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ORIGINAL PAPER

# Detection of QTLs for flowering date in three mapping populations of the model legume species *Medicago truncatula*

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**Abstract** Adaptation to the environment and reproduction are dependent on the date of flowering in the season. The objectives of this paper were to evaluate the effect of photoperiod on flowering date of the model species for legume crops, *Medicago truncatula* and to describe genetic architecture of this trait in multiple mapping populations. The effect of photoperiod (12 and 18 h) was analysed on eight lines. Quantitative variation in three recombinant inbred lines (RILs) populations involving four parental lines was evaluated, and QTL detection was carried out. Flowering occurred earlier in long than in short photoperiods. Modelling the rate of progression to flowering with temperature and photoperiod gave high $R^2$, with line-specific parameters that indicated differential responses of the lines to both photoperiod and temperature. QTL detection showed a QTL on chromosome 7 that was common to all populations and seasons. Taking advantage of the multiple mapping populations, it was condensed into a single QTL with a support interval of only 0.9 cM. In a bioanalysis, six candidate genes were identified in this interval. This design also indicated other genomic regions that were involved in flowering date variation more specifically in one population or one season. The analysis on three different mapping populations detected more QTLs than on a single population, revealed more alleles and gave a more precise position of the QTLs that were common to several populations and/or seasons. Identification of candidate genes was a result of integration of QTL analysis and genomics in *M. truncatula*.

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## Introduction

The model plant *Medicago truncatula* is close, from a phylogenetic point of view, to most fabaceous (legume) species cultivated in temperate regions (pea, alfalfa, clover, fababean). It is an annual, diploid and autogamous species, with a short cycle, and a genome size of approximately 0.55 pg/1C (Blondon et al. 1994). The currently available genetic and genomic resources (recombinant lines, banks of BACs, EST/cDNA, genetic and physical maps, mutants, genetic resources, sequences…) (Choi et al. 2004a) offer prospects to study this model species before investigating genetic bases of traits on the cultivated legume species (Bell et al. 2001) which often have complex genomes. There is a strong degree of synteny between the genome of *M. truncatula* and those of tetraploid alfalfa (Julier et al. 2003) and pea (Choi et al. 2004b; Aubert et al. 2006).

Aerial morphogenesis, which includes characters of growth, development and phenology, contributes to the ability of plants to withstand competition in canopies. For perennial species, it determines survival during cycles of regrowth and defoliation. For annual species, transition

from vegetative growth to reproductive growth ensures successful reproduction and permits a possible adaptation to the surrounding environments (Yoo et al. 2004). This developmental event is regulated by a complex pathway that responds to various signals including light, temperature, nutrient availability and developmental stage (Levy and Dean 1998). Depending on abiotic and biotic conditions, wild plant species evolved towards different strategies (Roux et al. 2006). An early flowering plant is adapted to a short or unpredictable growing season, whereas a late flowering plant is better suited to a long vegetative growth period. In breeding programs on cultivated species, flowering date is finely tuned to better fit the constraints of the environment.

The flowering process has been analysed in the model plant *Arabidopsis thaliana*. Four major pathways were identified using genetic and molecular dissections of flowering date mutants in *A. thaliana*: the photoperiod, the autonomous, the gibberelin and the vernalization pathways (Komeda 2004).

*Medicago truncatula* originates from the Mediterranean Basin (Ellwood et al. 2006). Under Mediterranean climates, vegetative growth predominantly occurs during late autumn, winter and early spring, under cool temperatures and short photoperiods. Flowering occurs in spring and seeds mature in late spring or early summer. Then the plant dies, and seeds, highly dormant, remain in the soil. In annual legumes (*Trifolium subterraneum*, *M. truncatula*, *M. scutella*, *M. polymorpha*, *M. littoralis*, *M. tornata*, *M. rugosa*), a long vernalization, a high temperature after vernalization and a long photoperiod were all shown to reduce the time of appearance of the first flower on the whole plant (Aitken 1955; Clarkson and Russell 1975). Recently, a model was developed to describe and analyse the response of *M. truncatula* flowering date to temperature, duration of vernalization, and photoperiod (Moreau et al. 2007).

Currently, a core-collection based on natural accessions of *M. truncatula* (Ronfort et al. 2006) is available for phenotypic characterization or for genetic analyses. Large genetic variation in flowering date was described (Delalande et al. 2004; Julier et al. 2007). Accessions from the Southern latitudes of the Northern hemisphere had an early flowering date, but accessions from Northern latitudes, had either late or early flowering date (Julier et al. 2007). Genetic control of flowering date investigated in a recombinant inbred lines (RILs) mapping population revealed a strong QTL on chromosome 7 (Julier et al. 2007). Other QTLs for flowering date were detected on other chromosomes in this population, depending on the experiments.

The integration of information from genomics and QTL analysis is a challenge, in order to identify sequences controlling the variation of important traits. The first objective of the present study was to explore genetic variation of the flowering date in response to two photoperiod durations. The data will be used to model flowering date as a function of temperature and photoperiod. The second objective was to evaluate the genetic variation for flowering date, in three RILs mapping populations of *M. truncatula*, the three populations being connected, and to locate QTLs. Colocation of QTLs across the populations and population-specific QTLs will be discussed. The benefit provided by the simultaneous study of three mapping populations on the number of detected QTLs and the precision of their position will be analysed.

## Materials and methods

### Materials and experiments

Two experiments were conducted at Lusignan (France) in 2005. The first one aimed at studying effects of photoperiod duration, genotype and their interaction on flowering date of *M. truncatula*. Eight lines from different origins were chosen: DZA315.16 (Thoquet et al. 2002) and DZA45.6 (Algeria), F83005.5 (France, Torregrosa et al. 2004) and TN6.18 (Tunisia, Aydi et al. 2004), Meiron and Levahim-B (Israel), Jemalong6 and A20 (Australia, T.Huguet, unpublished data). DZA315.16, DZA45.6, F83005.5 and Jemalong6 were provided by the Biological Resources Center of INRA Montpellier in France ( http://www.montpellier.inra.fr/BRC-MTR/), Meiron and Levahim-B were kindly provided by A. Samach, University of Jerusalem, Israel. Seeds were manually scarified and sown in Petri dishes on the 12 October 2005. After 24 h of imbibition at room temperature, they were transferred at 4°C for 7 days. Germinated seeds were transplanted into individual pots on the 20 October 2005 in two growth chambers. Experimental design included two photoperiod durations (12 and 18 h) and was arranged as a randomized block design with six replicates in each growth chamber (i.e. under each photoperiod), each replicate being composed of one plant. Pots were filled with 70% of a mixture of peat and clay, 20% of compost and 10% of sand. The differences in light energy between the treatments were minimized by using metal halid lamps for 12 h in both conditions and glow-lamps that produced little energy during six more hours under long-day treatment. Plants under short (12 h) photoperiod received 19,085 mmol/m$^2$/day of photon flux while plants under long (12 + 6 h) photoperiod received 19,177 mmol/m$^2$/day, i.e. differences among treatments in supplied energy was less than 0.5%. Temperatures were similar in both growth chambers: 20°C (light period) and 17.9°C (dark period) for short

