Theor Appl Genet (2006) 112: 1024–1041
DOI 10.1007/s00122-005-0205-y

ORIGINAL PAPER

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Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*

Received: 10 October 2005 / Accepted: 20 December 2005 / Published online: 14 January 2006

Abstract The identification of the molecular polymorphisms giving rise to phenotypic trait variability—both quantitative and qualitative—is a major goal of the present agronomic research. Various approaches such as positional cloning or transposon tagging, as well as the candidate gene strategy have been used to discover the genes underlying this variation in plants. The construction of functional maps, i.e. composed of genes of known function, is an important component of the candidate gene approach. In the present paper we report the development of 63 single nucleotide polymorphism markers and 15 single-stranded conformation polymorphism markers for genes encoding enzymes mainly involved in primary metabolism, and their genetic mapping on a composite map using two pea recombinant inbred line populations. The complete genetic map covers 1,458 cM and comprises 363 loci, including a total of 111 gene-anchored markers: 77 gene-anchored markers described in this study, 7 microsatellites located in gene sequences, 16 flowering time genes, the *Tri* gene, 5 morphological markers, and 5 other genes. The mean spacing between adjacent markers is 4 cM and 90% of the markers are closer than 10 cM to their neighbours. We also report the genetic mapping of 21 of these genes in *Medicago truncatula* and add 41 new links between the pea and *M. truncatula* maps. We discuss the use of this new composite functional map for future candidate gene approaches in pea.

Communicated by Y. Xue

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Introduction

The identification of the molecular polymorphisms giving rise to phenotypic trait variability—both quantitative and qualitative—is a major goal of the present agronomic research. Different approaches such as positional cloning or transposon tagging (for a review, see Morgante and Salamini 2003), as well as the candidate gene approach (Bhattacharrya et al. 1990; Martin and Smith 1995; Byrne et al. 1996; Harrison et al. 1998; Craig et al. 1998, 1999; Pelleschi et al. 1999; Frewen et al. 2000; Thornsberry et al. 2001; Osterberg et al. 2002; Page et al. 2002; Foucher et al. 2003) have been used to discover the genes underlying phenotypic variation in plants. The construction of functional maps including genes of known function is an important component of the candidate gene approach. In this approach, two types of candidates can be defined: (i) functional candidates are genes of known function whose expression may affect the trait of interest; or (ii) positional candidates are genes that are located in the region of a quantitative trait loci (QTL) or of a mutation affecting the trait of interest.

A functional map allows to verify for any mapped QTL or mutation, if any of the mapped genes is a good candidate. Numerous functional maps have been built in various species, using genes from specific pathways (Causse et al. 1995; Byrne et al. 1996; Chen et al. 2001), RGA genes (Pflieger et al. 1999; Wang et al. 2001; Pfaff and Kahl 2003; Lanaud et al. 2004), or ESTs expressed in specific organs and/or for specific treatments or stages (Ishimaru et al. 2001; Causse et al. 1996, 2004; Potokina et al. 2004; Ren et al. 2004).

In pea, there is a long history of genetic and mutation mapping (for a review see, McPhee 2005). The first mutation map was published by Wellensiek as early as 1925 (cited by Rozov et al. 1999), and numerous mutations were mapped on different segregating populations afterwards (for a synthesis see, Weeden and Wolko 1990). More recently, different types of molecular markers were used to map expressed genes in pea: firstly isozymes (Weeden and Marx 1987; Irzykovska et al. 2001), later cDNAs through the RFLP technique (Hall et al. 1997) and genes of known function or ESTs through PCR-based techniques (Gilpin et al. 1997; Weeden et al. 1999; Timmerman-Vaughan et al. 2000; Konovalov et al. 2005). A consensus map was calculated in order to place 46 genes (Weeden et al. 1998) and 104 mutations (Ellis and Poyser 2002) on the same map. However, the number of markers mapped in any single population was limited, and expressed sequences were not all of known function. Another opportunity for candidate gene approaches is now offered through advances in genome sequencing of the legume model species *Medicago truncatula* (Young et al. 2005). The annotation of the genome sequences will allow precise definition of the position of most of the genes in these model species. Establishing the syntenic relationship between pea and the model species *M. truncatula* will permit to infer gene positions in the pea genome from *M. truncatula* gene positions. Two recent comparative mapping studies reported a good conservation of marker order between pea and *M. truncatula* for a set of 57 genes (Choi et al. 2004), and between pea and *M. sativa*, a crop species closely related to *M. truncatula* for a set of 103 genes (Kalo et al. 2004).

In the present paper we report: (i) the development of 63 single nucleotide polymorphism (SNP) markers and 15 single-stranded conformation polymorphism (SSCP) markers for genes encoding enzymes mainly involved in primary metabolism, (ii) their genetic mapping in pea, and (iii) the genetic mapping of 21 of these genes in *M. truncatula* and the map correspondence for 44 genes between pea and *M. truncatula* genetic map. Our main objectives were to provide an enriched functional map of pea including 111 genes and mutations, and to add new links between the pea and *M. truncatula* maps, for future candidate gene approaches in pea.

Materials and methods

Plant material

In order to develop SNP markers in pea, DNA sequence data were obtained in three genotypes used as parents of two mapping populations: 'Térèse', 'Champagne', and K586. Then, the markers developed were genotyped in one of the two pea recombinant inbred line populations (RILs) developed by single seed descent from the cross between Térèse × K586 (Pop1, 139 F7 RILs, Laucou et al. 1998), and from the cross between Térèse × Champagne (Pop2, 164 F8 RILs, Loridon et al. 2005). Total DNA was extracted from leaf tissue harvested on plants grown in a glasshouse, following two methods (Dellaporta et al. 1983 for the parental genotypes and Pop1, and Murray and Thompson 1980 for Pop2). Some markers were also genotyped in an *M. truncatula* (*M. tr.*) recombinant line population derived from Jemalong × DZA315.16 (Thoquet et al. 2002) for an insight into the syntenic relationship between the two species. Total DNA was extracted using the Qiagen DNeasy 96 Plant Kit following the manufacturers instructions (Qiagen S.A, Courtaboeuf, France).

SNP and indel discovery

Gene sequences were selected from GenBank and EMBL databases, with a special focus on genes involved in carbon and nitrogen primary metabolism, transport, and proteolysis. PCR amplifications of the selected sequences were carried out on genomic DNA of the three parental genotypes for 49 genes and on DNA of K586 and Térèse only for 38 genes. Primer pairs were designed to amplify 0.3–3.0 kb sized fragments (Table 1), depending on the length and type of sequence available—genomic DNA or cDNA. PCR reactions were carried out in a total volume of 25 µl containing 20 ng of template genomic DNA, 0.2 mM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1X Taq buffer, and 1.5 U Taq polymerase (Eurobio, Les Ulis, France), in a PTC-200 thermocycler (MJ Research, Waltham, MA, USA). After an initial 3 min denaturation step at 94°C, 35 cycles each of 50 s denaturation at 92°C, and 50 s at the required temperature (Tm) (locus-dependent, not shown) and 3 min elongation at 72°C, were performed. These cycles were followed by a final 5 min elongation step at 72°C. PCR products were purified from 1–2% agarose gels using the NucleoSpin gel-extraction kit (Macherey-Nagel, Düren, Germany) and sequenced directly (MWG, Ebersberg, Germany). Sequences were aligned using ClustalW (http://www.infobiogen.fr/services/analyseq/cgi-bin/clustalw_in.pl). Sequence comparisons revealed insertions/deletions and SNPs among the parents.

