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ORIGINAL PAPER

Identification of quantitative trait loci influencing aerial morphogenesis in the model legume *Medicago truncatula*

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Abstract In many legume crops, especially in forage legumes, aerial morphogenesis defined as growth and development of plant organs, is an essential trait as it determines plant and seed biomass as well as forage quality (protein concentration, dry matter digestibility). *Medicago truncatula* is a model species for legume crops. A set of 29 accessions of *M. truncatula* was evaluated for aerial morphogenetic traits. A recombinant inbred lines (RILs) mapping population was used for analysing quantitative variation in aerial morphogenetic traits and QTL detection. Genes described to be involved in aerial morphogenetic traits in other species were mapped to analyse co-location between QTLs and genes. A large variation was found for flowering date, morphology and dynamics of branch elongation among the 29 accessions and within the RILs population. Flowering date was negatively correlated to main stem and branch length. QTLs were detected for all traits, and each QTL explained from 5.2 to 59.2% of the phenotypic variation. A QTL explaining a large part of genetic variation for flowering date and branch growth was found on chromosome 7. The other chromosomes were also involved in the variation detected in several traits. Mapping of candidate genes indicates a co-location between a homologue of Constans gene or a flowering locus T (FT) gene and the QTL of flowering date on chromosome 7. Other candidate genes for several QTLs are described.

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Introduction

Plant morphogenesis, including the growth and development of aerial organs, determines a large number of traits of agronomic importance in both grain and forage crops. In forage legumes such as alfalfa (*Medicago sativa*), aerial morphogenesis controls forage yield, forage yield distribution over seasons, persistency, cold and drought tolerance, lodging resistance, autumn dormancy and forage quality (protein concentration, dry matter digestibility)

(Julier et al. 1995). Plant molecular biologists have described many genes involved in aerial morphogenesis in model species, mainly *Arabidopsis thaliana* (for example on flowering date, (Komeda 2004). For breeding purposes with marker-assisted selection, it is important to identify the genes responsible for the natural genetic variations among genotypes, and to measure their respective effects. A strategy to reach this objective is to map quantitative trait locus (QTL) and to look for co-location of QTLs and candidate genes as an indication of the involvement of specific genes in the variation (Pflieger et al. 2001).

Medicago truncatula is now a model species for legumes (Barker et al. 1990). Genomic tools are being developed: EST sequences and the euchromatic DNA complete sequence will soon be available in databases (Young et al. 2005), microsatellite markers, genetic maps (Choi et al. 2004a; Eujayl et al. 2004; Thoquet et al. 2002). *M. truncatula* belongs to the *Trifolieae* tribe that includes major forage legumes such as alfalfa and clovers (*Trifolium* sp.) and is phylogenetically close to the *Vicieae* tribe that includes grain legumes such as pea (*Pisum sativum*) and fababean (*Vicia faba*). In this *Trifolieae–Vicieae* clade (Doyle and Luckow 2003), despite variation in DNA content and ploidy, conservation of the macro-synteny among species appears very good, as proven between *M. truncatula* and tetraploid or diploid alfalfa (Choi et al. 2004a; Julier et al. 2003), between *M. truncatula* and pea (Aubert et al. 2006; Choi et al. 2004c) and among six legume species (Choi et al. 2004b). The analysis of the genetic architecture of traits in *M. truncatula* should greatly help their analysis in crop species that is hampered by their complex biology and/or genetics (polyploidy and allogamy in alfalfa and clovers, genome size in pea). Genetic resources were collected, especially around the Mediterranean Basin from where thepecies originates. Models of vegetative development as a function of thermal time and of flowering date were recently proposed to help in giving more accurate and reproducible phenotyping data for genetic analysis (Moreau et al. 2005, 2007). The genetic variation in aerial morphogenetic traits has not been extensively studied until now (Delalande et al. 2004; Julier et al. 2002).

The objective of the present study was to evaluate the genetic variation in aerial morphogenesis among a set of *M. truncatula* accessions and to map QTLs for aerial morphogenetic traits in a mapping population of recombinant inbred lines of *M. truncatula* (Huguet et al. 2004). The position of candidate genes was determined from data mining on websites or gene mapping, and co-location between genes and QTLs was analysed.

Material and methods

Genetic variation for aerial morphogenesis in *M. truncatula*

A set of 20 natural populations collected around the Mediterranean Basin and nine lines involved in genomic studies (Table 1) chosen to represent the range of variation in morphology of the species was provided by INRA of Montpellier (France). This set included five *M. littoralis* accessions (MLI15, MLI108, MLI122, MLI377 and MLI518), as this species was proved to cross with *M. truncatula* species (Small and Brookes 1990; Small and Jomphe 1989). Due to the autogamy and an outcrossing rate under 1% (Bonnin et al. 2001), the within-population variation is very low. Within each of the accessions Jemalong, DZA45, DZA315 and F83005, two plants were identified and derived as pure lines. Eight of the nine lines were named after their respective population or cultivar followed by a number, which identifies a particular line. No genetic or phenotypic differences have been established between Jemalong-6 and Jemalong-A17 (Thoquet et al. 2002). Jemalong-6 was used as parent of mapping populations and Jemalong-A17 has been used for the construction of most BAC libraries and sequencing programs. The last line, MTR729, of unknown origin, is also named R108 and is often used for plant transformation and regeneration programs (d'Erfurth et al. 2003). The characteristics of the collection sites are given in Table 1. Seeds were scarified and sown in Petri dishes on7 April 2004, imbibed for 24 h at room temperature, and transferred for a vernalisation treatment of 7 days at 4°C. Germinated seeds were transplanted into individual pots in a greenhouse on13 April 2004 at INRA Lusignan (France) in a randomised block design with three replications, each replication being composed of one plant. The plants were grown under natural day-length that increased from 14 h 27 min to 17 h 07 min during the experiment. The plants received an application of 40 U of ammonitrate 1 month after transplantation. The temperature in the greenhouse averaged from 17 to 28°C, the minima varied from 12 to 22°C, the maxima from 18 to 36°C. The flowering date was recorded, and transformed into degree-days with a temperature basis of 0°C. Along the growing period, the length of the first two emerging primary branches (named as B0-1 and B0-2 in Moreau et al. 2007) was measured twice a week. The curve of branch elongation as a function of sums of degree-days from date of transfer in the greenhouse (Fig. 1) showed a short lag phase, followed by a linear phase. No plateau phase was observed, probably because the plants were harvested quite early. Each curve was

Table 1 Name and origin of 21 populations (names beginning by MTR or MLI) and eight lines of *M. truncatula* described for aerial morphogenetic traits, and description of the collection sites