photoperiod, 19.1°C (light period) and 18°C (dark period) for long photoperiod, leading to a similar mean temperature. Plants received NPK supplies. Flowering date was scored when a plant had one open flower on a primary branch. Along the flowering period, flowering date was scored three times a week. The flowering date was transformed into a sum of degree-days (°C.D) with temperature threshold of 0°C from transplantation into pots. This transformation makes it possible to compare the results of this experiment to those of the second one that was carried out in greenhouse conditions. An analysis of variance was carried out to test effects of line, photoperiod and interaction between line and photoperiod on flowering date (SAS Institute Inc. 2000). Contribution of each line to line × photoperiod interaction was measured by ecovalence criterion (Wricke and Weber 1986).

The second experiment was conducted in greenhouse at Lusignan (France) in spring and autumn 2005, aiming at a QTL detection for flowering date. Two RILs populations of *M. truncatula* were used: LR1 (196 RILs in F5 generation) and LR5 (173 RILs in F7 generation). They were obtained, respectively, from the crosses DZA315.26 × DZA45.6, and Jemalong6 × F83005.5. Another RILs population LR4 (199 RILs in F7 generation), from the cross Jemalong6 × DZA315.16 was studied in 2000, 2002, 2003 and 2004 (see Julier et al. 2007). The two lines DZA315.16 and DZA315.26 are supposed to be identical, as they were extracted from the same population and seem phenotypically identical, especially for flowering date (J.M. Prosperi, pers. comm.). Framework maps made with SSR markers were available for populations LR1 and LR5 (T. Huguet, unpublished) and LR4 (Julier et al. 2007). They included 91, 84 and 133 markers, and covered 562.3, 551.5 and 608.4 cM, respectively. In addition to the seven framework markers used for the LR4 map on chromosome 7 (Julier et al. 2007), 19 new markers were added between positions 53 and 65 cM (Table S1, Electronic supplementary material). Among these, 16 were SSR markers and three were marker genes (Constans, ABA and Sucrose synthase or mtic686). PCR was performed in a 25 µl volume containing 50 ng of genomic DNA, 0.2 µM of each primer, 200 µM dNTP, 1X of buffer 10X, 2 mM $MgCl_2$, and 0.625 unit of Taq polymerase. An MJ Research PTC-100TM was used for amplification with the following PCR profile: 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, optimal temperature melting for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min. PCR products were analyzed in 2% agarose gel in Tris-borate EDTA buffer. Experimental design for population LR4 was already described (Julier et al. 2007). For LR1 and LR5 there was no repetition except for 15 lines randomly taken per population and the parental lines that were repeated three times. The same vernalization treatment as described above was used. The flowering date was individually recorded and transformed into degree-days from the date of transfer into greenhouse after vernalization. For the spring experiment, the mean daily temperatures varied between 15.5 and 35.8°C. The photoperiod was 13 h57 at the beginning of the experiment (04 April) and 17h03 at the end (13 June). In the autumn, the mean daily temperatures varied between 12.7 and 20.3°C. The photoperiod was 13h21 at the beginning of the experiment (20 September) and 9h45 at the end (20 December). Additional light was provided to reach a 16-h photoperiod from 4th October to the end of the experiment.

## Modelling

Flowering date was modelled using a linear, multiple regression model (Summerfield et al. 1985; Erskine et al. 1990). The development rate to flowering ($1/f$, with $f$ the flowering date in days) was described as a function of mean temperature ($T$ in °C) and mean photoperiod ($P$ in h) :

$$1/f = a + bT + cP$$

with $a$, $b$ and $c$ genotype-specific constants. The model was applied to each of the four parental lines of the mapping populations, using the data collected in the photoperiod experiment and the autumn and spring 2005 experiments on the RILs. The PROC REG of SAS was used to estimate the parameters $a$, $b$ and $c$ of the model using FREQ option to indicate the frequency of each observation.

## Statistical and QTL analysis

Analyses of variance were carried out to test the effects of lines, seasons and lines × seasons on flowering date for the parents, and for the 15 lines per population that were repeated three times. SAS was used for all analyses (SAS Institute Inc. 2000). The PROC VARCOMP of SAS was used to estimate line $\left(\sigma_L^2\right)$ and error $\left(\sigma_R^2\right)$ variances. Broad sense heritabilities that represent the part of genetic variance in the total phenotypic variance were calculated using the variance components as: $h^2 = \frac{\sigma_L^2}{\sigma_L^2 + \sigma_R^2/b}$ with $b$ = number of repetitions.

QTL mapping was performed using QTLcartographer (Basten et al. 1994; Basten et al. 2002) using the composite interval mapping (CIM) procedure with five background parameters. The threshold, obtained by the permutation test method (Churchill and Doerge 1994), for adding a QTL was LOD $\geq$ 3 and allowed an experimentwise error rate close to 1%, as determined with 1,000 permutations. QTL positions were estimated where the LOD score reached its maximum in the region under consideration. A LOD support interval was constructed for each QTL based on a LOD drop-off equal to 1 (Lander and Botstein 1989).

A multi-population QTL analysis was carried out. The objective was to know whether QTLs identified for flowering date in one population corresponded to those detected in other populations. Results collected from QTL mapping experiments involved three genetic maps (LR1, LR4, and LR5) that shared common markers but also had their own markers. A projection of all maps and QTLs on the LR4 framework genetic map was carried out with Biomercator software (Arcade et al. 2004). Biomercator performs automatic compilations of several genetic maps. If two genetic maps share a sufficient number of common loci, those loci can be considered as bridges between maps. So, a consensus genetic map can be built from several maps by iterative projection of loci. A saturated map, which includes 133 SSR markers, built on LR4 was available (T. Huguet, unpublished). Markers of LR1 and LR5 were projected on the LR4 reference map using common markers of each map and the reference map. As three maps were used, the LR1 map was first projected on the reference LR4 map to produce a second map. The LR5 map was then projected on this map to produce a consensus map. This order was chosen because the LR1 map contained more markers than the LR5 map. The most likely position of each QTL and its support interval on LR1 and LR5 maps were projected on the LR4 map on the basis of their relative distance to flanking common markers with a homothetic function. The multi-population QTL mapping was performed with the MCQTL software package (Jourjon et al. 2005). The iterative QTL mapping method (iQTLm) using genetic cofactors was chosen. Cofactor selection and test of QTL effects were performed with $F$ tests. $F$ thresholds were determined by 1,000 permutation tests, to correspond to a global type I risk of 10% (across all populations and the total genome) for cofactor selection, and 10% for QTL detection. The threshold for adding a QTL was LOD $\geq 3$.