Table 1 Genotyping conditions for mapping in *Pisum sativum*

Function assignment	Species-accession number	Locus name	Primer 5′ to 3′	Temperature [Tm (°C)]	Marker assay[a]	Mapping pop
Starch metabolism						
ADP-glucose pyrophosphorylase; agpl1 gene	Ps-X96766	Agpl1	CGTCGAGAATGTATTGATCTTGG CAATCCATAATCAGATGCGCGGC	60	CAPs, Sna1	2
ADP-glucose pyrophosphorylase; agpS1 gene	Ps-X96764	AgpS1	GTTTTAGCAATTGGGTATATGCTG GCAGATATATTAACAATTAACCGTAA	55	ASP	2
ADP-glucose pyrophosphorylase; agps2 gene	Ps-X96765	AgpS2	CACTCAATCGACATCTTTCTCGCG GCACCAGGAATCATCTCACTCCC	60	CAPs, Sna1	2
Granule-bound starch synthase I	Ps-X88789	Gbsts1	GTCAACTGGCAACCCAATCT CACTGTAAGTCCACATTATGCC GACATGGAACATAGAAACTGA AAAATGACAGTTTTGATGAACAC	51	ASP	1
Granule-bound starch synthase II	Ps-X88790	Gbsts2	GCCGGTGTATCTGAAAGCAT ACACCGTTCACAATTCCCCG TTGCCTTTGTTGTTATGCACG AACGTGTGTCAGTGCGTATAT	57	ASP	1
Starch branching enzyme II	Ps-X80010	Sbe2	CCCCGATGCTGATGGAAATCC CTTTGGCCCACATCAAAGCCG	62	CAPS, Eco RI	1
Plastidial phosphoglucomutase	Ps-AJ250770	Ppgm	AACCTGCACCAGAATCCATC GCTTCCGAATTTCGTAACTCC	60	Size	1
Sucrose metabolism						
Sucrose phosphate synthase	Ms-AF322116/Vf-Z56278	Sps	GATGATTGTGATGTTGTGTTGTGG CTGGATTTAACCGAATCACCACCG	58	CAPS, Rsa I	1
Glucose-6-phosphate/ phosphate-translocator	Ps-AF020814	Gpt	GAATGCGTATCCTTACCCTTGGC CATTGCTGGTCCTTCCACGGCG	60	CAPs, Hin4I	2
Sucrose synthase	Ps-AJ012080	Sucsyn	CATGCTCTTGTGGTTGAAACTTTGC CAATGATTATAAAAACTGAATCGATTTG	51	CAPs, TasI	2
Second sucrose synthase	Ps-AJ001071	SS	GGGAAAGGGATTTTGCAAC CCATCAACTCACTTTTAACCGG	60	CAPS, Hpa II	1
Sucrose synthase isoform 3 (sus3 gene)	Ps-AJ311496	Sus3	GCCTCGATTCCGAAACCAACG GATGCGTTTGAGCATCTCCTC	60	CAPs, BalI	2
Cell-wall invertase I	Ps-Z83747	Cwi1	TCCCAAAGGAGCTGTTTGGGG GAATCGGGTGCTTGGCCCG	55	CAPS, Tha I	1
Cell-wall invertase II	Ps-Z83339	Cwi2	GCCATCTTTCCWTCTCAAGCGTCCG GGGTCAGATACATTTTTAGGGTATGC	60	CAPS, Taa I	1
Beta-fructofuranosidase	Ps-X85327	bfruct	GGCTTCATGAATCTGGGAAACCATTGG CTTGGTTGGCCAATTTACCGG	55	CAPs, TasI	2
Phosphate translocator	Ps-X68077	Ptrans	TCGTCGTGTTCCTTGATTTATGG CACCAAACAATACACCACTCCC	55	Size	1
Transport						
Amino-acid permease 1	M. Tegeder, personal communication	PsAAP1	GTCATGATCCTCTTTGCTTGGG CCAAGATTCGTATGAACAACTTCC	57	CAPS, Taq I	1
Amino-acid permease 2	M. Tegeder, personal communication	PsAAP2	TTTGGACCATATATGGCATATGC CAATAAAATGCAGCAATCACAGCC	55	Size	1
Putative peptide/amino-acid transporter	Ps-CD860432	Peptrans	GCCGTGATTCGGATCTGATGG CGGTCGTATAAAGGAATGACTAC	58	CAPs, PciI	1

Table 1 (Contd.)

Function assignment	Species-accession number	Locus name	Primer 5′ to 3′	Temperature [Tm (°C)]	Marker assay[a]	Mapping pop
Pore protein	Ps-Z73553	Pore	GCCTCGTAGCAGTTTTTCAGGG CAAAGACAACGGAGACCAAGTG CTGCAGATACCAGAGCCC CGGAGGTGCAGTGACAGA	55	ASP	1
Cationic amino-acid transporter-like protein	Ps-CD859399	catAAtrans	GCAATTGATCCTGATACACCG GGAACATCGACCAATATTTAGTAAT	54	dCAPs, SspI	1
Sugar transporter 1	Ps-AF109922	Sut1	GTCTATCTTTTTTGTCCCCG GTCTTCTACACAACGGCTTC	55	Size	1
Hexose transporter	Vf-Z93775	Htrans	GTTCGCTAATCCTACCCGACACGCC CGATTTATGGAGTTGATAAATGGGG	58	CAPS, Sca I	1
P54 protein, putative sucrose binding protein precursor	Ps-Y11207	P54	CTATGCTCAAACTTAGCCGTAGGC CAGAACCAGAAGAAGGTGGC	60	CAPS, AluI	1
Putative sugar transporter	Ps-CD859323	Sugtrans	GCATACTAGTATGGCTACTACCC CACAACCCATGGAACTGCTCCC	58	Size	1
Tonoplast intrinsic protein	Ps-AJ243309	Tip	CACCTTGAAAGCCGGTTTGGC CGGTGAAGGCTCCACCAACC	60	CAPs, HaeIII	1
Plasma membrane intrinsic protein 1	Ps-AJ548795	Pip1	CATTCGGTTTGTTCTTGGCGAGG GAGTCTCTGGCACTACGTTTGGC	65	CAPs, Bsp119I	1
Plasma membrane intrinsic protein 2	Ps-AJ243307	Pip2	GCCGGTATCTCTGGTATGATACC CAGGAACATGAGAGTCCCTAGCG	66	CAPS, HpaII	1
Putative tonoplast intrinsic protein	Ps-CD860138	PutTIP	CATGCTTTCTCACTATTTGCCGC GCAACCAAAGGTTGATGTTGAGG	60	CAPS, MaeII	1
Calvin cycle, photorespiration						
Ribulose biphosphate carboxylase small subunit	Ps-X04333	Rbcs	TGCTTCTACGGTGCAATCG GCTCACGGTACACAAATCC CCATTAATACTATTCAAATAGC ATTTGAGAACTAGAAGACTATA	53	ASP	1
Aldolase	Ps-M97476/Ps-M97477	Aldo	GAGAATTCCCCCTGCTGTCC CAGCATAATACTCAGTAGCACC	65	Size	1
Cytosolic malate dehydrogenase	Ps-CD858827	MDHc	mATGCTGCTTTCCCTCTTTTG TCTAGGGAATCCACCAACCA	55	CE-SSCP	2
Phosphoglycerate kinase	Ps-CD858627	PGK1	mCCATGGGAGTGTTCGAGTTT CGACGGAATCTCCTCCTACA	40	CE-SSCP	2
Phosphoglycerate kinase	Ps-CD858627	PGK2	mCCATGGGAGTGTTCGAGTTT CGACGGAATCTCCTCCTACA	40	CE-SSCP	2
Fructose-bisphosphate aldolase	Ps-P46256	FBPaldo	hGTCTGCCTTTGTTGGAAAGTATG GACACCAGAGAGGTATTGGAGTG	55	CE-SSCP	2
Nitrogen metabolism						
Asparagine synthase-1	Ps-Y13321	PsAS1	GCATCCATCACGTCCGTGTGACG CGATGTCACTACGATAAGGGCTGC CATCAAAGGCCTTTCTTAGAATCC	58	CAPS, Ehe I	1
Asparagine synthase-2	Ps-Y13322	PsAS2	CTAATCACACGTTTAGGACCGG CGAAATCCAAACCGAACCTAATCC	58	CAPS, Rsa I	1
Aspartate amino transferase	Ms-L25334	PsAAT1	CAGTTTCACAAGGAGTGTCGC CATAGCCTATGAGTTCTTAACCAG	57	Size	1
Chloroplast glutamine synthetase (GS2) gene	Ps-U22971	Gsp	CTTTTAATAGAAATCAATTATCCG ATTTTATTTATTTGGTGTTCTCACTC	50	Size	2

Table 1 (Contd.)