Name	Origin	Flowering date (°C.D)	Branch length (cm)	Main stem length (cm)	No primary branches	No secondary branches	Branch diameter (mm)	Branch elongation rate (cm/°C.D)	Dry weight (g)	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual rainfall (mm)
MLI15	Ex-Yugoslavia	1448.5	18.9	0.5	7.0	0.8	1.65	0.0249	5.6	42.4	17.5	30	450
MLI108	Egypt	957.5	64.8	38.7	5.7	6.3	1.41	0.0969	8.0	31.6	26.0	20	150
MLI122	Libya	841.5	66.5	59.5	4.0	6.5	1.31	0.0948	7.9	30.6	18.4	25	125
MTR144	Jordania	908.8	42.1	51.5	3.0	0.8	1.68	0.0738	9.7	32.0	35.0	23	100
MTR163	Syria	806.0	48.9	41.7	4.3	5.0	1.70	0.0770	10.5	35.0	37.1	500	250
MTR174	Cyprus	924.7	51.5	41.7	5.0	4.3	1.85	0.0699	9.3	34.8	33.2	550	500
MTR190	Morocco	806.0	50.1	40.7	3.0	2.8	1.64	0.0924	7.2	29.0	–9.0	1150	200
MTR243	Libya	806.0	99.9	80.0	3.3	6.0	1.84	0.1185	13.8	32.0	22.0	30	100
MTR341	Algeria	1070.3	61.2	8.8	8.7	7.5	2.32	0.0673	16.7	35.5	6.9	980	450
MTR365	Algeria	1001.5	47.6	0.5	9.0	8.3	2.08	0.0316	11.6	36.5	5.4	1080	1300
MTR368	Algeria	957.5	41.0	6.0	7.7	8.7	2.79	0.0376	14.0	36.5	3.2	200	833
MTR369	Portugal	806.0	62.0	43.5	5.0	5.5	1.64	0.0821	13.2	37.2	–8.9	30	350
MTR376	Greece	941.0	50.6	0.8	8.0	10.0	2.93	0.0449	17.1	38.3	22.4	5	350
MLI377	Greece	1110.3	76.0	0.5	9.3	11.3	2.10	0.0662	13.4	40.7	24.0	10	500
MTR430	Spain	1070.3	67.2	1.2	7.7	9.2	2.37	0.0788	17.0	37.9	–4.5	180	500
MTR450	France	1319.0	56.4	3.8	9.0	7.2	2.04	0.0546	10.5	42.6	2.6	80	500
MTR466	France	756.0	68.9	8.0	6.0	13.7	2.08	0.0650	12.4	41.4	9.1	195	500
MLI518	France	1510.2	45.5	0.5	9.0	4.5	1.87	0.0550	8.5	43.1	2.5	180	750
MTR537	Greece (Crete)	971.3	76.3	41.3	7.0	7.2	2.14	0.0946	16.6	35.1	25.4	350	750
MTR648	France	1186.7	46.3	3.4	8.5	9.3	2.17	0.0341	9.8	42.5	2.6	8	500
MTR729	Unknown	900.8	63.9	38.7	4.3	7.0	1.92	0.0933	14.2	–	–	–	–
DZA45.5	Algeria	1230.0	54.5	25.7	4.7	6.5	2.16	0.0487	9.7	36.9	7.7	100	800
DZA45.6	Algeria	1230.0	57.2	15.7	5.0	7.0	2.18	0.0495	10.1	36.9	7.7	100	800
DZA315.16	Algeria	841.5	43.8	2.0	6.0	8.5	2.11	0.0331	8.2	34.7	0.2	1070	400
DZA315.26	Algeria	948.5	39.4	1.5	7.0	9.0	2.15	0.0291	8.3	34.7	0.2	1070	400
F83005.5	France	1385.0	58.3	4.0	6.3	7.7	2.34	0.0580	11.0	43.6	6.2	261	750
F83005.9	France	1321.5	47.9	4.2	6.0	6.5	2.12	0.0535	10.7	43.6	6.2	261	750
Jemalong-6	Australia	981.3	74.8	39.3	5.7	6.5	1.71	0.1017	12.1	–	–	–	–
Jemalong-A17	Australia	1037.5	69.7	33.0	6.3	6.0	1.68	0.0710	10.2	–	–	–	–
Mean		1049.1	57.4	21.6	6.3	6.9	2.01	0.0674	11.4				
Mean square error		137.4	7.8	5.1	1.2	1.8	0.14	0.0114	2.7				
H^2		0.857	0.912	0.983	0.856	0.861	0.953	0.929	0.752				

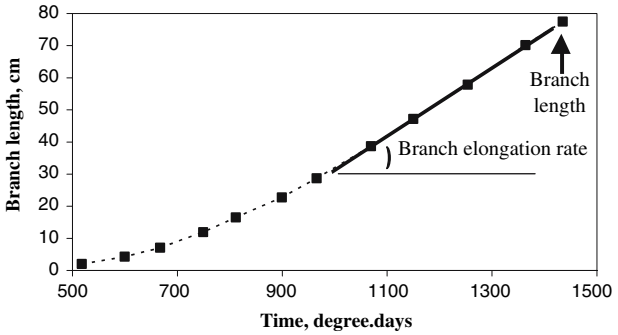


Fig. 1 Example of elongation curve of *M. truncatula* primary branch (line DZA315.16, at Lusignan in 2003) over time, and its description with two parameters, the branch elongation rate and the branch length

described by the slope of the linear phase, named as branch elongation rate, and the length of the branch at harvest date (Fig. 1). When all the plants of the trial had flowered (21 June 2004), the trial was harvested. The length of the main stem was measured and the number of primary branches was counted. The two primary branches that were measured were collected and their number of secondary branches was counted. In a basal portion of the primary branches, representing a mature part (1/6th, as determined in alfalfa stem; Guines et al. 2003), the diameter was measured. The dry weight of the aerial part of each plant was measured.

An analysis of variance was carried out to test the effect of accession on the traits. Genetic correlations among traits were calculated. Variances of accession, considered as a random effect, and error were estimated and broad sense heritability (h^2) was calculated as: $h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_R^2/b}$ with σ_G^2 the accession variance, σ_R^2 the error variance and b the number of blocks. A principal component analysis was carried out on the accession means, based on the correlation matrix. SAS statistical package, version 8.1, was used for all analyses (SAS Institute Inc. 2000).

QTL analysis

A population of 199 RILs in F6 generation, named LR4, was obtained from the cross Jemalong-6 × DZA315.16 (Huguet et al. 2004). The line Jemalong-6 was derived from one plant of the Australian cultivar Jemalong (registered in 1955, http://www.pi.csiro.au/ahpc/legumes/legumes.htm) selfed twice; the line DZA315.16 was derived from one plant of a wild Algerian ecotype and also selfed twice. The choice of these two parental lines was based on their numerous differences in terms of morphological, developmental or symbiotic traits. Jemalong-6 has an earlier flowering, fewer but longer primary branches and a more erect growth habit than DZA315.16.

The lines were sown five times in a greenhouse in France: on 22 February 2000 at INRA of Montpellier (122 lines, no replication, the seeds were placed at 5°C for 48 h after imbibition, natural vernalisation in the greenhouse), on 26 February 2001 at CNRS-INRA of Toulouse (109 lines, no replication, no vernalisation); on 02 April 2002 and 25 March 2003 at INRA Lusignan (three replications of 93 lines in alpha-lattice design, 2 weeks of vernalisation at 12/8°C D/N, photoperiod of 12 h, applied on seedlings with one trifoliolate leaf, then transferred to a greenhouse on 07 May 2002 and 24 April 2003, respectively), on 09 August 2004 at Lusignan (179 lines, no replication except for 15 lines with three replications, 3 weeks of vernalisation at 4°C applied on seeds germinated for 24 h, then transferred to a greenhouse on 30 August). In each year, day-length increased during the experiments, except in 2004 where it decreased from 14 h 31 min to 10 h 04 min. No supplemental light was added. A core set of 93 lines was present in each experiment. Average temperatures varied from 16 to 29°C except in 2004 where they varied from 12.5 to 27.5°C, with minimal temperatures reaching 6°C at the end of the experiment. The plants received 40 U of ammonitrate 1 month after transfer to a greenhouse.