### Bioinformatics

The current size for the chromosome 7 (http://www.medicago.org/genome/stats.php website data) is 24.3 Mbp (as of 09 July 2007) and is composed by 242 Bacterial Artificial Chromosomes (BACs). The list of BACs included within the support interval of the consensus major QTL was established from the position of the upper and lower bound markers, using the website http://www.medicago.org/genome/. Predictions of candidate genes on these BACs were analysed to identify those known to be involved in flowering date in other species. Gene sequences were compared to publicly available *M. truncatula* EST database of TIGR (The Institute for Genomic research) by BLASTn to see which genes are expressed. The IMGAG annotation of candidate genes was used to obtain coding DNA sequences that were compared to protein database of Swissprot by BLASTx. The genes that were already known to be involved in flowering date (Komeda 2004; Hecht et al. 2005; Imaizumi and Kay 2006) were selected.

## Results

### Variation in flowering date as a response to photoperiod

In analysis of variance, effects of line, photoperiod and line × photoperiod interaction were highly significant on the flowering date (Table 1). All lines had an earlier flowering date under long photoperiod than under short photoperiod (Table 2). F83005.5 had a flowering date more hastened by a long photoperiod than the other lines. Flowering date of Levahim-B was little affected by photoperiod. Contribution of each line to line × photoperiod interaction, measured by ecovalence criterion showed (Table 2) that DZA315.16, DZA45.6 and Meiron with low ecovalences contributed little to the interaction. Contrastingly, F83005.5, Levahim-B and Jemalong6 had high values of ecovalence, showing that they contributed to the line × photoperiod interaction.

### Quantitative variation in flowering date in three mapping populations

In analysis of variance on the four parental lines of the 2005 experiments (Table 3), season and line effects on the flowering date were highly significant. The repetition nested within season effect and the line × season interaction were not significant. For LR1 population, DZA45.6 flowered earlier than DZA315.26. For LR4 and LR5 populations, Jemalong6 always had an earlier flowering date than DZA315.16 and F83005.5 (Table 4).

Analyses of variance carried out on each population (LR1, LR5), for the lines that were repeated three times in spring and autumn 2005 are shown in Table 3. For the LR1 population, line and season (spring or autumn) effects were significant and mean squares were of similar range.

**Table 1** Analysis of variance (mean squares) for the flowering date of eight *M. truncatula* lines grown under two photoperiods

| Effect | Mean square |
|---|---|
| Photoperiod | 19,543*** |
| Block (Photoperiod) | 9 NS |
| Line | 2,118*** |
| Line × Photoperiod | 498*** |
| Error | 24 |

*NS* Not significant ($P = 0.05$)

*** Significant ($P < 0.001$)

**Table 2** Flowering date in °C.D for eight *M. truncatula* lines under photoperiods of 12 and 18 h, and evaluation of the contribution of each line to the line × photoperiod interaction (ecovalence of the lines expressed as a percentage of the total ecovalence)

| Line | Flowering date (°C.D) | | |
|---|---|---|---|
| | 12 h | 18 h | Ecovalence (%) |
| Levahim-B | 903 | 671 | 24.1 |
| A20 | 1,064 | 716 | 9.7 |
| Meiron | 1,186 | 741 | 2.7 |
| TN6.18 | 1,359 | 979 | 6.9 |
| Jemalong6 | 1,454 | 633 | 17.2 |
| DZA45.6 | 1,583 | 937 | 2.1 |
| DZA315.16 | 1,685 | 1,096 | 0.3 |
| F83005.5 | 1,995 | 1,049 | 37.0 |

**Table 3** Analysis of variance (mean squares) for the flowering date of parental lines and of the 15 RILS replicated three times in each of two mapping populations (LR1, LR5) of *M. truncatula*

| | Parents | | RILs | | | |
|---|---|---|---|---|---|---|
| | | | LR1 | | LR5 | |
| | Mean squares | *df* | Mean squares | *df* | Mean squares | *df* |
| Line | 112,262*** | 3 | 555,389*** | 13 | 69,873*** | 14 |
| Season | 495,022*** | 1 | 311,926*** | 1 | 2,005,316*** | 1 |
| Repetition (season) | 11,159 NS | 4 | 10,855 NS | 4 | 1,964 NS | 4 |
| Line × season | 22,777 NS | 3 | 9,493 NS | 13 | 20,501** | 14 |
| Error | 11,580 | 23 | 5,130 | 49 | 6,549 | 55 |

*NS* Not significant ($P = 0.05$)

** Significant ($P < 0.01$)

*** Significant ($P < 0.001$)

Line × season interaction was not significant. For the LR5 population, line and season effects were significant, but the mean square of the season effect was larger than the mean square of the line effect. Line × season interaction was significant. For both populations, replication effect, nested within season, was not significant. Analysis of variance was performed on LR4 (Julier et al. 2007). Mean of the RILs for flowering date was almost identical in spring and autumn for LR1 while it strongly differed for LR5. Differences among seasons were also noticed in LR4 (Table 4).

The broad sense heritability for flowering date was 82.3% in LR1 and 75.5% in LR5, it was 82.2% in LR4 (Julier et al. 2007).

### Modelling of flowering date

Times from sowing to flowering (*f*, in days) differed greatly among the four parental lines and also varied across photothermal regimes (Table 5). The $R^2$ of the linear, multiple regression model varied from 0.82 to 0.99, and all the parameters were highly significant. Parameters *b* and *c* were positive, indicating that long days and warm temperatures hastened flowering. Parameter *b* values indicate that Jemalong6 and F83005.5 had a flowering date that was more hastened by temperature than DZA315.16 and DZA45.6. Parameter *c* was higher for Jemalong6 than for the other three lines, showing that Jemalong6 was more affected by photoperiod duration.

### QTL identification

Nineteen markers were added on chromosome 7 in LR4 in the region of the QTL for flowering date previously detected (Fig. 1).

For LR1, we found QTLs at the same position (41.8 cM) in both spring and autumn on chromosome 7 that explained 31 and 34% of the variation, respectively (Table 6). There was another QTL in autumn on chromosome 4. DZA45.6 alleles for all QTLs had positive effects so they induced a late flowering date.

For LR4, QTLs on chromosome 7 were detected at similar positions in all 4 years (between 56.3 and 57.8 cM) with Jemalong6 allele inducing an early flowering date. They explained up to 60% of flowering date variation. On chromosome 8, QTLs were detected for each season at position between 4.0 and 62.0 cM, with a negative effect of Jemalong6 alleles that induced an early flowering date. QTLs were also found on chromosome 1, in springs 2000 and 2002 and on chromosome 5 in spring 2003. For the QTLs of chromosomes 1 and 5, Jemalong6 alleles showed positive effects on flowering date.