Function assignment	Species-accession number	Locus name	Primer 5′ to 3′	Temperature [Tm (°C)]	Marker assay[a]	Mapping pop
Glutamine synthase	Ps-U28925	Gs3B	GGGTCTTTCTTTTAAAGACCAAAGC GTATACTCTTGTTCAATACCATACC	57	Size	1
Dihydrodipicolinate synthase	Gm-L36436/Pd-U70960	DHPS1	GCTGARGGTGTKRTWGTTGGTGG TACTCTTTGATCCGGTCATTTCCC	55	CAPs MboI	2
NIP protein	Ps-U15036	Nip	GGATTCTACTAACCACATGGACC CTGCATGTCAGAGGTCCATAGG	60	CAPS, Mbo II	1
Phosphoenolpyruvate carboxylase	Ps-D64037	PepC	CCGTACCGATGAAATCAAGAGG GCATCCATCACGTCCGTGTGACG	57	CAPS, Hinf I	1
Proteolysis						
Thiol protease	Ps-X66061	ThiolP	CCGAAGAGGATTACCCCTAYCGTGC GCTTCTCCCCAGCTACCACCCC	55	CAPS, Mbo II	1
Clp sérine protéase	Ps-AJ276507	Clpser	GTATTGGAGGAGGATTTTAGGG GCTTATGAGTTTGGAGGGGAGGGG	58	Size	1
Elsa protease	Ps-AJ278699	Elsa	CAAGCACGGTGTTAATCGTATGAG GCAGAGCAATTTTGAGCAGCACC	55	dCAPS, Alu I	1
Dipeptidyl peptidase IV-like protein	Ps-CD859478	DipeptIV	CATACAGCTGTGGATGGTTACCC CTTATCACTCTGCTTGGTTCTGTGG	55	CAPS, AluI	1
Cysteine protease homolog	Ps-U44947	NTH1	GAGTTGGGTTGAACGAGTTTTC TAGTCCCCCATGAGTTCTTCAC ATTGGAGAGGGGCTCTTAC AACTTCCTTGATTTTTTATAGGACC	51	ASP	1
Cell wall metabolism						
Reversibly glycosylated polypeptide	Ps-U31565	Rgp	GTATCTCCTTCAAGGATTCGGC CCCAAATGGTCACAGATAACC	55	CAPS, Hae III	1
Beta-1,3-glucanase	Ps-AJ251646	Gns2	CTCTTCTTGCTGAGGGTATTACCG CGCAGGATTCCAGCATCATAAACCG	55	Size	2
Endo-1,4-beta-glucanase 1	Ps-L41046	Egl1	TTCACTCGGTTCCGCTGCTTG CAGCTCCCAAAGATTTCACCGATTTG	69	Size	1
Xyloglucan fucosyltransferase	Ps-AF223643	Xyft	GTAGATCGGATCAGCATCGTGG CTCTGCAATGAGATGCAGTTGC	57	Size	1
Caffeic acid *O*-methyltransferase	Ps-CD858528	cOMT	mTTAACTTTGATTTGCCTCATGTT ATGGTCGTCGCTCCAGTC	55	CE-SSCP	2
Hormone metabolism						
Gibberellin 2-beta-hydroxylase	Ps-AF101383	Ga2ox	GGAGAAGACATAGACAAATTTAGG ATATTTAGGGTTTCGGATTTAGGG	55	Size	2
GA20 oxidase	Ps-AF138704/Ps-U70471	Ga20ox	TTAAGARTTTAGAAWGTTGTTTGGC GTTTGAACCCACTACATCTCC	50	dCAPS, Hae III	1
Abscissic-insensitive3	Ps-AB080195	Abi3	GTGTATCAGCTAATGATTTGAGG CTGCCAATACATCCAGTTAGC	55	CAPs, PciI	1
Ent-kaurenoic acid oxidase (KAO2)	Ps-AF537322	PsKao2	GAACCTGTTATGGAAGCTTTGG CGGAAAAATTCAGGATGCCTCTGC	55	CAPs, SecI	1
Seed storage						
Fabatin-like protein	Ps-CD859908	FabatinL	GTTATAACTTGTCATCATGGAGAGG CTCTGAACTTGGTGCTCTTAACC	55	CAPS, Hpy188I	1
Homologue to seed maturation protein PM34	Ps-CD860048/CD860075	PM34like	GAAGCAAGACACGCAACCTGG CAGTTATGTAAGAGGAGCACTGG	60	CAPS, TaqI	1
Putative peptidyl-prolyl *cis–trans*-isomerase	Ps-CD860684	PPIlike	hTTTTGAGCATGGCGAATG GCTTGCTTGTCTCCTGTGATT	55	CE-SSCP	2

Table 1 (Contd.)

Function assignment	Species-accession number	Locus name	Primer 5′ to 3′	Temperature [Tm (°C)]	Marker assay[a]	Mapping pop
bZip similar to O_2 transcription factor	Mt-BI311616	O_2like	CTTGTGAACAAAGTACAAACCC CACAAACTTGGATATTCTAACTGG	55	CAPS, Taq I	1
Nodulation						
Sym29 gene for serine-threonine protein kinase	Ps-AJ495759	Sym29	TAATCTCTAGAATCAGATAAATAACCAG GAAAATATTTCCAATCACTCAACGTG	50	dCAPS, Bme1390I	1
Nodulin26-like major intrinsic protein	Ps-AJ243308	Nlm	GCTTTTCTCTGTTCTTCACAAGCC GAGAGAAGCAGTGGCATGTGCGG	60	CAPS, MaeII	1
Nodule inception protein (sym35)	Ps-AJ493066	Nin	CCATTGAGAAGTGTCTACACTGGC GATGCAGTAGTAGCAGCATCGG	60	CAPs, NruI	1
N_3-like protein	H. Weber, personal communication	N_3like	GAGCCCATGCTTCTATCAACTC GAGCCCATGCTTCTATCAACTC	55	CAPs, HinfI	1
Early nodulin 12B	Ps-X57232	Eno12B	GAGGATCCTTACTAGTATAAAACC GTTTATCATAAACGTGCAAATGTGTCTC	60	CAPS NmuCI	1
Miscellaneous						
1-aminocyclopropane-1-carboxylic acid oxidase	Ps-CD859427/CD859452	ACCox	GAATCATGGCATACCTCATGACC GAGAAGGATGATCCCGCCAG	55	CAPs, MseI	1
RNA Helicase	H. Weber, personal communication	RNAhel	GGGTTTGGTAGGTTTGGTAGAGG GCTATCAAAATTGTAGTGGGTGGG	60	CAPS, AluI	1
UDP-glycose:flavonoid glycosyltransferase	H. Weber, personal communication	Ugfgt	CAAGTGCGATAGAAGCATCGGG AATCTGCCTTAATCGCTTAAGCTC	55	CAPs, AvaII	1
Superoxyde dismutase	Ps-CAA39819	SOD9	hCCAATTCAGTCGTTGGGAGA ATCTTCCACCAGCATTTCCA	60	CE-SSCP	2
Translational elongation factor	Ps-AY444796	eEF1Bb	hCAGCTTTGTCATCAGTTCCATC AGTGGCAACAGCCTCTTCAG	65	CE-SSCP	2
Violaxanthine de-epoxydase precursor	Ps-CD859115	Viola1	mTTCAAACCCGTCATGTGCT GAATTCATCAACCACGCTGT	52	CE-SSCP	2
Violaxanthine de-epoxydase precursor	Ps-CD859115	Viola2	mTTCAAACCCGTCATGTGCT GAATTCATCAACCACGCTGT	52	CE-SSCP	2
Ribosomal protein S13	Ps-CD860523	TE002L09	hAACGGCCACTTCTCCAATC TCAACACCACCAACACGAAC	58	CE-SSCP	2
Ribosomal protein L2	Ps-CD860545	TE002M14	mAGCCTCCGGTGATTATGCTA CCAGCCTTCAACATAGGCTTC	55	CE-SSCP	2
Cell-division cycle protein	Ps-CD860570	TE002O11	hTGACAAAGCTCGTGGATCTG GATTCAAAACCCTGTCAGCA	55	CE-SSCP	2
Chalcone isomerase	Ps-CD860583	TE002P07	hTTTCCACCTGGTGCTTCTGT TTGCATCCTCCTTTTCTGGT	55	CE-SSCP	2
Thaumatin-like protein	Ps-CD860773	ThaumatinL	hTCATTTGTTTAACTCACTCCCAAA GACGATGTCAAACCTTGCTG	55	CE-SSCP	2

[a]Marker assay and if necessary, enzyme used for restriction

Looking for putative orthologous genes in *M. truncatula*

In order to map putative orthologous genes in *M. tr.*, a number of strategies were used. When possible, we amplified the genomic DNA of the two *M. tr.* parental lines using the same primer pairs as used in pea. PCR conditions used for pea were tried initially, and PCR conditions were optimized in order to obtain a single band in the electrophoretic profile (Table 2). In eight cases, where there was no amplification using the pea primers, we looked for the orthologous sequence of the pea gene in the *M. tr.* EST databases (http://www.medicago.toulouse.inra.fr/Mt/EST/ or http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=medicago), and designed specific primers for *M. tr.* (Table 2). The amplification products were sequenced directly and screened for polymorphism between Jemalong and DZA315.16. In the remaining cases we looked for putative orthologous genes in *M. tr.* BAC sequences, and their linkage group assignment and position when available, on http://www.medicago.org/genome/ (Table 4).

We also used the reverse strategy, starting from *M. tr.*-mapped gene markers to design putative orthologous gene markers in pea. EST-derived microsatellite markers have been designed and mapped in *M. tr.* in the Jemalong × DZA315.16 genetic map (T. Huguet, unpublished data, http://www.medicago.toulouse.inra.fr/Mt/GeneticMAP/LR4_MAP.html). We searched the EMBL database and a pea EST database (http://www.gene-exp.ipk-gatersleben.de/pea_ests.html, H. Weber, personal communication) to find homologous sequences in pea. Where good homology was found, primer pairs were designed in order to amplify, sequence, and detect polymorphism between the corresponding Térèse and K586 sequences (Table 3).

Design of gene-anchored markers and population genotyping

Depending on the type of polymorphism found, different strategies were used to design new primers in order to optimize polymorphism scoring (Table 1).