At Montpellier and Toulouse, the flowering date was individually recorded and, in the first location, expressed in C.D above 0°C from sowing. At Montpellier, when 90% of the lines had flowered (03 May 2000), the longest primary branch was sampled, the secondary branches were removed, the leaves were separated from the stem, both leaves and stem were dried and weighed. The leaf to stem dry weight ratio and the leaf plus stem dry weight were calculated. At Lusignan, the same traits as in the analysis of the 29 lines were measured with a few changes: the length of the first two emerging primary branches was measured three times a week in 2002 and twice a week in 2003 and 2004. The trial was harvested when most of the lines (95% in 2002 and 2003, 90% in 2004) had flowered (19 June in 2002, 12 June in 2003, 29 November in 2004). For the non-flowered lines, it was considered that the plants would have flowered 1 week after the harvest, as follows: 1282°C.D in 2002, 1500°C.D in 2003 and 2120°C.D in 2004. The number of primary branches was counted in 2003 and 2004. The number of internodes and the number of secondary branches on the two measured primary branches were counted in 2002 and 2004. In 2002 and 2003, on the two stems that were measured, the secondary branches were discarded, the leaves were separated from the stems, both leaves and stems were dried and weighed. The leaf to stem ratio and the leaf plus stem weight were calculated.

For the data from Lusignan in 2002 and 2003, analyses of variance were carried out to test the effects of lines, years and replications and the interaction between year and line. Line effect was considered as random, year and

replication effects as fixed. Variances of line, year × line and error were estimated and broad sense heritability (h^2) was calculated as: $h^2 = \frac{\sigma_L^2}{\sigma_L^2+\sigma_{Y.L}^2/y+\sigma_R^2/b.y}$ with σ_L^2 the line variance, $\sigma_{Y.L}^2$ the line × year variance, σ_R^2 the error variance, b the number of replications and y the number of years. Genetic correlations among traits were calculated. On the 15 lines that were replicated three times in 2004 experiment, an analysis of variance was performed with the effects of line and replication to estimate the error variance.

A framework genetic map of LR4 population based on microsatellite markers was available (T. Huguet et al., unpublished). QTL mapping was performed using QTL-Cartographer (Basten et al. 1994, 2002) with the composite interval mapping (CIM) procedure with five background parameters. The threshold for adding a QTL was set to 11.8 (LOD ≥ 2.6), as determined with 1,000 permutations for giving a probability of 5%. The limits of the confidence interval of QTL position were estimated as the positions where the LOD value decreased by 1 unit relative to that of the most likely position. The software BioMercator (Arcade et al. 2004) was used to draw the QTLs on the map.

Mapping of candidate genes

The literature was searched for candidate genes involved in flowering date, plant height and branching among those known in pea, *Arabidopsis* or other species. Flowering genes (Hecht et al. 2005; Komeda 2004) belonged to the gibberellin pathway (as Gibberellic Acid Insensitive), the autonomous pathway (as Lumini-Dependens), the vernalisation pathway (as Vernalisation IndePendent 2), the light-dependent pathway (as CRYptochrome 1 and 2, PHYtochrome A, Constans, GIgantea) and the integration pathway (as Flowering locus T, LeaFY, Terminal FLower 1). Stem height is mainly determined by genes related to gibberellin biosynthesis (as Gibberellic Acid hydroxylase) and brassinosteroid (as BRassinosteroid Insensitive1, Brassinosteroid insensitive1-Associated receptor Kinase 1 and brassinosteroid receptor) pathways (Kwon and Choe 2005; Müssig 2005; Wang and Li 2006). Genes related to branching (McSteen and Leyser 2005; Ward and Leyser 2004) are related to meristem initiation, meristem outgrowth (More AXillary growth in *Arabidopsis* or RaMoSus in pea). The sequences of these genes in *M. truncatula* were searched in databases through keyword queries or blast between *A. thaliana* or pea gene sequence and *M. truncatula* EST or BAC sequences.

Five genes were partially sequenced on Jemalong-6 and DZA315.16: gibberellic acid 3β hydroxylase (G3H) that is responsible for stem dwarfism in pea (Le locus) (Martin et al. 1997), corresponding to LAX (Lateral AXillary) in *Arabidopsis*, TFL1a, an homologue of TFL1 that corresponds to DETERMINATE in pea (Foucher et al. 2003), Cry1, GAI and LD. In addition, Rms1 was sequenced on DZA45.5 and DZA315.26, parents of another RILs population (LR1, T. Huguet and J.M. Prosperi, unpublished).

Degenerated primers designed on pea were used to amplify and sequence TFL1a (Foucher et al. 2003) (Table 2). For the other genes, *M. truncatula* EST sequences, available on websites, were aligned on pea or *Arabidopsis* genes. Primers were defined to amplify gene portions of 500–1,000 bp for both parents. After sequencing and alignment, detected polymorphism was used to map the genes on the mapping populations, with specific primers that led to length or restriction site polymorphism (Table 2). In addition, Phospho Enol Pyruvate Carboxylase (PEPC), a gene coding for a protein involved in carbon fixation was mapped (Thoquet et al. 2002), and is one marker of this framework map (MTIC890). The genes were mapped using MAPMAKER software (Lander et al. 1987).

The positions of other genes were inferred indirectly. The BACs on which given genes were annotated were identified on the website of Okhlahoma University devoted to *M. truncatula* sequencing data (http://www.genome.ou.edu/medicago.html) and the website developed by Kazusa DNA Research Institute in Japan for genomic development on *Lotus japonicus* (http://www.kazusa.or.jp/lotus/). The chromosomal location of the BACs was determined (http://www.medicago.org/genome/BACregistry) on the integrated genetic map of University of Minnesota (UMN) (http://www.medicago.org/genome/map.php). SSR markers within the BACs or adjacent BACs were genotyped on our mapping population, to get the position of the candidate genes.

Results

Genetic variation for aerial morphogenesis in *M. truncatula*

A large significant variation was observed for all traits (Table 1). The mean square error that included within-population variation was low compared to the between population mean square. Accessions differed in flowering date, branch morphology (length, diameter) and branch elongation rate. In these conditions, some accessions were as a rosette, with an extremely reduced main stem, whereas other accessions had long main stem, reaching 80 cm. Branch length varied from 18.9 cm in MLI15, an accession with a very slow branch elongation rate, to 99.9 cm for MTR243 that had a rapid branch elongation rate. The accessions with short main stems had either short (as MLI15) or long (as MLI377) branches. The flowering date varied from 756 to 1510°C.D, a delay representing 29 days, for two accessions originating from France (MTR466 and

Table 2 Primers and conditions used to detect polymorphism between the two parents, and chromosome location for some genes involved in aerial morphogenesis