For LR5, a QTL was found in spring and autumn 2005 on chromosome 7 at the positions 62.8 and 70.4 cM and explained 11 and 20% of the variation, respectively. In spring 2005, a QTL was also detected on chromosome 8 and explained 17%, and in autumn 2005, a QTL was detected on chromosome 4 explaining 10% of the variation. For all QTLs in this population, Jemalong6 alleles had a negative effect.

### Map projection and multi-population QTL analysis

A consensus map was calculated from the projection of the LR1 and LR5 maps on the LR4 reference map. The most likely position of each QTL and its support interval were projected on the consensus map (Fig. 1). At this stage, the positions of the QTLs can be visually compared (Fig. 1) and are indicated in Table 6.

QTL mapping in this multi-cross design, using MCQTL software showed two QTLs (Table 7). On chromosome 7, a QTL that explained 25% of the total variation in the three

mapping populations was found at 57.8 cM with a support interval of 0.9 cM. The additive effects of this QTL were negative for Jemalong6 and F83005.5 but positive for DZA315.16/DZA316.26 and DZA45.6. On chromosome 8, a QTL that explained 3% of the total variation was found at 10.0 cM with a support interval of 16.1 cM. The additive effects of this QTL were negative for Jemalong6 and positive for the other parental lines. No QTL was found on chromosomes 1, 4 and between 52 and 68 cM on chromosome 8 with this multi-population analysis although they were present in the LR1, LR4 or LR5 populations. The effects of the genetic background varied among the populations: it was on average 1229.0°C.D for the LR1 population, 1399.5°C.D for the LR4 population and 1446.8°C.D for the LR5 population. The global model explained 80% of the variation.

The support interval of 0.9 cM for the QTL on chromosome 7 is surrounded by the markers mtic760 and mtic714 that correspond to mtgsp004g09 and mtgsp002g05 on the integrated genetic map of University of Minnesota (http://www.medicago.org/genome/map.php), respectively. The genetic distance between mtic760 and mtic714 is 4.2 cM and is covered by 24 BACs (http://www.medicago.org/genome/show_BAC_chr2.php?chr = 7) that comprised 507 genes. Six genes presented high homologies with genes involved in flowering in *A. thaliana* (Table 8): Constans (CO), three copies of flowering locus T (FT), FD and phytochrome kinase substrate (PKS). CO was mapped at 57.4 cM on the LR4 map

**Fig. 1** Position of the QTLs for flowering date in three mapping populations, projected on the reference map of LR4 population of *M. truncatula*. The chromosomes without QTLs were omitted. *Vertical bars* on the left side of chromosomes indicate the support interval of the QTLs. The *horizontal bars* represent the position of the QTLs and the bar length is proportional to $R^2$ value. The traits are named by the code of the population, the season (s for spring, a for autumn), the year and the site of experiment

**Table 4** Mean and range of variation for flowering date, expressed in °C.D from sowing, for three RILs population of *M. truncatula* and their parents

| Population | Location/Year | Parents | | RILs | |
|---|---|---|---|---|---|
| | | Names | Flowering date (°C.D) | Mean (°C.D) | Range (°C.D) |
| LR1 | Lusignan 2005 spring | DZA45.6 | 1,132 | 1,288 | 913–1,784 |
| | | DZA315.26 | 1,389 | | |
| | Lusignan 2005 autumn | DZA45.6 | 1,440 | 1,401 | 1,040–1,613 |
| | | DZA315.26 | 1,502 | | |
| LR4 | Montpellier 2000 spring | Jemalong6 | 740 | 790 | 639–1,181 |
| | | DZA315.16 | 848 | | |
| | Lusignan 2002 spring | Jemalong6 | 968 | 1,023 | 878–1,282 |
| | | DZA315.16 | 1,093 | | |
| | Lusignan 2003 spring | Jemalong6 | 1,069 | 1,147 | 899–1,500 |
| | | DZA315.16 | 1,123 | | |
| | Lusignan 2004 autumn | Jemalong6 | 1,050 | 1,402 | 867–2,120 |
| | | DZA315.16 | 1,364 | | |
| LR5 | Lusignan 2005 spring | F83005.5 | 1,104 | 1,012 | 831–1,568 |
| | | Jemalong6 | 872 | | |
| | Lusignan 2005 autumn | F83005.5 | 1,379 | 1,315 | 1,022–1,554 |
| | | Jemalong6 | 1,277 | | |

**Table 5** Modelling of the rate of progression to flowering ($1/f$) for four *M. truncatula* lines depending on mean temperature ($T$) and mean photoperiod ($P$)

| Line | Parameters for the model $1/f = a + bT + cP$* | | | | | | |
|---|---|---|---|---|---|---|---|
| | $a$ | SE of $a$ | $b$ | SE of $b$ | $c$ | SE of $c$ | $R^2$ |
| Jemalong6 | −0.0358 | 0.00318 | 0.000914 | 0.000162 | 0.00269 | 0.000093 | 0.98 |
| DZA315.16 | −0.0067 | 0.00158 | 0.000408 | 0.000081 | 0.00091 | 0.000058 | 0.96 |
| DZA45.6 | −0.0139 | 0.00197 | 0.000693 | 0.000107 | 0.00117 | 0.000082 | 0.95 |
| F83005.5 | −0.0187 | 0.00387 | 0.000985 | 0.000210 | 0.00102 | 0.000165 | 0.83 |

The values of the parameters are followed by standard error

* All parameters were significant ($P < 0.001$)

8
mtic086 (0)
mtic1089 (10)
mtic1087 (14)
mtic665 (23)
mtic308 (28.6)
mtic492 (32)
mtic135 (35.5)
mtic248 (41)
mtic299 (50)
mtic178 (51.6)
mtic537 (53.3)
mtic750 (62)
mtic364 (68)
mtic049 (75)
mtic523 (75.6)
mtic517 (82.4)
mtic629 (86)
mtic080 (86.8)
LR4_a04_Lusignan
LR4_a03_Lusignan
LR5_s05_Lusignan
LR4_s00_Montpellier
LR4_s02_Lusignan

7
mtic507 (-8.5)
mtic744 (0)
mtic503 (2.2)
mtic240 (4)
mtic147 (4)
mtic891 (9.1)
mtic644 (18)
mtic521 (18)
mtic226 (29.7)
mtic572 (34)
mtic432 (45)
mtic518 (51.2)
mtic642 (53)
mtic724 (53)
mtic246 (54.3)
mtic470 (55)
mtic175 (55.2)
mtic040 (55.2)
mtic705 (55.7)
mtic719 (55.9)
mtic705 (55.9)
mtic783 (56.3)
mtic720 (56.5)
mtic502 (56.6)
mtic509 (56.6)
mtic760 (56.7)
Constans (57.4)
mtic252 (57.5)
mtic701 (57.6)
ABA (57.8)
mtic714 (60.9)
mtic508 (61.7)
mtic1172 (62.2)
mtic639 (62.2)
mtic1090 (65)
mtic514 (66.6)
mtic516 (67.5)
mtic241 (68.6)
mtic333 (72.4)
mtic686 (73.3)
LR4_a03_Lusignan
LR1_s05_Lusignan
LR1_a05_Lusignan
LR4_s02_Lusignan
LR4_s00_Montpellier
LR4_a04_Lusignan
LR5_s05_Lusignan
LR5_a05_Lusignan