1. If insertion/deletion events (indels) were present in the sequenced fragments, PCR products showing length polymorphism were separated directly on either 1–2.5% agarose gels, or on 6% polyacrylamide gels for genes containing a microsatellite motif.
2. If polymorphic restriction sites were present, CAPS markers (cleaved amplified polymorphic sequence) were designed: polymorphic profiles were obtained after cleavage by a restriction enzyme following the manufacturers' instructions (http://www.rebase.neb.com). Alternatively, a modified primer was designed when it was possible to create a restriction site at the level of the SNP for one of the parental genotypes, leading to deviated CAPS (dCAPS) markers (Neff et al. 1998). Restriction products were electrophoresed on 1–2.5% agarose gels and and visualized after Ethidium Bromide staining.
3. Alternatively, bi-directional allele-specific PCR was performed as described by Délye et al. (2002): two internal allele-specific primers (ASP) were designed (the 3′ end of the primer corresponding to a polymorphic region) and added to the two external primers of the PCR reaction, producing three amplimers (two specific for each genotype and one common to both parents). PCR products were electrophoresed on 1–2.5% agarose gel and visualized after Ethidium Bromide staining.

PCR reactions were carried out with the protocol described above. The markers were then genotyped in the different segregating populations. Mapping conditions are summarized in Tables 1, 2 and 3.

Detection of SNP/indel and genotyping using CE-SSCP assay

We also used the CE-SSCP technique (Andersen et al. 2003)—SSCP on Applied Biosystems Capillary Electrophoresis Systems (CE)—to detect sequence polymorphism between Térèse and Champagne. Primers were designed from EST sequences from the databases.

Table 2 PCR amplification conditions for allele sequencing in *Medicago truncatula*

Function assignment	Species-accession number	*Medicago* marker	Locus	Primer 5′ to 3′	Tm (°C)
RNA helicase	H. Weber, personal communication	MTIC 243	RNAhel	AAGGTTGACGGGGTGCAAGGTC CGACCTAATTCTTACAATTTCCTG	53
UDP-glycose: flavonoid Glycosyltransferase	H. Weber, personal communication	MTIC 75	Ugfgt	CAAGTGCGATAGAAGCATCGGG AATCTGCCTTAATCGCTTAAGCTC	55
N_3-like protein	H. Weber, personal communication	MTIC 97	N_3like	GAGCCCATGCTTCTATCAACTC TATATCACCATCTGAATTATGCC	57
Malate dehydrogenase	H. Weber, personal communication	MTIC135	MalDH	CCATGCAGGAGTTACTATTCTTCC GGTTTTAATTTCAATCCCTGCGGC	54
Unknown protein	H. Weber, personal communication	MTIC153	Mp153	GCTTTTGAAGCTGTTGCTGCTGTC CTGAGCTAATCAAACCACTCCC	55
Mp312	H. Weber, personal communication	MTIC312	Mp312	GTTCAAATATCGCCCTGATCCC CCAATTGCAATTACATATCAGCC	57
Early nodulin 12B	Ps-X57232	MTIC 459	Eno12B	GAGGATCCTTACTAGTATAAAACC CCTGAAACACATATACCAAAAGAC	57

Table 3 Genotyping conditions for mapping in *M. truncatula*

Locus	Primer 5′ to 3′	Tm (°C)	Marker assay
PsAAT1	GTTGTGACTTGTGAAGCTTACGC CATTAGTGTCGTGTCCGATGTCC	60	Size
Agpl1	GCCACAAGATGCATTGAACTCGCC CAAATATTGTGTGTTCTCTATTTACG	66	ASP
Bfruct	GGACCAATGAGATATGGAGG CTTGGTTGGCCAATTTACCGG	60	CAPS, HpyF10VI
Elsa	GAATGGTCTCCGACGTGGAGG GCCACACTAACAGGCCGAGC	65	CAPS, BspCNI
Gpt	TGCACACACAATTGGACTTGTCG CTTACCTCAAGCTTCTACCTTGCC	65	CAPS, NlaIII
PsAAP1	CCAAGAGCTTGCAAACTCCCCCAAGC ACATGATTTCATTTGGTGCAGTGC	65	dCAPs, HindIII
Rgp	CAATTTGAATAGATCCAGCTGAG GTATCTCCTTCAAGGATTCGGC	55	CAPs, Bsp119I
Sbe2	CCCCGATGCTGATGGAAATCC CTTTGGCCCACATCAAAGCCG	62	CAPs, NdeI
PepC	CTCATCCTACTCAGTCGGTTCGTCG ACACGTAGCTCATCGTTGCAACGCC	60	CAPs, RsaI
Agps2	CACTCAATCGACATCTTTCTCGCG GCACCAGGAATCATCTCACTCCC	60	CAPs, Tru9I
Clpser	GCATTTCCCACCTTCACTATCGC CTGCATCTGGATCAATAGTAGCC	55	CAPs, MboII
Cwi1	TCCCAAAGGAGCTGTTTGGGG GAATCGGGTGCTTGGCCCG	55	CAPs, MboII
Egl1	CAGTTTGCTTTGCAGTATCAAGG CAGGTAACTTGAACATGAGTCC	60	CAPs, AluI
Ga20ox	CAGGGGAACATTGTGGTTATGC GTCTTGTTGTTCACCACTGCC	60	CAPs, Tru9I
Cwi2 (TC72333)	CTGATCTTGTTAACTGGACCCC TAATCCATTCCCTTAGAAAAGGGCC	57	dCAPs, HpaII
Gbsts1 (TC59742)	GATCAATACAAGGATGCATGGG CACACCTTTCATATTCCAAATGCC	55	CAPs, TaqI
SS (TC62069)	GGCATGATGTTCAAATAATGC CTCGGCAGCAATTTCACTGGC	64	ASP
ThiolP (TC59458)	GAGAAATGAATGGAAAGTGTCGCAATT GAAGGATAGTTAGAGCAAAACC	57	dCAPS, MunI
Xyft (TC66794)	GTGTTCCCAAACGATAAACCAATT CTTAAATGGCCAGAATTGAGATCC	57	dCAPS, MunI
PsAS2 (TC67975)	CTAATCACACATTCCGAACGGG GGTGTTGTGGGGGTAGATATTGCC	60	Size
Gns2 (TC60980)	CATTCTGCTCTTGTTGCTGAGGG CTCTGTGCATTGCGCAAGATTCC	60	Size

Prior to primer design, sequences alignment between pea EST sequences and homologue genomic DNA from full sequenced species (*Arabidopsis thaliana* and/or *Oriza sativa*) were performed to determine the expected intron position along the pea sequence. Primers were then selected in order to surround the hypothetical intronic region of pea genomic sequence. For CE-SSCP analysis, one primer was labelled with the fluorescent dyes 6-FAM or HEX. PCR assay was performed in 15 µl containing 1×PCR buffer with 1.5 mM $MgCl_2$, 330 µM of each dNTP, 0.170 µM of reverse and forward primers, 0.625 U Taq polymerase (Biolabs) and 40 ng of template DNA. PCR conditions were 4 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 52–65°C (depending on the primers Tm), and 30 s at 72°C. PCR cycling was terminated by a 72°C extension step of 10 min. Denaturation and CE-SSCP fractionation was as described in the ABI3100Avant Reference Guide (Applied Biosystems). The labelled fragments were visualized on an Applied Biosystems DNA analyzer. The GeneScan™-500 LIZ® or ROX™ size standard used in all samples served as an internal ladder to align data from different capillaries and eliminate capillary-to-capillary or run-to-run variability. Peaks with peak height under 10 U in fluorescence intensity were excluded from the analysis. Polymorphic markers were genotyped in Pop2.

Map construction

The markers derived from genes described in Table 1 were added to other molecular markers (RAPD, RFLP, SSR) and placed onto the individual maps of Pop1 and Pop2 described in Loridon et al. (2005) using the try command of MAPMAKER/EXP version 3.0b (Lander et al. 1987; Lincoln et al. 1992). Markers exhibiting segregation distortion ($\alpha = 0.01$) were discarded for this first mapping step. Afterwards, the consensus map was built and marker order was refined using the 'annealing'

Table 4 Linkage group assignment for 45 genes in pea and *M. truncatula* (*M. tr.*)

LG Pea	Locus Pea	Locus *M tr.*	LG *M tr.*
I	AgpS2	AgpS2	5
	ACCox	mth2-11j1	5
	PsAS2	PsAS2	5
	Rgp	Rgp	5
II	ThiolP	ThiolP	1
	Gbsts1	Gbsts1	1
	P54	mth2-166m22	1
	PPIlike	mth2-34o21	7
	Cwi1	Cwi1	1
	MDHc	mth2-116k17	1
	pPpgm	mth2-49g13	1
III	Sbe2	Sbe2	3
	PsAAT1	PsAAT1	3
	NIP	mth2-10n2	3
	PsAAP2	mth2-15m12	3
	DiPeptIV	mth2-6c16	3
	PsAAP1	PsAAP1	3
	PsAS1	PsAS1	3
	Gpt	Gpt	3
	Eno12B	MTIC459	3
	Egl1	Egl1	3
	PepC	PepC	2
	PGK1	mth2-11n13	2
	bfruct	bfruct	2
IV	Elsa	Elsa	8
	Sucsyn	mth2-13h21	8
	Ugfgt	MTIC75	8
	SPS	mth2-16a6	8
	Xyft	Xyft	3
	Cwi2	Cwi2	8
V	Viola1	mth2-144j8	7
	PM34like	mth2-6g4	7
	TE002M14	mth2-27l9	7
	SS	SS	7
	PsGAPA1	mth2-24f15	7
	Gns2	Gns2	7
VI	Ga20oxal	Ga20oxal	6
	RNAhel	MTIC243	6
VII	N3like	MTIC97	4
	SOD9	mth2-11o4	4
	Htrans	mth2-32m20	4
	Sym29	mth2-181h2	4
	Nlm	mth2-76i7	4
	Clpser	Clpser	4
	FabatinL	mth2-24n16	4

algorithms using CarthaGene software (Schiex et al. 2001). Finally, markers exhibiting segregation distortion were placed on the map. The Haldane function was used to calculate centiMorgan (cM) distances. The *M. tr.* map was built as described in Thoquet et al. (2002). Map were drawn using MapChart © Version 2.1 (Voorrips 2002, Plant Research International, Wageningen).

