days and heights observed

19 transgenic lines. Two lines, which were genotyped as positive for the *PvTFL1y* transgene, remained determinate. A plausible explanation could be the random nature of transgene insertion; the transgene could insert into heterochromatic or highly methylated regions leading to gene silencing or low expression (Pröls and Meyer 1992; Nocarova and Fischer 2009). Supporting this hypothesis is the observation that transformed plants showed more phenotypic variability in plant height and DUF than nontransformed ones, which could again be attributed to random insertion into regions of the host genome with variable expression. Additionally, the overall lateness of DUF could be caused by the constitutive promoter driving the *PvTFL1y* transgene expression (Guo et al. 2006).

In this paper, we demonstrated the utility of comparative genomics in the identification of novel agronomic genes. *PvTFL1y* was identified as a candidate gene for determinate growth habit based on its homology with the *TFL1* gene in *Arabidopsis*. There are now several genes for determinate growth habit within the legume family, *PvTFL1y*, *GmTFL1*, *LjCen1* and *PsTFL1b/c* that have been shown to be functional homologs of *TFL1* (Foucher et al. 2003; Guo et al. 2006; Tian et al. 2010). These genes will likely be instrumental in identifying further determinacy candidates in crop legumes such as *P. coccineus* (runner bean), *P. lunatus* (lima bean), and *Cajanus cajan* (pigeon pea), all of which have varieties bred for determinate growth habit (Huyghe 1998; van Rheenen et al. 1994; Waldia and Singh 1987). Additionally, more information needs to be obtained about the effect of the different *PvTFL1y* haplotypes and their correlated effects on other plant characteristics, such as photoperiod sensitivity, plant height or internode length. *fin* is tightly linked to the photoperiod gene, *ppd*, on chromosome 01(Kelly 2001). Exploring the interaction and linkage of these two loci may expand the geographic locations in which novel adaptation traits can be evaluated. This information can be obtained through backcrossing the different *PvTFL1y* haplotypes into a common genetic background or by association analysis in a sample with known flowering phenotypes. Finally, knowing the molecular basis for determinate growth habit will facilitate the establishment of different breeding strategies, involving determinacy in particular, for the different gene pools of common bean.

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