Gene	Forward and reverse primers	Restriction enzyme	Tm (°C)	Bands in Jemalong-6	Bands in DZA315.16	Location LG/cM
TFL1a	GAT GTT CCW GGW CCT AGT GAY CC	–	61	506	520	7/64.1
	CTT GCA GCR GTY TCY CTY TG					
G3H	TAA CCA CAA GCA CCC TGA TTT CA	*Mbo*II	59	323, 232, 91	232, 91	2/72.2
	TCC CTG CTA ACT TTT TCA TGG CT					
Cry1	GAA ATG CAA ATG TGG AGA GAG	–	57	516	466	5/60.4
	CGC TAT GAA AAT ACC ATT GCA					
GAI	GTC ACC GGA AAC GGA ATG	–	77	108	–	4/79.3
	GGA TAA CAA TTT CAC ACA GGG CCG AAT CTG ACG AAT AAC AG					
LD	ATG TCT GGA TAT AAG CCC	–	63	265	–	7/73.5
	ATC AGT AAG TCT TCG TTG					
PEPC	CTCATCCTACTCAGTCGGTTCGTCG	*Rsa*I	60	125, 200, 250	200, 250, 360	2/60.1
	ACACGTAGCTCATCGTTGCAACGCC					
Rms1	CTA TGC TTG TGG AGC ACA GCG	*Hae*III	52	417[a]	171, 246[b]	
	TTA CTG TTT TGG AAC CCA ACA					3/bottom

[a] In DZA315.26

[b] In DZA45.5

MLI518). For all lines but one, in these growing conditions, the first two emerging primary branches were longer than the main stem. The *M. littoralis* accessions had similar range of variation than the other accessions, except MLI15 that was very late flowering and had a very limited vegetative growth. When two lines were extracted from one accession (DZA45, DZA315, F83005, Jemalong), their differences were limited. Broad-sense heritabilities were high, especially for main stem length (Table 1).

Genetic correlations among traits were calculated (Table 3). Late-flowering genotypes had on average shorter main stem and branches and slower branch elongation rate than early flowering genotypes, but they had more primary branches. Branch length was positively correlated to branch elongation rate. Main stem length was positively correlated to branch length but negatively correlated to the number of primary or secondary branches. Stem diameter was weakly negatively correlated to stem length, strongly

Table 3 Genetic correlations between aerial morphogenetic traits, calculated over 29 *M. truncatula* accessions, and correlations between the traits and the annual rainfall of collection sites

	Flowering date	Branch length	Main stem length	No primary branches	No secondary branches	Branch diameter	Branch elongation rate	Dry weight
Flowering date		–0.310	–0.695	0.606	0.073	0.362	–0.659	–0.262
Branch length			0.519	–0.263	0.072	–0.326	0.724	0.203
Main stem length				–0.939	–0.740	–0.698	0.873	–0.302
No primary branches					0.764	0.606	–0.773	0.475
No secondary branches						0.584	–0.531	0.380
Branch diameter							–0.691	0.732
Branch elongation rate								0.083
Annual rainfall	0.483*	NS	–0.567**	0.527**	NS	0.486**	–0.542**	NS

NS Not significant

* Significant ($P < 0.05$)

** Significant ($P < 0.01$)

negatively correlated to stem elongation rate and positively correlated to the number of branches.

For flowering date, number of primary and secondary branches, branch diameter and dry weight, the parents of the mapping population, Jemalong-6 and DZA315.16 were included in the range of variation covered by this set of accessions. But Jemalong-6 had extremely long branches and a high branch elongation rate. DZA315.16 had a very short main stem. Surprisingly, Jemalong-6 that is described as an early flowering line flowered slightly later than DZA315.16 in this experiment.

The accessions were plotted on the first two axes of a principal component analysis (Fig. S1, Electronic supplementary material). There was no clear structuration of the material, but the accessions from Europe tended to flower later, to have more branches, shorter main stem and branches than the accessions from North Africa and Near East.

The correlations between traits and characteristics of the collection sites (Table 3) showed that accessions collected in sites with a higher annual rainfall flowered later, had shorter main stem and slower stem elongation rate, but more primary branches and larger branch diameter. Correspondingly, the first axis was positively correlated to annual rainfall and to the latitude. The correlations of the axes with the other site characteristics were non significant or low.

QTL analysis

Quantitative analysis of the traits

A large variation in LR4 population was recorded for all traits in each year (Table 4). In most cases, a difference was observed between the parents, and the distribution of the lines followed a normal-shaped curve around the mid-parent values (Fig. 2). Each year, DZA315.16 flowered later than Jemalong-6. But there was a shift in the distribution of the RILs in favour of early lines. Possible cases of transgression (i.e. lines with lower values than the lowest parent or higher values than the highest parent) were observed. The means and range values markedly differed among years. For instance, mean branch length was around 80 cm in 2002 and 2003, but 42 cm in 2004. It should be noticed that the conditions of vernalisation, photoperiod and temperature were not the same every year.

In analyses of variance on the data collected in 2002 and 2003, the variance of the line effect was much larger than the variance of the interaction line × year (Table 5). The error variance was of similar range to that of line variance. The broad sense heritabilities

Table 4 Mean and range of variation of the traits recorded in five locations in LR4 population of *M. truncatula*

Trait	Montpellier 2000		Toulouse 2001		Lusignan 2002		Lusignan 2003		Lusignan 2004	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Flowering date[a]	790	639–1181	91.6	58–138	1023	878–1282	1147	899–1500	1503	867–2120
Branch length, cm	–	–	–	–	81.6	57.7–100.2	77.9	35.0–97.7	42.3	7.7–73.3
Branch elongation rate, cm/°C.D	–	–	–	–	0.149	0.112–0.190	0.109	0.065–0.138	0.041	0.008–0.076
Branch diameter, mm	–	–	–	–	2.29	1.88–2.80	2.35	1.67–2.80	1.83	1.36–2.35
No internodes	–	–	–	–	15.7	13.0–17.2	16.5	10.5–18.2	14.8	10.0–17.5
No primary branches	–	–	–	–	–	–	9.54	7.0–14.7	5.1	3.0–8.0
No secondary branches	–	–	–	–	11.7	8.5–13.2	–	–	8.0	0.0–12.5
Stem + leaf dry weight, g	5.79	2.36–15.13	–	–	1.49	0.83–2.17	1.71	0.66–2.37	2.25	0.2–6.5
Leaf to stem ratio	1.19	0.80–1.64	–	–	0.646	0.421–1.238	0.66	0.36–1.11	–	–
Main stem length	–	–	–	–	–	–	–	–	14.0	0.1–57.5

[a] In days from 1st January at Toulouse, in °C.D from sowing in the other locations

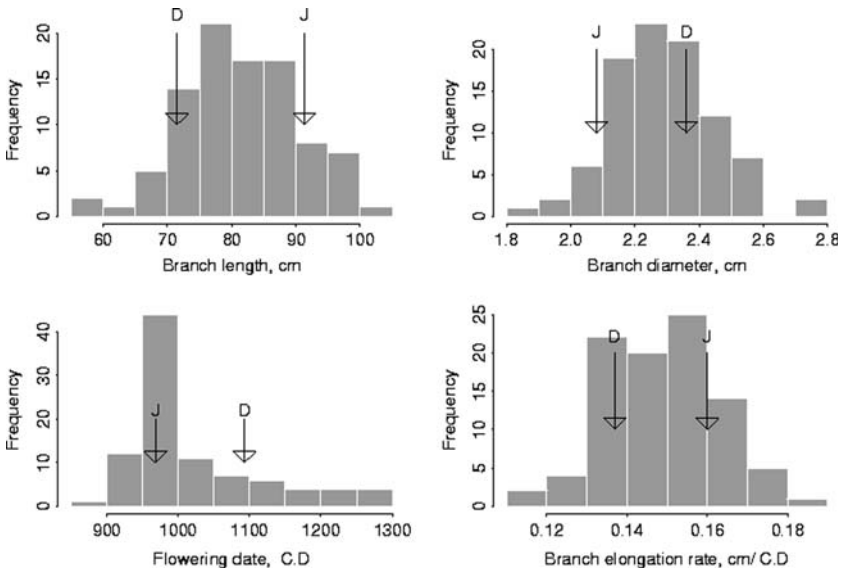


Fig. 2 Distribution of *M. truncatula* lines of LR4 population for several quantitative traits related to aerial morphogenesis in 2002. Mean values for the Jemalong-6 (J) and DZA315.16 (D) are indicated by *arrows*