5
mtic180 (-8.3)
mtic078 (-5.6)
mtic204 (-3.2)
mtic768 (0)
mtic148 (6)
mtic512 (11.1)
mtic520 (11.7)
mtic511 (13)
mtic893 (14.9)
mtic764 (25)
pp14 (28.7)
mtic1072 (39)
mtic525 (43.5)
mtic524 (52)
pp04 (54.9)
mtic238 (60.7)
mtic1067 (64.8)
mtic670 (67)
mtic048 (72)
pp06 (73)
mtic1073 (80)
mtic439 (80.6)
mtic448 (81.5)
mtic059 (88)
mtic536 (94)
LR4_a03_Lusignan

4
mtic348 (-0.6)
mtic559 (0)
mtic332 (1.5)
mtic331 (5)
mtic510 (9.7)
mtic528 (10.7)
mtic529 (14.4)
mtic234 (16.4)
mtic229 (20.1)
mtic736 (21)
mtic090 (29)
mtic895 (30.4)
mtic089 (36)
mtic506 (39.2)
mtic677 (43)
mtic490 (53)
mtic303 (58)
mtic1169 (62.3)
mtic488 (68)
mtic085 (70.4)
mtic761 (71)
mtic326 (71.5)
mtic430 (74.1)
LR1_a05_Lusignan
LR5_a05_Lusignan

1
mtic578 (0)
mtic325 (11.8)
mtic068 (13)
mtic746 (30)
mtic504 (38)
mtic312 (58)
mtic142 (64)
mtic534 (66.3)
mtic125 (71)
mtic468 (77)
mtic419 (77)
mtic530 (84.2)
mtic584 (86)
mtic531 (87.2)
mtic061 (88.6)
mtic192 (89.8)
mtic532 (90)
mtic314 (90)
mtic539 (90.1)
mtic369 (90.4)
LR4_s00_Montpellier
LR4_s02_Lusignan

**Table 6** QTL for flowering date revealed by composite interval mapping in *M. truncatula* recombinant inbred lines of DZA315.26 × DZA45.6 (LR1), Jemalong6 × DZA315.16 (LR4) and Jemalong6 × F83005.5 (LR5) populations. Position and support intervals are indicated on the map of each cross and on a consensus obtained from the projection of LR5 and LR1 maps onto LR4 reference map

| Population | Location, year, season | Chromosome | Position and support interval on each map (cM) | Position and support interval projected on consensus map | LOD score | Effect[a] | $R^2$ |
|---|---|---|---|---|---|---|---|
| LR1 | Lusignan 2005 spring | 7 | 41.8 (34–44) | 58.3 (42–62) | 9.4 | 91.4 | 0.31 |
| | Lusignan 2005 autumn | 4 | 45.0 (42–50) | 62.1 (59–67) | 3.6 | 49.5 | 0.16 |
| | | 7 | 41.8 (38–43) | 58.3 (51–59) | 9.9 | 73.5 | 0.34 |
| LR4 | Montpellier 2000 spring | 1 | 64.0 (58–69) | 64.0 (58–69) | 3.6 | 27.1 | 0.05 |
| | | 7 | 57.5 (55–58) | 57.5 (55–58) | 18.6 | −74.1 | 0.42 |
| | | 8 | 18.0 (12–23) | 18.0 (12–23) | 4.3 | −31.4 | 0.08 |
| | Lusignan 2002 spring | 1 | 68.0 (64–77) | 68.0 (64–77) | 3.1 | 28.1 | 0.08 |
| | | 7 | 56.7 (55–61) | 56.7 (55–61) | 9.4 | −58.6 | 0.37 |
| | | 8 | 62.0 (53–68) | 62.0 (53–68) | 3.7 | −31.8 | 0.10 |
| | Lusignan 2003 spring | 5 | 0.0 (0–4) | 0.0 (0–4) | 3.8 | 33.0 | 0.06 |
| | | 7 | 56.3 (56–57) | 56.3 (56–57) | 18.5 | −99.9 | 0.60 |
| | | 8 | 58.0 (52–62) | 58.0 (52–62) | 5.3 | −43.6 | 0.10 |
| | Lusignan 2004 autumn | 7 | 57.8 (56–59) | 57.8 (56–59) | 14.1 | −213.0 | 0.32 |
| | | 8 | 4.0 (0–12) | 4.0 (0–12) | 4.3 | −115.0 | 0.10 |
| LR5 | Lusignan 2005 spring | 7 | 62.9 (47–65) | 61.3 (41–64) | 4.2 | −56.5 | 0.11 |
| | | 8 | 2.0 (0–11) | 1.6 (0–8) | 5.7 | −67.3 | 0.17 |
| | Lusignan 2005 autumn | 4 | 58.3 (55–60) | 70.4 (65–72) | 3.8 | −45.2 | 0.10 |
| | | 7 | 63.8 (58–67) | 62.3 (55–65) | 5.5 | −66.8 | 0.20 |

[a] Effect of Jemalong6 allele for LR4 and LR5, DZA315.26 allele for LR1

**Table 7** QTLs obtained after a multi-population QTL analysis with MCQTL software for flowering date in the three mapping populations of *M. truncatula*

| Chromosome | Position (cM) | Support interval (cM) | LOD score | Allele effect | | | | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | | | | Jemalong6 | DZA315.16 | DZA45.6 | F83005.5 | |
| 7 | 57.8 | 57.2–58.1 | 82.7 | −195.8 | 54.7 | 214.0 | −72.8 | 0.25 |
| 8 | 10.0 | 0.0–16.1 | 7.9 | −64.3 | 8.8 | 15.3 | 40.2 | 0.03 |

(Fig. 1). It is involved in perception of daylength in *A. thaliana* (Hayama and Coupland 2004). FT is supposed to be the florigen signal and speeds up the floral transition (Huang et al. 2005). Protein FD interacts with FT gene and induces the expression of floral identity genes (Abe et al. 2005; Blazquez 2005). PKS protein modulates the kinase activity of Phytochrome B (Fankhauser et al. 1999).