Results

Development of SNP/indel markers into genes

Public databases were searched for plant gene sequences encoding mainly proteins involved in carbon/nitrogen metabolism and transport, with a focus on available legume sequences. The first strategy was to obtain pea sequence information on the parents of the mapping population in order to develop PCR-based marker assays. In most cases, Genbank information was used directly to design gene-specific primers. Nevertheless, sequence comparisons were necessary to design gene-specific primers for members of gene families or to define the most conserved regions for sequences available in several legumes but not in pea. Specific primer pairs were designed for 104 genes and used to amplify DNA for the pea genotypes: Champagne, K586, Térèse. Seventeen primer pairs produced many bands, smears, non-reproducible bands, or no amplimer at all, despite several attempts to amplify with different primers and PCR conditions. The remaining 87 genes gave unique and reproducible fragments for all samples. These PCR products were systematically sequenced (approximately 500–700 bp for each fragment) to identify polymorphism. Altogether, sequence data were obtained for 49 genes in 3 genotypes and additional sequence data were obtained for a further 38 genes in 2 genotypes. Out of the 49 gene fragments sequenced in 3 genotypes, 7 were monomorphic, 31 were polymorphic in Pop1, and 33 in Pop2, including 22 polymorphic in both populations. Out of the 38 gene fragments sequenced in 2 genotypes, 21 were polymorphic in Pop1. Polymorphism consisted of indels or SNP. New primers were designed around or close to the polymorphic sites, leading PCR-based markers to easily score for segregation analyses (Table 1). The primer pair designed for gene *Agps1* yielded a 500 bp allele for Térèse and a null allele (i.e. no product) for Champagne. Fourteen genes were scored by direct length polymorphism, with indels varying from a few nucleotides (polymorphic microsatellite in gene *Sut1*) to hundreds of base pairs (*Gns2*). In 43 cases, PCR products had to be digested by a restriction enzyme to generate polymorphic fragments (CAPS and dCAPS markers). For the five remaining genes, internal allele-specific primers were added to the PCR reaction, producing three products for each sample, two specific of each parental genotype (their size had to be different) and one common to both parents. All the markers described in Table 1 were genotyped in the mapping populations 1 (52 loci) or 2 (11 loci). Using the CE-SSCP technique, about one-third of the genes tested for polymorphism appeared polymorphic. Fifteen genes were genotyped in Pop2 using this technique. Altogether, 52 genes were genotyped in Pop1 and 26 in Pop2.

The pea composite map

The addition of gene-anchored markers allowed us to improve the individual maps obtained for Pop1 and Pop2, by joining two linkage groups corresponding to LGI in Pop1, two linkage groups corresponding to LGIV in Pop2 and two linkage groups corresponding to LGVI in Pop2 (Loridon et al. 2005). As reported in Loridon et al. (2005), there was a good alignment of the

two maps obtained for these two populations except for LGII which showed an inversion of marker order in the fragment Y02_1200–C01_580. Bridge markers, that were mapped in the two populations, were distributed throughout the genome : 7 on LGI, 13 on LGII, 24 on LGIII, 9 on LGIV, 5 on LGV, 8 on LGVI, and 7 on LGVII. A 'composite' genetic map based on the two segregating populations Pop1 and Pop2 (303 RILs) was built using mapping information from a previously published composite map (Loridon et al. 2005). This new composite map included gene-anchored markers described in the present paper, RAPD, RFLP, SSR markers (Laucou et al. 1998; Loridon et al. 2005), and other gene-anchored markers described elsewhere. The complete genetic map covers 1,458 cM (Haldane) or 1,351 cM (Kosambi) and comprises 363 loci (Fig. 1), including a total of 111 gene-anchored markers: 77 gene-anchored markers described in this study, 7 microsatellites belonging to genes (Loridon et al. 2005; Burstin et al. 2001), 16 flowering time genes (Hecht et al. 2005), the *Tri* gene (Page et al. 2002), 5 morphological markers, and 5 other genes published in Laucou et al. (1998). The mean spacing between adjacent markers is 4 cM and 90% of the markers are closer than 10 cM. Because we wanted to place a maximum of gene-anchored markers, we placed markers exhibiting distorted segregation in the second step (Fig. 1). This resulted in an increase of the map length, from 1,370 to 1,458 cM (Haldane).

Pea–*M. truncatula* marker synteny

Twenty five genes were mapped both in pea and *M. truncatula*. Forty primer pairs designed for pea were tested on DNA from the two parents of an *M. tr.* mapping population. Twenty primer pairs gave single bands in PCR and clear sequence data after direct sequencing of the PCR products. In all cases, the sequencing revealed a single amplified product and a high similarity of the sequences between the two species. Fifteen of these primer pairs revealed polymorphic loci, 11 of which have been mapped in the *M. tr.* mapping population. A second approach to map putative orthologues of the mapped pea genes in *M. tr.* involved using homologous sequences, obtained after searching the *M. tr.* EST databases, to design new primer pairs for detecting polymorphism between Jemalong and DZA315.16. Ten additional polymorphic loci were mapped in this way. Finally, the reverse approach was taken: we looked for putative orthologues in pea of ESTs already mapped in *M. tr.* Four polymorphic loci showing clear sequence data out of six genes amplified were mapped in Pop1. We also used the genomic data available from the *M. tr.* sequencing and physical mapping project to look for orthologues of mapped pea genes in *M. tr.* BACs that were assigned to contigs and linkage groups. This allowed us to compare the linkage group assignment for another 20 putative orthologues of the mapped pea genes. Linkage group assignment as revealed by the 45 gene-specific markers, mapped both in pea and *M. tr.*, showed a high level of conservation between the two species (Table 4): all genes mapped on the pea LGI were placed on LG5 in *M. tr.* Similarly, all markers from pea LGV mapped to *M. tr.* LG7, those from LGVI to LG6, and those from LGVII to LG4. Gene markers of pea LGII mapped to *M. tr.* LG1 except for TE005I14 whose putative orthologue was found on *M. tr.* LG7, and gene markers of pea LGIV had their putative orthologues located on *M. tr.* LG8, except for *Xyft* whose homologous locus mapped on LG3. The putative orthologues of 10 out of the 13 genes mapping on LGIII in pea were located on LG3 in *M. tr.* The three remaining genes (*PepC*, CE007D21a, and *bfruct*) that mapped to the same extremity of pea LGIII, mapped on LG2 in *M. tr.* We also observed an overall conservation of marker orders between pea and *M. tr.* maps. Marker order was conserved for three markers from pea LGI and *M. tr.* LG5 (*Agps2*, *PsAS2*, and *Rgp*). For pea LGIII and *M. tr.* LG2 and LG3, marker order was generally well conserved, except for the closely linked *Sbe2* and *PsAAT1* (1.4 cM distant in *M. tr.*). For pea LGV and LGVII and *M. tr.* LG7 and LG4, respectively, there was a good conservation of marker order for four markers common between pea and UMN *M. tr.* map. For pea LGII and *M. tr.* LG1, marker orders were inverted for *Gbsts1* and *Cwi1* (LIPM *M. tr.* map) and for *P54* and *MDHc* (UMN *M. tr.* map).

Discussion

Correspondence of the composite functional map with previous maps

The functional composite map developed in the present study (Fig. 1) is intrinsically related to two previously published maps (Laucou et al. 1998; Loridon et al. 2005) : all three maps were built using the RILs mapping population derived from Térèse × K586 (Pop1) and all three are based on common RAPD and RFLP markers. The microsatellite composite map described in Loridon et al. (2005) and the functional composite map were also built using the RIL mapping population derived from Térèse × Champagne (Pop2), and are based on common microsatellite markers. Thus, marker content and order are very similar among the three maps.

The functional composite map shares 45 common microsatellite markers with the map from Prioul et al. (2004), and numerous RAPD markers with that of Laucou et al. (1998), allowing to approximately place the genes mapped in these studies on the functional map. Several gene-anchored markers are also shared with other pea genetic maps. Some markers corresponding to the same genes may appear under different names: pID5 and *Gbsts*1 (granule-bound starch synthase 1) on LG II, *TPPA* and *ThiolP* (thiol protease) on LG II, pID18 and *Gbsts*2 (granule-bound starch synthase 2) on LG VII, *Gsn1* and *GS3B* (glutamine synthase) on LG VII.