Table 5 Variance components (line, line × year and error) and broad sense heritability (h^2) for a RILS population of *M. truncatula* in two years (2002 and 2003) at Lusignan. All factors in analysis of variance were significant, except those indicated as NS ($P > 0.05$)

Trait	Variance			h^2 (%)
	Line	Line × year	Error	
Flowering date	8665.4	1846.4	5720.0	82.2
Branch length	68.54	9.02	58.01	82.9
Branch elongation rate	1.28 E-04	0.07 E-04 (NS)	1.52 E-04	81.6
Branch diameter	0.0198	0.0012 (NS)	0.0303	77.8
No internodes	0.273	0.161	0.945	53.4
Stem + leaf dry weight	0.0527	0.0131	0.0634	75.5
Leaf to stem ratio	0.0109	0.0033	0.0157	71.9

Table 6 Genetic correlations among traits in LR4 population of *M. truncatula*, calculated over 2 years (2002 and 2003) at Lusignan

Trait	Branch length	Branch elongation rate	Branch diameter	Dry weight	Leaf to stem ratio
Flowering date	–0.69	–0.29	–0.13	–0.16	0.59
Branch length		0.80	0.38	0.55	–0.81
Branch elongation rate			0.26	0.48	–0.55
Branch diameter				0.83	–0.22
Stem + leaf dry weight					–0.31

were high, especially for branch length, flowering date and branch elongation rate. The number of internodes had the lowest heritabilities. Error variances in 2004, evaluated on 15 lines, were similar to error variances in 2002 and 2003 (not shown).

Genetic correlations calculated among traits observed in 2002 and 2003 are indicated in Table 6. Flowering date was strongly negatively correlated to branch length and positively correlated to leaf to stem ratio. Its correlation with branch elongation rate, branch diameter and dry weight was low. Branch length was positively correlated to branch elongation rate and less intensively to branch diameter. Dry weight was positively correlated to all growth traits, branch length, branch elongation rate and especially to branch diameter. Correspondingly, leaf to stem ratio was negatively correlated to branch length and elongation rate, but its correlation to branch diameter and dry weight was low.

QTL identification

The QTLs found by CIM procedure are presented in Table 7 and in Fig. 3. The R^2 for each QTL varied from 5.2 to 59.2%. For the flowering date that was recorded over 5 years, a QTL explaining from 33.9 to 59.2% of the variation was detected on chromosome 7, between 51 and 59 cM. Each year, the Jemalong-6 allele had a negative effect on flowering date. The Jemalong-6 allele in this region of chromosome 7 was also associated with increased branch length in 2002–2004, increased main stem length in 2004, increased branch elongation rate in 2003, decreased branch diameter in 2004, decreased number of primary branches in 2003–2004 and decreased leaf to stem ratio in 2000, 2002 and 2003. Variation explained by the QTLs of this region was generally large. Other QTLs for flowering date, explaining from 5.2 to 16.9% of the variation were

Table 7 QTL for aerial morphogenetic traits revealed by composite interval mapping in *M. truncatula* recombinant inbred lines of LR4 population

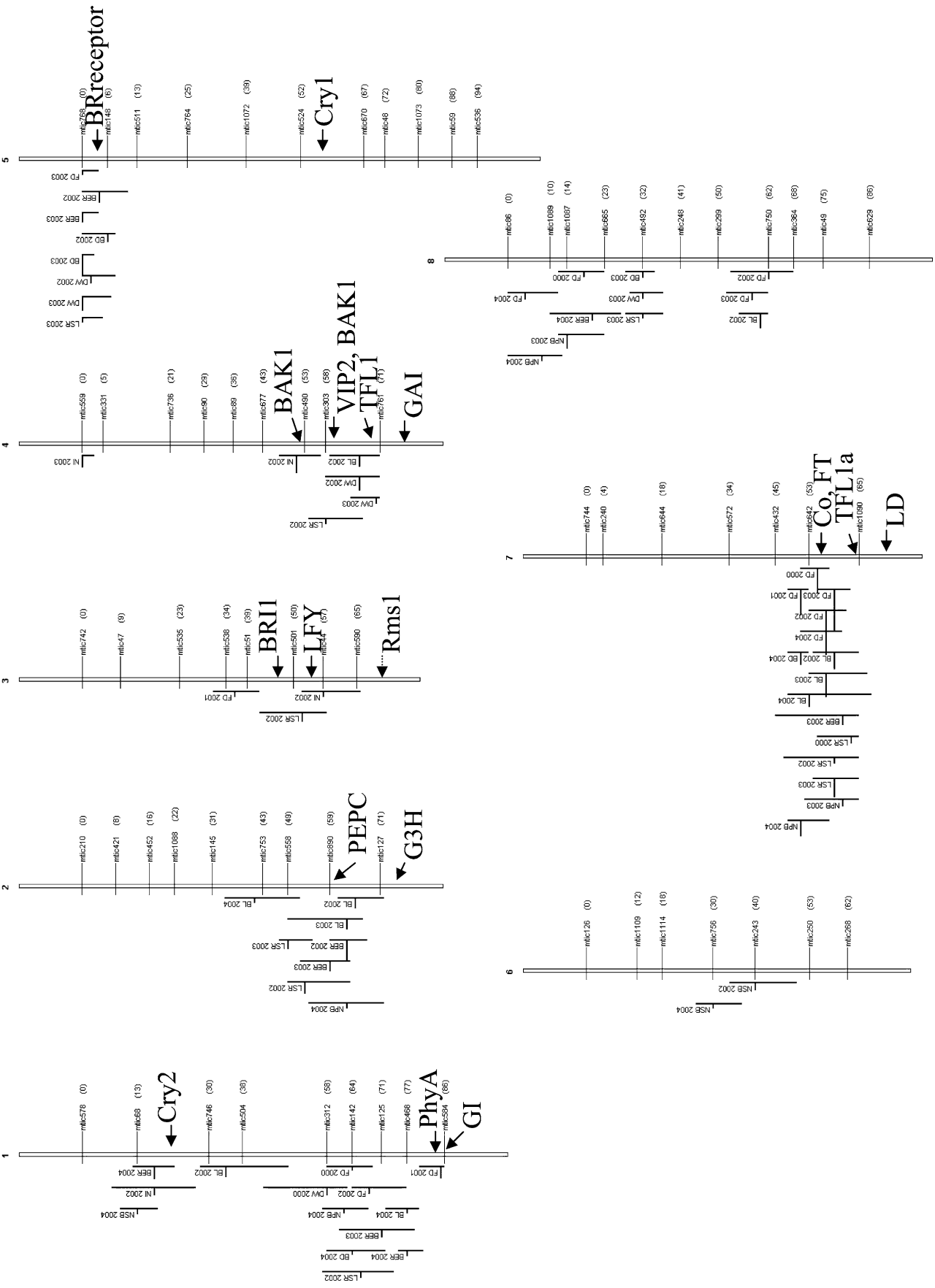
Trait	Chromosome	QTL position and confidence interval (cM)	LOD	Effect of Jemalong-6 allele	R^2
Montpellier 2000					
Flowering date	1	64 (58–69)	3.6	27.1	5.2
	7	55 (51–58)	17.1	–69.2	35.8
	8	18 (12–23)	4.3	–31.4	7.5
Dry weight	1	58 (43–63)	2.6	0.92	10.8
Leaf to stem ratio	7	63 (55–65)	3.1	–0.057	9.8
Toulouse 2001					
Flowering date	1	85 (80–86)	7.0	8.7	16.9
	3	36 (31–42)	3.0	–0.1	7.1
	7	51 (48–53)	12.7	–13.1	36.9
Lusignan 2002					
Flowering date	1	68 (64–77)	3.1	28.1	8.2
	7	57 (53–62)	10.9	–64.6	42.3
	8	62 (53–68)	3.7	–31.8	10.1
Branch length	1	34 (28–49)	3.8	3.64	14.0
	2	65 (61–72)	3.7	3.60	14.3
	4	66 (59–71)	3.9	–3.72	15.7
	7	59 (54–65)	6.3	4.53	23.8
	8	60 (55–62)	5.4	4.36	20.5
Branch elongation rate	2	63 (59–68)	7.4	0.0080	29.5
	5	4 (0–11)	4.8	–0.0057	15.3
Branch diameter	5	6 (0–8)	3.4	–0.0640	11.1
No internodes	1	17 (7–27)	2.9	0.276	10.6
	3	57 (52–66)	2.6	0.305	7.2
	4	51 (47–57)	7.3	–0.398	24.2
No secondary branches	6	40 (34–50)	2.7	0.299	10.3
Dry weight	4	66 (58–71)	4.8	–0.120	19.1
	5	2 (0–8)	3.1	–0.092	10.9
Leaf to stem ratio	1	66 (57–74)	2.7	0.0380	8.6
	2	53 (49–64)	5.7	–0.0557	16.8
	3	52 (42–58)	4.2	–0.0594	12.3
	4	58 (54–67)	2.9	0.0365	7.4
	7	59 (47–65)	2.9	–0.0379	8.3
Lusignan 2003					
Flowering date	5	0 (0–4)	3.8	33.0	5.6
	7	59 (55–63)	18.9	–103.3	59.2
	8	58 (52–62)	5.3	–43.6	10.4
Branch length	2	63 (49–67)	3.9	4.57	14.4
	7	57 (53–67)	9.1	6.72	33.3
Branch elongation rate	1	71 (61–79)	3.0	0.0044	10.3
	2	59 (52–64)	3.9	0.0054	14.3
	5	0 (0–4)	3.9	–0.0051	13.6
	7	61 (45–65)	3.9	0.0051	13.3
Branch diameter	5	0 (0–3)	10.2	–0.106	28.4
	8	32 (28–35)	3.8	0.063	8.1
No internodes	4	0 (0–3)	4.2	–0.449	16.2
No primary branches	7	61 (52–65)	2.6	–0.520	9.9
	8	14 (12–23)	5.1	–0.680	19.0