## Discussion

### Effect of photoperiod on flowering date

The alteration of flowering date in response to photoperiod corresponds to the interactions between environmental signals and the circadian clock (endogenous rhythm with a period of 24 h) (Hayama and Coupland 2004). Annual medics are known to be vernalizable long-day plants (Clarkson and Russell 1975). In growth chamber experiments where photoperiod varied only from 12.2 to 16 h, photoperiod had less impact on flowering date of *M. truncatula* Jemalong-A17 than vernalization (Moreau et al. 2007). Other authors also observed that the effect of vernalization on flowering date of Jemalong is of large importance under long days, but can be secondary to the effect of photoperiod for other genotypes (Chabaud et al. 2006). Indeed, a genetic variability for the flowering date in response to photoperiod was observed in *A. thaliana* (Schmitz and Amasino 2007).

Flowering date influences the possibility to complete reproduction. Photoperiod varies with the season and the geographic latitude. It induces changes in plant

**Table 8** The candidate genes present in the support interval of QTL for flowering date on chromosome 7, and their similarity with *A. thaliana* flowering date genes (Swissprot database) and EST (TIGR)

| Gene | Accession | Similarity *with A. thaliana* genes | E value | EST |
|---|---|---|---|---|
| CONSTANS | AC133780_7.1 | CONSTANS-LIKE Zinc finger protein COL14 | 6,00E-48 | AW696837 |
| FD | AC149134_18.2 | Protein FD (BZIP transcription factor 14) | 8,00E-15 | TC99455 |
| FT | AC123593_3.2 | Protein FLOWERING LOCUS T | 4,00E-72 | TC101901 |
| FT | AC123593_7.2 | Protein FLOWERING LOCUS T | 6,00E-32 | TC101901 |
| FT | AC123593_9.2 | Protein FLOWERING LOCUS T | 2,00E-53 | TC101901 |
| PKS | AC149134_39.2 | Phytochrome kinase substrate 1 | 7,00E-04 | BF644398 |

morphogenesis, as a response to the timing of light and darkness that represents implicitly the total light energy and the perception of the day length (Thomas and Vince-Prue 1997). Little knowledge was available on the effect of photoperiod duration on flowering date of *M. truncatula*, and on genetic variation for its response to photoperiod. In this study, flowering date of eight *M. truncatula* lines sampled from the Mediterranean Basin and Australia was earlier under long than under short photoperiod. Thus it clearly shows that photoperiod duration which only corresponded here to a perception of the day length as a change of light and dark cycle, is of great influence on phenological development. A significant interaction between line and photoperiod was observed: flowering date of Jemalong6 and F83005.5 was more hastened by long photoperiod duration than that of other lines. It was already shown (van Heerden 1984) that flowering date of Jemalong was more sensitive to day length in the range of 13–15 h than in 9-h treatment.

The differences between lines for the flowering date in response to photoperiod treatment could indicate an adaptation to the original environment. Indeed in rice (*Oriza sativa*), tropical varieties are long day flowering plants whereas the non-tropical varieties were selected for photoperiod insensitivity (Thomson et al. 2006). For maize (*Zea mays*), exposure to long days delays the initiation and development of the terminal staminate inflorescence, but some variation between hybrids were noticed (Krüger 1984). Compared to rice, we can conceive that some populations of *M. truncatula* were naturally selected for high/low photoperiod sensitivity to ensure flowering at an optimal time depending on climatic constraints. The lines with early flowering under short photoperiod (early flowering in spring) could suffer from late cold periods under natural conditions, whereas the lines with late flowering under short photoperiod (late flowering in spring) avoid cold periods but can be affected by water stress. In a collection of *M. truncatula* accessions from the Mediterranean Basin, a correlation was established between flowering date and the annual rainfall in the sites of collection, itself related to the latitude (Julier et al. 2007). This adaptation could partly result from a differential response of the accessions to the photoperiod.

### Quantitative analysis and QTL detection in three mapping populations

The four lines used as parents of the three mapping populations differed for flowering date. The RILs derived from crosses DZA315.26 × DZA45.6 (LR1), Jemalong6 × DZA315.16 (LR4) and Jemalong6 × F83005.5 (LR5) showed a large variation for flowering date. In LR1 and LR5 populations, flowering date of the RILs was normally distributed. In contrast, LR4 RILs had a skewed distribution, due to the loss of late flowering RILs during inbreeding generations (Julier et al. 2007). In all three populations, transgressive lines for flowering date (RILs that flowered either earlier or later than the parents) were observed, as in LR4 population for morphogenetic traits (Julier et al. 2007).

An effect of season (spring vs. autumn) was observed. Indeed the sum of degree-days needed to reach flowering was higher in autumn than in spring. It is important to notice that day length increased in spring and decreased in autumn with an amplitude from 3 to 4 h (see "Materials and methods"). Flowering date of Jemalong6 and its progenies was probably more hastened by long photoperiod in spring 2005 than flowering of the other lines, as already shown in the photoperiod experiment.

This QTL analysis is the first one on *M. truncatula* to gather information from several populations. It provides insight into the genetic control of flowering date in *M. truncatula* and environmental effect. QTLs were found on chromosomes 1, 4, 5, 7 and 8 depending on the population and the year or season. A major QTL present on chromosome 7 in all three populations in all environments explained from 20 to 60% of the total variation. This genomic region had thus a high effect in genetic variation of flowering date, but this effect was higher in LR1 and LR4 ($R^2$ between 30 and 60%) than in LR5 ($R^2 < 20\%$). Other QTLs were found on other chromosomes depending on the season. These QTLs of less important effect could

be related to effects of environmental conditions which varied among experiments and to the available polymorphism in each population. Another genomic region was common to two populations (between 0 and 16.1 cM on chromosome 8 for LR4 and LR5). Alleles at each QTL can have contrasting effects on flowering date. Jemalong6 alleles induced either a negative effect (early flowering date), as on chromosomes 7 and 8 (LR4 and LR5 populations) or a positive effect (late flowering date), as on chromosomes 1 and 5 (LR4 population). The DZA315.16/DZA315.26 alleles had positive effects in LR1 population but positive or negative effects in LR4. F83005.5 alleles had positive effects in LR5 population. Finally, the effect of the alleles at the QTL on chromosome 7 is in accordance to the ranking for flowering date of each parental line: Jemalong6 flowered earlier than both DZA315.16 and F83005.5, and DZA45.6 flowered earlier than DZA315.26. The presence of transgressive lines can be explained by an accumulation of positive or negative alleles coming from both parents. A similar situation was found in *A. thaliana* (El Lithy et al. 2004; Loudet et al. 2005).