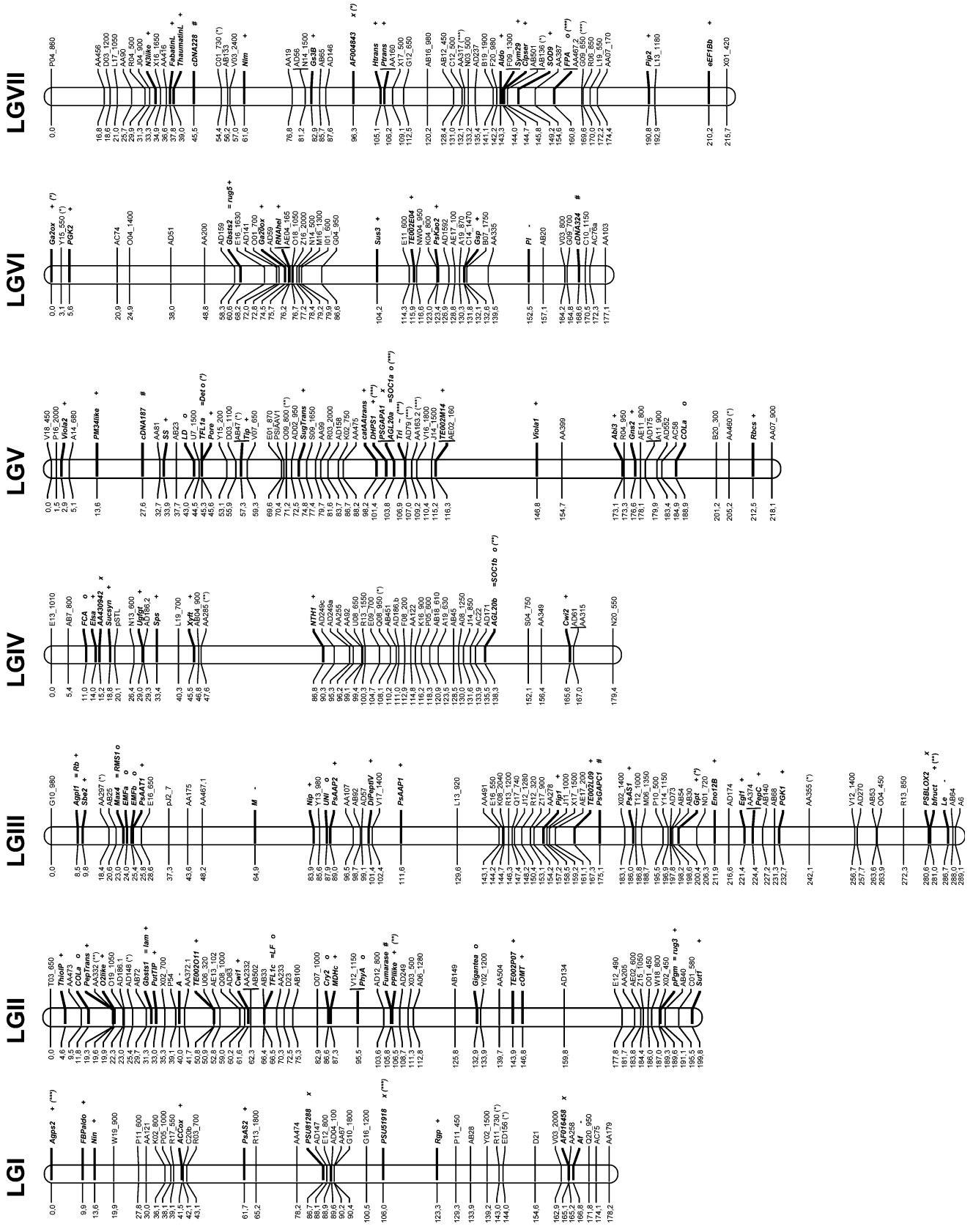

Fig. 1 Composite gene-anchored marker map in pea. Gene-anchored markers are indicated in *bold*. For markers that show a distorted segregation, the levels of the distortion as revealed by a chi-square test in are indicated *within brackets* (significant at *5%, **1%, ***0.1%). Distances are in cM (Haldane). The origin of the markers is indicated as follows: + markers from present study, - morphological markers, x Loridon et al. 2005, o Hecht et al. 2005, # Laucou et al. 1998 or C. Rameau, personal communication, ~ Page et al. 2002

We have tried to use names that are easy to decipher, in order to facilitate the use of the functional map. Three genes located on LGIII—*Rb*, *PepC*, and *Egl1*—are common with Konovalov et al. (2005), one gene—*GSp*—is common with Hall et al. (1997), and six genes—*TPP*, pID5, pID68, *Glucanase*, *GSn1*, and *GSp*—are common with Gilpin et al. (1997). The functional map is connected to the pea consensus map (Weeden et al. 1998) by common morphological and gene markers : *Af* located on LG1, *TPPA*, pID5, *A*, *Fum*, *Pgmp* on LGII, *Aatc*, *Rb*, *M*, *UNI*, *Enod12*, and *Le* on LGIII, none on LGIV, *Det*, *Tri*, *Rbcs* on LGV, *Gsp* and *Pl* on LGVI, and *Gsn1* and *Aldo* on LGVII. Except for LGIV, linkage groups on Fig. 1 were oriented according to Weeden et al. (1998). It is also connected to the mutation consensus map (Ellis and Poyser 2002) by *af* on LGI, *a*, *lam* (= *Gbsts*1), *rug3* (= *Pgmp*), *LF* (= *Tfl1c*) on LGII, *Rb* (= *Agpl1*), *M*, *Uni*, *Le* on LGIII, , *DET* (= *Tfl1a*) on LGV, and *rug5* (= *Gbsts*2) and *Pl* on LGVI. Thirteen genes are common to the flowering time gene map developed by Hecht et al. (2005). In most cases, markers mapped in the same order.

The functional map as a tool for candidate gene approaches in pea

Waddington (1943) suggested in a controversial letter to Nature that even though there was a true difference between polygenic variation "determined by numerous genes" and oligogenic variation "determined by few genes", that did not imply that the kind of genes involved was different. Much later, Robertson (1985) emphasized this idea and suggested that oligogenic variation could be the result of large effect mutations, and polygenic variation the result of minor effect mutations, in the same genes. Indeed, recent studies aimed at identifying QTL report that genes known to have drastic effects in mutant phenotypes may also control quantitative trait variation. Thévenot et al. (2005) showed that several genes involved in carbon metabolism and identified as starch-deficient mutants in maize (*mn1*, *sh1*, *bt1*, and *sh2*) are good candidates for controlling QTLs of the corresponding enzyme activities as well as kernel carbohydrate composition. Among others, *Sh1*, which encodes sucrose synthase, is significantly associated with kernel filling traits. Prioul et al. (1999) showed that *Sh2*, a gene encoding ADPglucose-pyrophosphorylase, was a good candidate for controlling maize kernel starch content. A potato functional map including 69 genes, corresponding to 85 loci involved in carbohydrate metabolism and transport, revealed associations between several of these genes and QTLs for tuber starch content. Fridman et al. (2000) showed that a mutation within an invertase gene correspond to a QTL for tomato sugar content.

In pea, five genes have been shown to determine the wrinkled-seed character of well-known mutants. The five genes encode enzymes involved in starch synthesis: starch branching enzyme I (*r*, Bhattacharrya et al. 1990), ADPglucose pyrophosphorylase (*rb*, Martin and Smith 1995), plastidial phosphoglucomutase (Harrison et al. 1998), sucrose synthase (*rug4*, Craig et al. 1999), and granule bound starch synthase II (*rug5*, Craig et al. 1998). Other genes, including *AGPase*, *PepC*, and *AAP1*, had drastic effects on seed size and composition when their expression was altered in transgenic legumes (Rolletschek et al. 2004; Weber et al. 2005). In the present paper, we have developed and mapped markers for genes involved in primary carbon metabolism and transport, as well as genes for which mutant or transgenic seed phenotypes have been observed. Mapping bridging markers on maps that have served for mapping QTLs for size and seed protein content (Timmerman-Vaughan et al. 1996; Tar'an et al. 2004) could suggest some positional candidates for some of these QTLs. In Timmerman-Vaughan et al. (1996) a major QTL for seed size was located around the marker M27 on LGIII. This marker was not polymorphic in our populations. The genes located in this region (*Pip1, PepC, Egl1*) could be mapped on the 'Primo' × 'OSU442-15' map to confirm their role as good candidates. *PepC* which has been shown to control seed size by transgenic approaches in *Vicia* and pea could well correspond to this QTL for seed size that is conserved in different legume species. In Tar'an et al. (2004), a QTL for seed protein content was located on LGVI between markers G4_2000 and B7_1750. Three genes are located in this interval on our composite map: *Sus3*, *PsKao2*, and *Gsp*. *Sus3* which encodes a sucrose synthase and *Gsp* which encodes a plastidial glutamine synthetase are good candidates and should be mapped on the 'Carneval' × 'MP1401' map.