Table 7 continued

Trait	Chromosome	QTL position and confidence interval (cM)	LOD	Effect of Jemalong-6 allele	R^2
Dry weight	4	70 (64–71)	2.8	–0.100	8.4
	5	0 (0–4)	6.8	–0.153	19.3
	8	32 (29–37)	3.0	0.099	8.5
Leaf to stem ratio	2	49 (47–55)	4.9	–0.0574	12.2
	5	0 (0–5)	2.6	0.0395	6.1
	7	59 (54–65)	9.2	–0.0806	26.3
	8	32 (28–37)	2.7	–0.0403	6.4
Lusignan 2004					
Flowering date	7	57 (51–61)	13.5	–218	33.9
	8	4 (0–12)	4.3	–115	9.5
Branch length	1	77 (72–80)	3.1	–3.2	6.0
	2	41 (34–52)	3.0	3.5	6.8
	7	53 (48–68)	7.9	5.3	17.0
Branch elongation rate	1	17 (12–22)	4.6	0.0073	17.0
	1	77 (75–81)	3.1	–0.0037	7.2
	8	20 (10–27)	3.3	0.0042	9.2
Branch diameter	1	64 (58–72)	3.0	0.052	5.6
	7	51 (48–53)	7.7	–0.090	17.4
No primary branches	1	62 (57–68)	3.3	0.32	6.5
	2	63 (54–72)	3.5	–0.35	7.9
	7	51 (48–58)	10.2	–0.57	22.5
	8	8 (0–13)	3.2	–0.32	7.1
No secondary branches	1	13 (9–18)	3.2	1.01	7.5
	6	30 (26–37)	2.6	0.80	6.0
Main stem length	7	55 (50–62)	11.6	5.94	21.0
	8	2 (0–7)	6.4	4.68	11.0
	8	36 (27–48)	3.8	3.51	6.7

observed on chromosomes 1 (in 2000–2002) with a positive effect of Jemalong-6 allele, 5 (in 2003) with a positive effect of Jemalong-6 allele, and 8 (in 2000, 2002–2004) with a negative effect of Jemalong-6 allele. In the region of chromosome 1 involved in flowering date, QTLs were observed for elongation rate in 2003–2004 (with a positive effect of Jemalong-6 allele in 2003 and a negative effect in 2004), branch diameter in 2004 (with a positive effect of Jemalong-6 allele), explaining up to 17.0% of variation. Higher on chromosome 1, QTLs were detected for branch length and number of internodes in 2002, number of secondary branches and branch elongation rate in 2004, in all cases. The bottom part of chromosome 2 was involved in variation in several traits involved in branch growth: branch length in 2002–2004, branch elongation rate in 2002–2003, leaf to stem ratio in 2002 and 2003, number of primary branches in 2004. The R^2 explained by the QTL of chromosome 2 were generally large, reaching 29.5% for branch elongation rate in 2002. The bottom of chromosome 4 was involved in branch length, number of internodes, number of secondary branches and leaf to stem ratio in 2002 and dry weight in 2002–2003, with negative effects of Jemalong-6 alleles for all QTLs except for leaf to stem ratio. The top of chromosome 5 carried QTLs for branch

Fig. 3 Linkage map of *M. truncatula* and QTL positions for aerial morphogenetic traits in LR4 population (*BD* branch diameter, *BER* branch elongation rate, *BL* branch length, *DW* dry weight, *FD* flowering date, *LSR* leaf to stem ratio, *NI* number of internodes, *NPB* number of primary branches, *NSB* number of secondary branches). On the right side of the chromosomes, the marker names are followed by their position indicated within *brackets*. *Vertical bars* on the left side of chromosomes indicate the confidence interval of the QTLs. The *horizontal bars* represent the position of the QTLs and the bar length is proportional to R^2 value. The candidate genes are shown by arrows (*Cry2* Cryptochrome 2, *PhyA* Phytochrome 1, *GI* Gigantea, *PEPC* Phospho Enol Pyruvate Carboxylase, *G3H* Gibberellic acid 3β Hydroxylase, *BRI1* Brassinosteroid Insensitive 1, *LFY* Leafy, *Rms1* Ramosus 1, *BAK1* BRI1 associated receptor kinase 1, *VIP2* Vernalisation Independence 2, *TFL1* Terminal Flowering 1, *GAI* Gibberellic Acid Insensitive, *BR* receptor: brassinosteroid receptor, *Cry1* Cryptochrome 1, *CO* Constans, *FT* Flowering locus T, *TFL1a* Terminal Flowering 1a, *LD* Lumini Dependens)


BRreceptor
Cry1
BAK1
VIP2, BAK1
TFL1
GAI
BRI1
LFY
Rms1
PEPC
G3H
Co, FT
TFL1a
LD
Cry2
PhyA
GI

elongation rate, branch diameter and dry weight in 2002–2003 with negative effects of Jemalong-6 alleles, but also flowering date and leaf to stem ratio in 2003 with positive effects of Jemalong-6 alleles. QTLs for number of secondary branches in 2002 and 2004 were found on chromosome 6. On chromosome 8, aside flowering date, many traits showed QTL distributed over the whole chromosome: branch length in 2002, branch elongation rate in 2004, main stem length in 2004, branch diameter, dry weight in 2003, with positive effects of Jemalong-6 alleles and leaf to stem ratio in 2003, number of primary branches in 2003–2004 with negative effects of Jemalong-6 alleles.