Precision of QTL mapping depends on several factors such as trait heritability, number of QTLs involved, distribution of QTLs over the genome, number of marker loci and their distribution over the genome and population size (Knapp and Teuber 1990). Precision of QTL position depends more on population size than on the number of markers. Flowering date in *M. truncatula* was shown to have a high heritability. Thus, a design with multiple mapping populations offers the possibility to increase population size in gathering several QTL experiments, and to combine QTLs. The addition of markers in the region of a QTL in chromosome 7 was also made to get a more precise position of the QTL. A striking result in our study on eight populations × seasons situations was the single QTL on chromosome 7 that explained 25% of the variation over the design. Its position was estimated within a support interval of 0.9 cM only, as a result of the size of the global population which reached ~600 RILs. Another QTL on chromosome 8 explained 3% of the variation. These two QTLs and the population background explained 80% of the global variation. Some population-specific QTLs were not detected in the multi-population analysis, probably because they had a small contribution to the variation over the three populations. The combination of information from multiple crosses has been proved to be powerful for QTL detection in theoretical (Rebai and Goffinet 1993; Muranty 1996) and experimental studies (Chardon et al. 2004; Blanc et al. 2006). It increases the chances that polymorphic alleles are present in the parental lines, permitting the detection of QTLs that are undetectable in a single cross where the two parents are fixed for the same allele. It also allows the estimation of QTL effects over a large set of parents, and finally, it increases the power of QTL detection, giving a more accurate position of the QTLs. The main advantage given by this study of three different populations compared to a single one (LR4 as described by Julier et al. 2007) was to reduce the support interval of a major QTL. It also provided more genomic locations that contribute to genetic architecture of flowering date.

This QTL on chromosome 7 was present in all three populations, and had a strong effect on flowering date in all seasons. A list of six candidate genes involved in flowering was proposed. Among them, CO and FT are involved in the response to photoperiod (Searle and Coupland 2004). The CO protein is accumulated in the leaves during the light phase and degraded during the night phase. The coincidence of light at the end of a long day and high CO protein levels in leaves activates the FT gene, which promotes flowering (Valverde et al. 2004; Baurle and Dean 2006; Zhou et al. 2007). FD gene encodes a transcription factor that interacts with protein FT to initiate the expression of floral identity genes (Abe et al. 2005; Blazquez 2005). The CO gene has been shown to encode a transcription factor that plays a central role in the photoperiod detection mechanism (Putterill et al. 1995). In *M. truncatula* databases, four CO-like sequences were identified distinguishing MtCOLa in one hand and MtCOLb to MtCOLd in other hand (Hecht et al. 2005). They have essentially the same domain structure (zinc finger region and a CCT domain). COLa gene is located on chromosome 7 of *M. truncatula.* Three genes homologous to *Arabidopsis* FT gene are on a single same BAC (AC123593) in *M. truncatula* chromosome 7, and the three FT have a syntenic relationship with the GIGAS gene on pea chromosome 5. *Gigas* pea plants were late flowering and they showed an enhance response to photoperiod and vernalization (Beveridge and Murfet 1996). In the flowering pathway, PKS gene acts at an upstream level in interaction with Phytochrome B (Fankhauser et al. 1999). QTLs with large effects are often controlled by single genes that may be cloned as observed in rice with Hd1 or EDI in *A. thaliana* (Yano et al. 2000; El-Assal et al. 2001). So, the hypothesis of a single gene among the list of six genes identified in the QTL is anticipated. However, two different genes could control the QTL (Kole et al. 2002), the genes implied in a given function being sometimes clustered (Lee et al. 2000). If ever two genes were involved in the QTL, it would complicate positional cloning of the QTL.

Despite of the reduction of the support interval of this QTL, it was impossible to eliminate one or the other gene. The identification of the gene involved in the QTL on chromosome 7 requires different strategies such as fine mapping, transcriptomic studies and analysis of transgenic or mutant plants. A supplementary work of fine mapping for this major QTL on chromosome 7 is under

development. This "positional candidate genes" (Pflieger et al. 2001; Kottapalli et al. 2006) strategy should make it possible to find the gene(s) responsible for the flowering date variation in these populations.

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## References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309:1052–1056

Aitken Y (1955) Flower initiation in pasture legumes. I. Factors affecting flower initiation in *Trifolium subterraneum*. Aust J Agric Res 26:212–244

Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326

Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-Henaut I, Huguet T, Burstin J (2006) Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. Theor Appl Genet 112:1024–1041

Aydi S, Drevon JJ, Abdelly C (2004) Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Medicago truncatula-Sinorhibobium meliloti* symbiosis. Plant Physiol Biochem 42:833–840

Basten CJ, Weir BS, Zeng ZB (1994) Zmap—a QTL cartographer. Guelph, Ontario, pp 65–66

Basten CJ, Weir BS, Zeng ZB (2002) QTL Cartographer, Version 1.16 North Carolina State University, Department of Statistics, Raleigh, NC

Baurle I, Dean C (2006) The timing of developmental transitions in plants. Cell 125:655–664

Bell CJ, Dixon RA, Farmer AD, Flores R, Inman J, Gonzales RA, Harrison MJ, Paiva NL, Scott AD, Weller JW, May GD (2001) The Medicago Genome initiative: a model legume database. Nucleic Acids Res 29:114–117

Beveridge CA, Murfet IC (1996) The gigas mutant in pea is deficient in the floral stimulus. Physiol Plant 96:637–645

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet 113:206–224

Blazquez MA (2005) The right time and place for making flowers. Science 309:1024–1025

Blondon F, Marie D, Brown S, Kondorosi A (1994) Genome size and base composition in *Medicago sativa* and *M. truncatula* species. Genome 37:264–270

Chabaud M, Lichtenzveig J, Ellwood S, Pfaff T, Journet EP (2006) Vernalization, crossings and testing for pollen variability. http://www.noble.org/MedicagoHandbook/pdf/Vernalization.pdf

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168:2169–2185

Choi HK, Kim D, Uhm T, Limpens E, Lim H, Mun JH, Kaló P, Penmetsa RV, Seres A, Kulikova O, Roe BA, Bisseling T, Kiss GB, Cook DR (2004a) A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. Genetics 166:1463–1502

Choi HK, Mun JH, Kim DJ, Zhu H, Baek JM, Mudge J, Roe B, Ellis N, Doyle J, Kiss GB, Young ND, Cook DR (2004b) Estimating genome conservation between crop and model legume species. Proc Natl Acad Sci USA 101:15289–15294

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Clarkson NM, Russell JS (1975) Flowering responses to vernalization and photoperiod in annual medics (*Medicago* ssp.). Aust J Agric Res 26:831–838

Delalande M, Ronfort J, Prosperi JM (2004) Diversity for flowering time in a large collection of *Medicago truncatula* Gaertn. Québec, http://www.naaic.org/Meetings/National/2004NAAIC&TC/2004abstracts/jprosperi.pdf

El Lithy ME, Clerkx EJM, Ruys GJ, Koornneef M, Vreugdenhil D (2004) Quantitative trait locus analysis of growth-related traits in a new *Arabidopsis* recombinant. Plant Physiol 135:444–458

El-Assal SED, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M (2001) A QTL for flowering time in *Arabidopsis* reveals a novel allele of CRY2. Nat Genet 29:435–440