The functional map as a tool for investigating synteny between pea and *M. truncatula*

The difference in genome size between pea (5×10^9 bp per haploid genome) and *M. truncatula* (5×10^8 bp per haploid genome) makes the latter species a potentially very useful legume model where the sequencing of the gene space is possible. *M. truncatula* data can be used to reinforce functional map development in pea, provided there is a good conservation of synteny between the species. To check this last point, we have mapped 21 genes both in pea and *M. truncatula* using different approaches. Using pea sequences, 50% of the primer pairs could amplify a fragment in *M. tr*. The presence of a single amplified product and the similarity of the sequence data between the two species strongly suggests that the fragments amplified correspond to orthologous gene loci. One-third of the amplified products were polymorphic among the parents of the*M. tr*. mapping population (Jemalong × DZA315.16, Thoquet et al. 2002). Eleven markers were mapped following this approach. The second approach involved designing new primer pairs after searching putative orthologues of the mapped pea genes in the *M. tr*. EST databases.

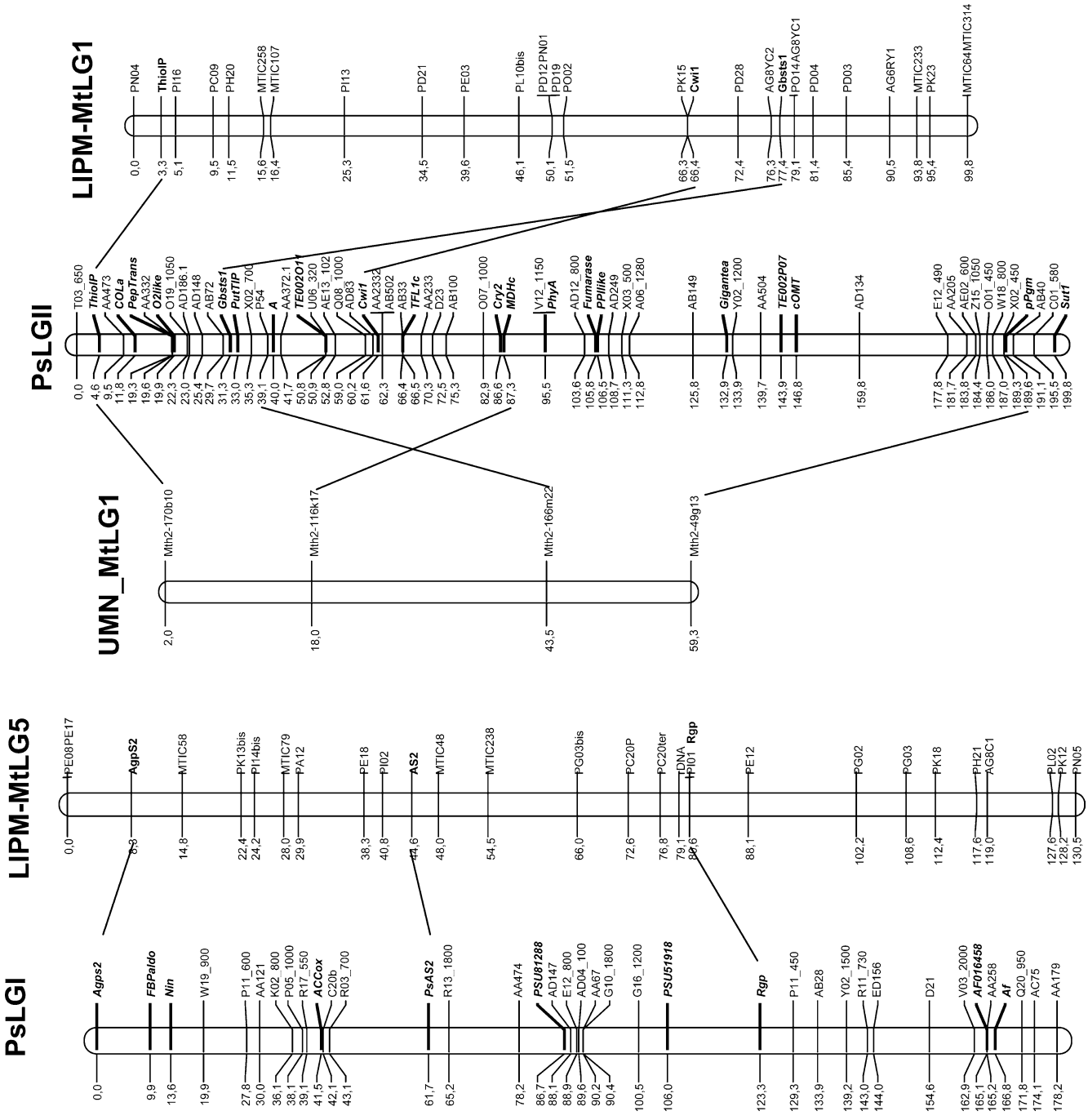

Fig. 2 Correspondence between the pea map and the *Medicago truncatula* map, as revealed by 42 gene-anchored markers. Gene markers were mapped both on the composite gene-anchored pea map (this study) and on linkage maps of *M. truncatula* (UMN, available at http://www.medicago.org/genome/ and LIPM, this study)

Ten additional polymorphic loci were mapped this way. This approach, which was used by Zhu et al. (2003) to evaluate the synteny between *M. tr.* and *Arabidopsis thaliana*, should be more powerful when the sequencing of the genespace of *M. tr.* is achieved (Young et al. 2005): the number of sequences available will be dramatically increased and the risk of choosing a homologue which is not the orthologue will be reduced. Finally, we looked for putative orthologues in pea of ESTs already mapped in *M. tr.* Four polymorphic loci with clear sequence data were mapped in this way in pea. This permitted us to check for the conservation of the synteny among the two species (Fig. 2). We observed a conservation of marker orders in most cases, for example, between pea LGI and *M. tr.* LG5 and for most of markers on pea LGIII and *M. tr.* LG3. However, we also found some inversion of marker order (for example on pea LGII and *M. tr.* LG1). No inversion of

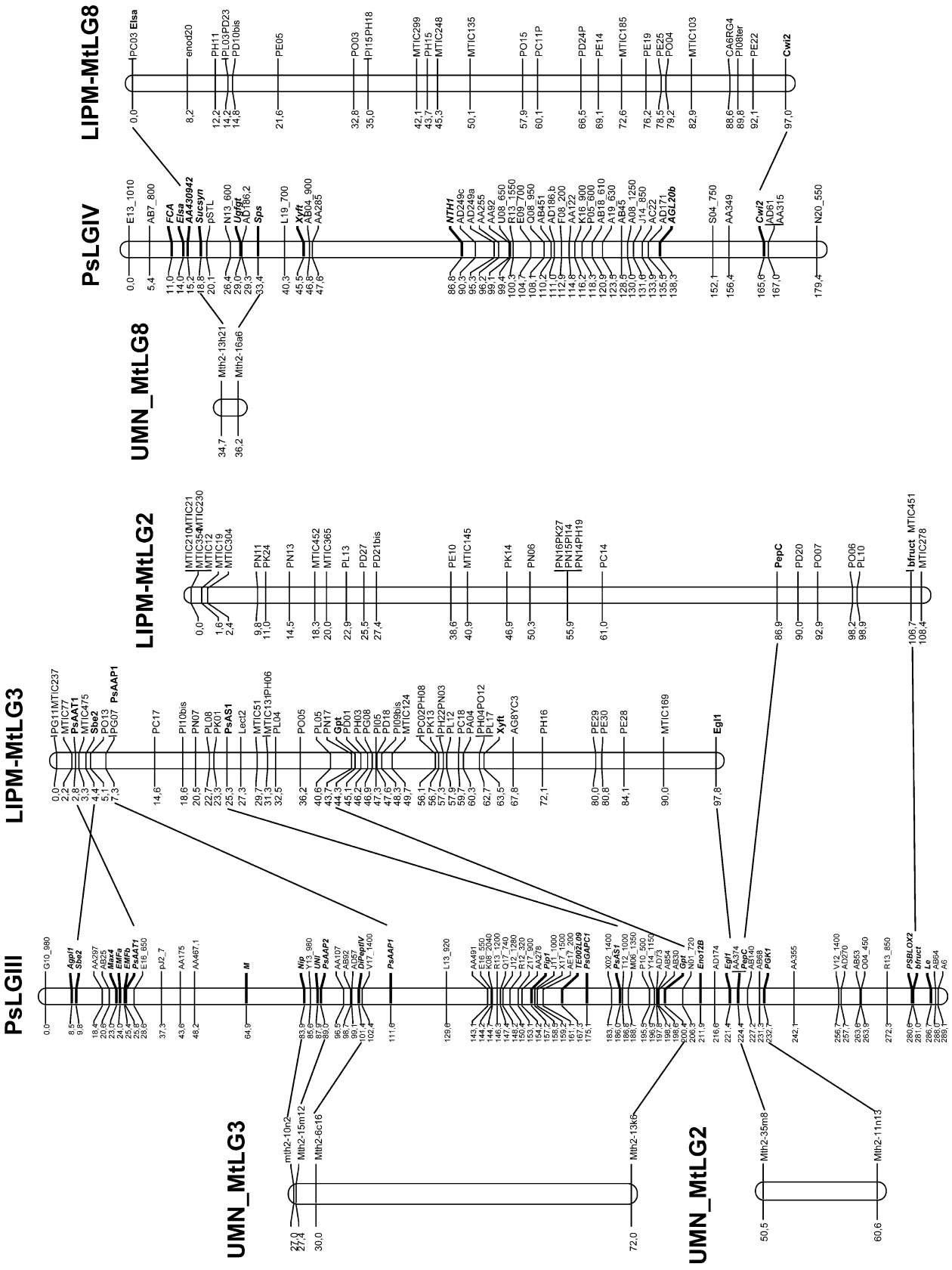
PsLGIII
LIPM-MtLG3
LIPM-MtLG2
PsLGIV
LIPM-MtLG8
UMN_MtLG3
UMN_MtLG2
UMN_MtLG8

Fig. 2 (Contd.)