The traits that showed co-locating QTLs had significant correlations (for example, flowering date and branch length, or dry weight and branch diameter). The traits that were positively correlated (respectively, negatively correlated) had QTLs with Jemalong-6 alleles in the same direction (respectively, opposite direction): for example, branch length and branch elongation rate were positively correlated and QTLs for these traits on chromosome 2 showed a positive effect of Jemalong-6 allele. But branch length and flowering date that were negatively correlated showed opposite QTL effects on chromosome 7. For traits controlled by several QTLs, both parents contributed alleles of positive and negative effects. For example, for flowering date, Jemalong-6 allele on chromosome 7 had a negative effect, but Jemalong-6 allele on chromosome 1 had a positive effect.

Mapping of candidate genes and co-location with the QTLs

Seven genes were mapped after designing specific primers (Table 2). PEPC and G3H were mapped at 60.1 and 72.2 cM on chromosome 2, respectively; Cry1 was mapped on chromosome 5 at 60.4 cM; GAI was mapped on chromosome 4 at 79.3 cM; TFL1a and LD were mapped on chromosome 7, at 64.1 and 73.5 cM, respectively. In population LR1, Rms1 was mapped at the bottom of chromosome 3.

On chromosome 1, three genes involved in flowering date, Cry2, PhyA and GI, were found in adjacent positions to QTLs (Table 8, Fig. 3). On chromosome 2, PEPC gene was closer to the position of QTLs related to growth than gibberellic acid 3β hydroxylase, a gene involved in dwarfism in pea. On chromosome 3, LFY, a gene involved in flowering date and BRI1, a gene related to the brassinosteroid pathway, known to be involved in plant height, were mapped at the same position as QTLs for growth, but the branching gene Rms1 was located in a lower position than these QTLs. On chromosome 4, genes related to plant height and flowering date (two copies of BAK1, VIP2 and TFL1) showed a co-location with QTLs for growth. GAI, a flowering gene, was outside the confidence interval of these QTLs. At the top of chromosome 5, a gene related to a brassinosteroid receptor was mapped in the region of QTLs for stem growth. On chromosome 7, a gene encoding for an homologue of the flowering gene Constans and three copies of flowering locus T (FT) were found at the same position as the major QTLs involved in flowering date and stem growth. Two other genes controlling flowering (TFL1a and LD) were mapped in adjacent positions but were outside the support interval of these QTLs. No candidate genes were found to explain the QTLs on chromosome 8.

Discussion

Natural variation available in *M. truncatula* for aerial morphogenetic traits has not yet been described, except

Table 8 Position of candidate genes in the regions containing QTLs, identified by data mining

Chromosome	Gene name	Pathway	Accession number	Position on UMN map	Position on LR4 map
1	Cry2	Flowering	AC122161	19.2	37.8
1	PhyA	Flowering	AC148406	49.2	83.7
1	GI	Flowering	AC148397	58.5	101.3
3	LFY	Flowering	AC139708	69.6	54
3	BRI1	Height	AC137827	69.6	47.8
3	Ap3	Flowering	AC151483	72.5	51.6
4	BAK1	Height	AC151739	54.6	50.7
4	BAK1	Height	AC140029	59.0	58.9
4	VIP2	Flowering	AC148344	45.5	42.0
4	TFL1	Flowering	AC139526	61.1	61.8
5	BR receptor	Height	AC147178	4.4	5.0
7	Co	Flowering	AC133780	50.4	58.3
7	FT	Flowering	AC123593	50.4	58.3

for flowering date and stem morphology (Delalande et al. 2004; Julier et al. 2002). Such an analysis is a way of identifying contrasting lines that are useful for genetic analysis of traits. The present QTL analysis is an essential step towards the identification of zones of the genome involved in the phenotypic traits variation. As a contribution to the worldwide investment on the model legume *M. truncatula* (VandenBosch and Stacey 2003), this study is the first QTL analysis published on this species.

Trait variation

The variation in the set of 29 accessions was large for all aerial morphogenetic traits: flowering date, main stem and branch length, dynamics of branch elongation, and branch diameter. The late-flowering accessions have, on average, shorter main stem and branches, but more branches than the early flowering ones. A strong negative correlation was observed between main stem length and the number of primary and secondary branches. Depending on their genotype, carbon from photosynthesis is either allocated to the stem elongation or to the elaboration of more axes. Logically, plant dry weight was correlated to the traits that contributed to plant size: mainly branch diameter but also branch length and number of branches. Trait variation in this set of accessions was correlated to the climatic conditions of collection sites. Accessions collected in areas with high rainfall had later flowering, shorter main stem but more branches. In southern regions, the accessions had early flowering, although both early and late accessions were evidenced in northern regions. Indeed, most French lines were late flowering, one of them (MTR466) flowered early. Under short growing season, due to low rainfall, the accessions had an early flowering and rapid branch elongation that is detrimental to the elaboration of numerous axes. Correspondingly, under long growing seasons, the populations flowered later, built more branches that grew more slowly. In both cases, with either a few numbers of long axes or many axes of short length, the plants display a large number of reproductive sites, i.e. the flowers that are borne at leaf axillaries. When two lines coming from the same population were studied (DZA45.5 and DZA45.6, DZA315.16 and DZA315.26, F83005.5 and F83005.9, Jemalong-6 and Jemalong-A17), the difference between the two lines was low. Contrastingly, a difference was observed for *Aphanomyces euteiches* resistance between F83005.5 and F83005.9 (Moussart et al. 2006). This natural variation, related to its adaptation to ecogeographical conditions is available for genetic studies. Ten accessions among the 29 studied here belong to the core-collection of *M. truncatula* (Ronfort et al. 2006). The two parental lines chosen to build the mapping population were not very different for flowering date, compared to the variation available in the species, but showed large differences in aerial morphogenesis.