Ellwood SR, D'Souza NK, Kamphuis LG, Burgess TI, Nair RM, Oliver RP (2006) SSR analysis of the *Medicago truncatula* SARDI core collection reveals substantial diversity and unusual genotype dispersal throughout the Mediterranean basin. Theor Appl Genet 112:977–983

Erskine W, Ellis RH, Summerfield RJ, Roberts EH, Hussain A (1990) Characterization of responses to temperature and photoperiod for time to flowering in a world lentil collection. TAG Theor Appl Genet 80:193–199

Fankhauser C, Yeh KC, Lagarias JC, Zhang H, Elich TD, Chory J (1999) PKS1, a substrate phosphorylated by phytochrome that modulates light signaling in *Arabidopsis*. Science 284:1539–1541

Hayama R, Coupland G (2004) The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. Plant Physiol 135:677–684

Hecht V, Foucher F, Ferrandiz C, Macknight R, Navarro C, Morin J, Vardy ME, Ellis N, Beltran JP, Rameau C, Weller JL (2005) Conservation of *Arabidopsis* flowering genes in model legumes. Plant Physiol 137:1420–1434

Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) The mRNA of the *Arabidopsis* gene FT moves from leaf to shoot apex and induces flowering. Science 309:1694–1696

Imaizumi T, Kay SA (2006) Photoperiodic control of flowering: not only by coincidence. Trends Plant Sci 11:550–558

Jourjon MF, Jasson S, Marcel J, Ngom B, Mangin B (2005) MCQTL: multi-allelic QTL mapping in multi-cross design. Bioinformatics 21:128–130

Julier B, Flajoulot S, Barre P, Cardinet G, Santoni S, Huguet T, Huyghe C (2003) Construction of two genetic linkage maps in cultivated tetraploid alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. BMC Plant Biol 3:9

Julier B, Huguet T, Chardon F, Ayadi R, Pierre JB, Prosperi JM, Barre P, Huyghe C (2007) Identification of quantitative trait loci

influencing aerial morphogenesis in the model legume *Medicago truncatula*. Theor Appl Genet 114:1391–1406

Knapp E, Teuber L (1990) Environmental factors and plant phenotype affect alfalfa floret tripping. Crop Sci 30:270–275

Kole C, Williams PH, Rimmer SR, Osborn TC (2002) Linkage mapping of genes controlling resistance to white rust (*Albugo candida*) in *Brassica rapa* (syn. campestris) and comparative mapping to *Brassica napus* and *Arabidopsis thaliana*. Genome 45:22–27

Komeda Y (2004) Genetic regulation of time to flower in *Arabidopsis thaliana*. Annu Rev Plant Biol 55:521–535

Kottapalli KR, Sarla N, Kikuchi S (2006) In silico insight into two rice chromosomal regions associated with submergence tolerance and resistance to bacterial leaf blight and gall midge. Biotechnol Adv 24:561–589

Krüger GHJ (1984) Effect of photoperiod on initiation of staminate inflorescence in different hybrids of *Zea mays*. S Afr J Bot 3:81–82

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lee S, Reth A, Meletzus D, Sevilla M, Kennedy C (2000) Characterization of a major cluster of nif, fix, and associated genes in a sugarcane endophyte, *Acetobacter diazotrophicus*. J Bacteriol 182:7088–7091

Levy YY, Dean C (1998) The transition to flowering. Plant Cell 10:1973–1989

Loudet O, Gaudon V, Trubuil A, Daniel-Vedele F (2005) Quantitative trait loci controlling root growth and architecture in *Arabidopsis thaliana* confirmed by heterogeneous inbred family. Theor Appl Genet 110:742–753

Moreau D, Salon C, Munier-Jolain N (2007) A model-based framework for the phenotypic characterisation of the flowering of *Medicago truncatula*. Plant Cell Environ 30:213–224

Muranty H (1996) Power of tests for quantitative trait loci detection using full-sib families in different schemes. Heredity 76:156–165

Pflieger S, Lefebvre V, Causse M (2001) The candidate gene approach in plant genetics: a review. Mol Breed 7:275–291

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The Constans gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc-finger transcription factors. Cell 80:847–857

Rebai A, Goffinet B (1993) Power of tests for QTL detection using replicated progenies derived from a diallel cross. Theor Appl Genet 86:1014–1022

Ronfort J, Bataillon T, Santoni S, Delalande M, David JL, Prosperi JM (2006) Microsatellite diversity and broad scale geographic structure in a model legume: building a set of nested core collection for studying naturally occurring variation in *Medicago truncatula*. BMC Plant Biol 6:28

Roux F, Touzet P, Cuguen J, Le Corre V (2006) How to be early flowering: an evolutionary perspective. Trends Plant Sci 11:375–381

Schmitz RJ, Amasino RM (2007) Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants. Biochim Biophys Acta Gene Struct Expr 1769:269–275

Searle I, Coupland G (2004) Induction of flowering by seasonal changes in photoperiod. EMBO J 23:1217–1222

Summerfield RJ, Muehlbauer F, Roberts EH (1985) *Lens culinaris* medic. In: Havely A (ed) A handbook of flowering. CRC Press, Florida, pp 118–124

Thomas B, Vince-Prue D (1997) Photoperiodism in plants. Academic Press, London

Thomson MJ, Edwards JD, Septiningsih EM, Harrington SE, McCouch SR (2006) Substitution mapping of dth1.1, a flowering-time quantitative trait locus (QTL) associated with transgressive variation in rice, reveals multiple sub-QTL. Genetics 172:2501–2514

Thoquet P, Ghérardi M, Journet EP, Kereszt A, Ané JM, Prosperi JM, Huguet T (2002) The molecular genetic linkage map of the model legume *Medicago truncatula*: an essential tool for comparative legume genomics and the isolation of agronomically important genes. BMC Plant Biol 2:1

Torregrosa C, Cluzet S, Fournier J, Huguet T, Gamas P, Prosperi JM, Esquerre-Tugaye MT, Dumas B, Jacquet C (2004) Cytological, genetic, and molecular analysis to characterize compatible and incompatible interactions between *Medicago truncatula* and *Colletotrichum trifolii*. Mol Plant Microbe Interact 17:909–920

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303:1003–1006

van Heerden JM (1984) Influence of temperature and daylength on the phenological development of annual *Medicago* species with particular reference to *M. truncatula*, cv. Jemalong. S Afr J Plant Soil 1:73–78

Wricke G, Weber WE (1986) Quantitative genetics and selection in plant breeding. Walter de Gryter, Berlin

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene CONSTANS. Plant Cell 12:2473–2483

Yoo SY, Kardailsky I, Lee JS, Weigel D, Ahn JH (2004) Acceleration of flowering by overexpression of MFT (MOTHER of FT and TFL1). Mol Cells 17:95–101

Zhou Y, Sun XD, Ni M (2007) Timing of photoperiodic flowering: light perception and circadian clock. J Integr Plant Biol 49:28–34