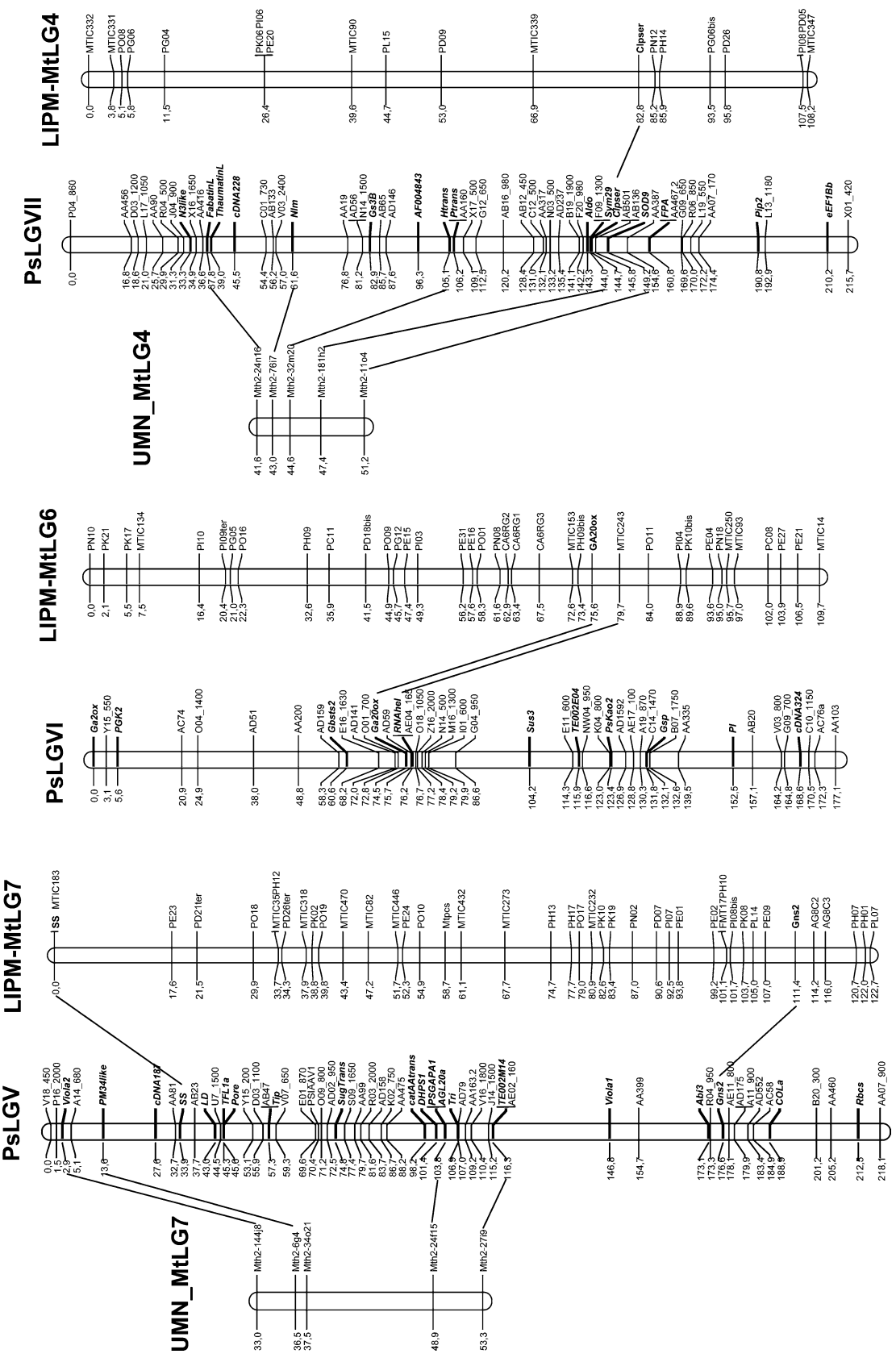
PsLGV
LIPM-MtLG7
UMN_MtLG7
PsLGVI
LIPM-MtLG6
UMN_MtLG6
PsLGVII
LIPM-MtLG4
UMN_MtLG4

Fig. 2 (Contd.)

order was identified by Choi et al. (2004) between pea LGII and *M. tr.* LG1. However, the position of markers HRIP and TUP appeared closer to SCP marker in *M. tr.* and to CYSP in pea, suggesting a possible rearrangement in this region between the two species, and emphasizing the need for a denser coverage of linkage groups with bridge markers to clarify the syntenic relationship between the two species. Moreover, this region may also be subjected to rearrangements in pea (Ellis and Poyser 2002). To compare the linkage group assignment of other gene markers in the two species, we looked for orthologues in *M. tr.* of the genes mapped in pea BACs which were assigned to contigs and linkage groups. This added linkage group assignment information for another 20 putative orthologues of the mapped pea genes, and provided a connection to the UMN *M. tr.* map. Linkage group assignments, as revealed by 45 gene-specific markers localized both in pea and *M. tr.* (Table 4), showed a high conservation of the genome structure between the two species and indicated a correspondence between linkage groups of pea and *M. tr.*: the pea LGI corresponded to *M. tr.* LG5, LGII to LG1 (except for marker TE005I14), LGIV to LG8 (except for the marker *Xyft*), LGV to LG7, LGVI to LG6, and LGVII to LG4. The pea LGIII corresponded to *M. tr.* LG3 for ten markers placed at one extremity of LGIII, and to *M. tr.* LG2 for the three markers placed at the other extremity of LGIII. These results confirm a good conservation of gene content and linkage group assignment between *M. tr.* and pea genomes. These are consistent with those obtained by Gualtieri et al. (2002) and Choi et al. (2004) with *M. tr.* and with those obtained by Kalo et al. (2004) with *M. sativa*. They add 41 further gene correspondences between pea and *Medicago*. Of the 45 gene correspondences described in this study, 2 were described by Kalo et al. (2004)—*PepC* and *AAT1* (*Aatc*), and 2 by Choi et al.(2004)—*Sucsyn* (*susy*) and *PsAS2* (*asn2*). The data also suggested an overall conservation of marker orders, with some inversions (for example middle of pea LGII and *M. tr.* LG1). Additional markers need to be mapped in both species for a clearer vision.

In the present study, we developed and mapped new gene-anchored markers in pea. Using bridge markers, such as microsatellite markers, to connect our map with other pea maps would allow the construction of a new consensus map in pea, including a larger number of expressed sequences, and would be useful for future candidate gene approaches. We also developed and mapped gene-anchored markers in *M. truncatula* that constitute new links between the maps of the pea and *Medicago* species, adding to the links published by Choi et al. (2004) and Kalo et al. (2004). The task of connecting the pea genetic map to the genetic and/or physical map of *M. truncatula* should be pursued, to allow transfer of information collected in the model plant to the pea crop. This will be enhanced by the completion of the *M. tr.* sequencing project.

Acknowledgments We acknowledge the excellent and reliable technical assistance of J. Potier, A. Chauveau for gene markers genotyping in pea and some gene markers in *M. tr.*, and G. Cardinet, M. Gherardi, for genotyping in *M. tr.* We are very grateful to H. Weber who gave us access to his pea sequence resources. We warmly thank K. Lievre, C. Rochat, J.P. Boutin, C. Masclaux, C. Damerval, and J.L. Prioul for helpful discussion on the choice of genes to be mapped. Thanks to J.M. Prosperi for providing seeds of the *Medicago truncatula* mapping population. Thanks also to P. Touzet for providing us with a copy of Waddington's paper a long-time ago. This work was supported by the French national program Genoplante GOP-PEAA and by INRA program ATS Medicago. F. J. and K. L. were appointed on Genoplante grants. Thanks to R. Thompson for his suggestions on the manuscript.

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