Consequently, a large genetic variation was also observed for all traits related to aerial morphogenesis in the LR4 Jemalong-6 × DZA315.16 RILs population. In *Arabidopsis* also, a large range of variation in flowering date was observed among RILs obtained from a cross between relatively similar parents (El Lithy et al. 2004). The LR4 lines were not observed in the same conditions as the 29 accessions, so it is difficult to compare the range of variation in the two sets of material. But the variation available among the 29 accessions was larger than among the RILs. Each year, Jemalong-6 flowered earlier than DZA315.16, even if the opposite was unexpectedly observed in the trial with 29 accessions. Limited differences in vernalisation conditions and day-length between this trial and the trials in 2002 and 2003 and an interaction between genotype and growing conditions could have produced this significant effect on flowering date. For most traits, the distribution of the lines for the traits was normal. For flowering date, the shift of the distribution towards earliness could indicate that some late lines were lost during the construction of the RILs population. The LR4 population was studied over 5 years, under contrasted environmental conditions: vernalisation intensity varied from none (Toulouse 2001) to very high (Lusignan 2004), day-length duration increased in all experiments except in Lusignan in the autumn 2004 in which day-length decreased during the experiment. Average growth temperatures were not controlled and varied according to the year of experiment, even if cold and chilling temperatures were avoided. In spite of this, the heritability of most traits was high with a low level of line × year interaction. Correlations among traits indicated that flowering date was negatively related to vegetative development, and in the same direction as in the trial with 29 accessions. Nevertheless, the branch diameter was positively correlated to branch length in the RILS population, but negatively correlated in the trial with 29 accessions. As observed in alfalfa (Julier and Huyghe 1997), leaf to stem ratio and stem dry weight were correlated to stem length.

To better understand the differences among the trials and the growing conditions, more data are needed on the effects of environmental conditions (vernalisation, temperature, photoperiod) on aerial morphogenesis. Vernalisation is known to reduce the delay to flower (Moreau et al. 2007), but photoperiod and temperature effects have to be analysed when they vary in large extent. Furthermore, the effect of environmental conditions has to be tested on a range of genotypes, as genotype × environment interactions are likely to be detected.

QTL identification

QTLs were found for a majority of traits, the major one explaining 59.2% of the variation of flowering date. In this experiment as observed in *Arabidopsis* (El Lithy et al. 2004), the traits with the highest heritabilities tended to give the highest R^2 for the QTLs. All chromosomes were involved in the variation of the traits under study. The co-locations among years were numerous, despite variation in experimental conditions. Co-locations among traits were consistent with genetic correlations. Indeed, flowering date and stem growth were correlated, and the QTL for flowering date on chromosome 7 was also close to QTLs for stem growth. It is interesting to notice that although flowering date has a skewed distribution, with more early flowering lines (as Jemalong-6) than late ones, the markers close to the QTL of chromosome 7 were also distorted, with more lines carrying the Jemalong-6 allele than those carrying the DZA315.16 allele. Correlation between flowering date and stem growth, together with co-location of QTLs could indicate a common genetic regulation. An underlying gene involved in both flowering and growth traits can be assumed, as it is known that some genes, such as those involved in the gibberellin pathway (Komeda 2004), have an effect on both flowering date and plant growth. Similarly, a coordinated regulation of flowering and branching in response to photoperiod was evidenced in pea (Beveridge et al. 2003). The other possibility for explaining the QTLs for several traits (as in chromosome 7) would be the presence of two or more distinct genes in close positions. In *Arabidopsis* and maize (*Zea mays*), QTLs involved in both flowering date and morphological traits were also identified (Bandaranayake et al. 2004; Chardon et al. 2004; El Lithy et al. 2004).

We found a major QTL for flowering date in the cross Jemalong-6 × DZA315.16. In most QTL studies in *Arabidopsis*, several QTLs were found, as expected from the quantitative nature of flowering date (Koornneef et al. 2004). The analysis of other crosses in *M. truncatula* would be interesting to know if other strong QTLs could be found. This major QTL was detected in all years, even if the experimental conditions varied a lot. On Jemalong-A17, flowering date appeared to be affected more by vernalisation duration than by photoperiod and temperature (Moreau et al. 2007). But in our study, the QTL on chromosome 7 was present whatever the vernalisation intensity. This region is also involved in QTL of many other traits that showed significant correlations to flowering date. A common genetic regulation could be anticipated (i.e. a single gene with pleiotropic effects), a hypothesis more probable than different genetic regulation, because the direction of QTL effects were in accordance to the genetic correlation between traits. Another major QTL position was identified at the top of chromosome 5, and explained a part of variation for several traits of branch diameter, dry weight, leaf to stem ratio, branch elongation rate and flowering date. A gene regulating branch diameter, with a pleiotropic effect on flowering date could be involved. Increase in branch diameter is related to increase in dry weight, so this gene would also have an effect on dry weight variation.

For all traits, several QTLs were identified. For flowering date, alongside the major QTL on chromosome 7, QTLs were found on other chromosomes according to the year. These minor QTLs could be related to the effect of environmental conditions that varied among the experiments (vernalisation duration, photoperiod and temperature).

In most cases, when several QTLs were found for a given trait, both parents contributed alleles to positive and negative effects. This situation may explain the presence of transgressive RILs lines that carried all positive or all negative alleles, and flowered then either earlier than the earliest parent or later than the latest one.

Gene and QTL co-locations

A number of genes involved in flowering date, plant height or branching were mapped. These genes were listed from published studies on *Arabidopsis* or other species in which the effect of gene disruption was found to have an effect on phenotypic traits (Komeda 2004; Wang and Li 2006; Ward and Leyser 2004). A co-location between genes and QTLs is a first step towards the identification of the genes responsible for the QTLs. An interesting co-location was observed on chromosome 7 between the QTL for flowering date and two groups of genes: Constans and three copies of FT, both described as belonging to floral pathway. CO is functional for integrating the light pathway and FT belongs to the integration pathway, and seems responsible for the switch towards flowering (Komeda 2004). CO and FT genes directly interact (Samach et al. 2000). In *Arabidopsis*, the major genes involved in natural variations of flowering date (Frigida and FLC, (Alonso-Blanco et al. 2005) do not include CO or FT. Similarly, comparing the regulation of flowering time in *Brassicaceae* and *Poaceae*, it was shown that the response to photoperiod and vernalisation involved allelic variation at different genes (Alonso-Blanco et al. 2005). The effect of CO and FT genes on vegetative growth is not clearly described, but can be assumed, given (1) the relationship between floral and growth pathways, and (2) the high genetic correlations between flowering date and growth. So on the bottom part of chromosome 7, presumably a single gene could explain variations in many traits (flowering date and stem growth). On chromosome 2, the gene encoding for a major protein of photosynthesis (PEPC) is located in the same region as

QTLs for traits related to stem growth. At the top of chromosome 5, where QTLs for several traits related to branch elongation rate, plant dry weight and branch diameter were located, a candidate gene involved in brassinosteroid perception was mapped. Brassinosteroid mutants are described to be dwarf, sometimes with thicker stems (Kwon and Choe 2005). On chromosome 4, the QTLs involved in stem growth were mapped in the same region as genes involved in flowering date or brassinosteroid pathways: VIP 2, TFL1 and BAK1.

This study indicated genomic regions in *M. truncatula* involved in the variation for quantitative traits related to aerial morphogenesis. The small size of *M. truncatula* genome gives the possibility of positional cloning where a QTL is found (Ané et al. 2004; Endre et al. 2002). We are developing a program aiming at evidencing the gene explaining the QTL of flowering date on chromosome 7. Innear future, when *M. truncatula* genes involved in traits of agronomic interest for legume crops are found, it will be possible to analyse allelic variation available in legume crops and to test their effects on agronomic traits. The methods of association genetics (Flint-Garcia et al. 2003) will be useful. The underlying hypothesis that remains to be tested is that the genes explaining genetic variation in the model legume would also explain variation in crop legumes. All these developments would add to the model legume status of *M. truncatula*, and would be especially important for species with very complex genomes and biology such as alfalfa, clovers and peas.

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