A Sequence-Based Genetic Map of *Medicago truncatula* and Comparison of Marker Colinearity with *M. sativa*

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ABSTRACT

A core genetic map of the legume *Medicago truncatula* has been established by analyzing the segregation of 288 sequence-characterized genetic markers in an F_2 population composed of 93 individuals. These molecular markers correspond to 141 ESTs, 80 BAC end sequence tags, and 67 resistance gene analogs, covering 513 cM. In the case of EST-based markers we used an intron-targeted marker strategy with primers designed to anneal in conserved exon regions and to amplify across intron regions. Polymorphisms were significantly more frequent in intron *vs.* exon regions, thus providing an efficient mechanism to map transcribed genes. Genetic and cytogenetic analysis produced eight well-resolved linkage groups, which have been previously correlated with eight chromosomes by means of FISH with mapped BAC clones. We anticipated that mapping of conserved coding regions would have utility for comparative mapping among legumes; thus 60 of the EST-based primer pairs were designed to amplify orthologous sequences across a range of legume species. As an initial test of this strategy, we used primers designed against *M. truncatula* exon sequences to rapidly map genes in *M. sativa*. The resulting comparative map, which includes 68 bridging markers, indicates that the two Medicago genomes are highly similar and establishes the basis for a Medicago composite map.

THE genus Medicago contains in excess of 54 characterized species (Lesins and Lesins 1979; Small and Jomphe 1988), with the majority of species being either diploid annuals or tetraploid perennials. The most important economic species of Medicago is the tetraploid perennial *Medicago sativa*, or alfalfa, although several annual Medicago species are of regional agricultural importance either as forage crops or for intercropping as a means to enhance soil nitrogen. *M. truncatula* is native to the Mediterranean basin, where the existence of numerous native populations has provided an important resource for population biology and surveys of natural phenotypic variation (Bonnin *et al.* 1996a,b). In addition to its native distribution, *M. truncatula* has been cultivated for close to 1 century in Australia, where it was developed on a limited scale as a winter forage and for use in ley rotation with wheat (Davidson and Davidson 1993). Also known by the common name "barrel medic," *M. truncatula* is well suited as a crop in areas of nonacidic soils and low winter rainfall.

As a consequence of its native distribution in the Mediterranean basin and agronomic use particularly in Australia, *M. truncatula* has great potential for the study of both basic and applied aspects of plant biology. The natural attributes of *M. truncatula* that make it desirable as an experimental system include its annual habit, diploid and self-fertile nature, abundant natural variation, relatively small 500-Mbp genome, and close phylogenetic relationship to the majority of crop legume species (Barker *et al.* 1990; Cook 1999). Moreover, over the past decade several research groups have developed the tools and infrastructure for basic research, including efficient transformation systems (Trieu and Harrison 1996; Trinh *et al.* 1998; Kamaté *et al.* 2000), collections of induced variation (Penmetsa and Cook 2000), well-characterized cytogenetics (Cerbah *et al.* 1999; Kulikova *et al.* 2001), and a collaborative research network (http://www.medicago.org). Research efforts on *M. truncatula* encompass a broad range of issues in plant biology, ranging from studies of population biology (Bonnin *et al.* 1996a,b) and resistance gene evolution (Cannon *et al.* 2002; Zhu *et al.* 2002) to the molecular basis of symbiotic interactions (*e.g.*, Penmetsa and Cook 1997; Catoira *et al.* 2000, 2001; Harrison *et al.* 2002; Ben Amor *et al.* 2003; Limpens *et al.* 2003; Liu *et al.* 2003; Mathesius *et al.* 2003) and micronutrient homeostasis

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(Nakata and McConn 2000; McConn and Nakata 2002; Ellis *et al.* 2003). Of importance to these hypothesis-driven investigations is the parallel development of tools for genome analysis, including a large collection of expressed sequence tags (ESTs) and an ongoing physical map and whole-genome sequencing effort, as well as corresponding activities on metabolic profiling and proteomics.

A key resource for both classical genetic and genomics efforts in *M. truncatula* is a genetic map composed of well-characterized molecular markers. Thoquet *et al.* (2002) have produced a genetic map based primarily on the analysis of anonymous sequence polymorphisms [*i.e.*, amplified fragment length polymorphism (AFLP) and randomly amplified polymorphic DNA (RAPD) markers]. However, the increasing sequence information for *M. truncatula* provides an opportunity to map sequence-characterized loci. We have used such a strategy in previous studies to describe the organization and distribution of resistance gene analog sequences (Zhu *et al.* 2002) and as the basis for examining genome conservation between *M. truncatula* and *Arabidopsis thaliana* (Zhu *et al.* 2003).

The goal of the current study was to develop codominant genetic markers for the transcribed region of the *M. truncatula* genome. Ancillary goals included providing a community resource for genetic mapping in *M. truncatula* and developing a set of conserved genetic elements for comparative map analysis within the Fabaceae. We have emphasized the development of sequence-based genetic markers, as these are anticipated to have wider application among populations within a species and between related species. Toward this end, we used the extensive collection of ESTs for *M. truncatula* (*e.g.*, Fedorova *et al.* 2002) to develop genetic markers for genes that exhibit high sequence conservation with other legumes or with Arabidopsis. In parallel to the EST approach, we used DNA hybridization and sequence information to identify and genetically map bacterial artificial chromosome (BAC) clones containing genes of special interest [*e.g.*, *M. truncatula* resistance gene analogs, genes expressed during symbiosis, or homologs of mapped soybean restriction fragment length polymorphism (RFLP) clones]. The majority of the genetic markers (BAC and EST) are anchored to BAC clone contigs, providing an important opportunity to use fluorescence *in situ* hybridization (FISH) to resolve ambiguities in the genetic map, as well as to increase the integration of genetic, cytogenetic, and physical map data. The resulting genetic map defines eight linkage groups (LGs), corresponding to the eight cytogenetically defined *M. truncatula* chromosomes (Kulikova *et al.* 2001). To test the utility of these genetic markers for cross-species comparison, we analyzed 68 sequence-based markers in a diploid *M. sativa* (alfalfa) population. The results demonstrate that the two species are essentially colinear, with the exception of the notoriously variable 5S rDNA loci and two ESTs that appear to have been the focus of lineage-specific expansion or contraction.

MATERIALS AND METHODS

Identification of expressed sequence tags for genetic marker development: *M. truncatula* EST sequences were obtained from the National Center for Biotechnology Information (NCBI) dbEST and used to query the NCBI databases using blastx, blastn, or tblastx. *M. truncatula* ESTs with high similarity to genes discovered in other organisms (principally Arabidopsis and/or other legumes) were selected for further analysis. Analyses were conducted against public domain sequences available at NCBI in February 2000. In the initial attempt, we screened ~2700 *M. truncatula* ESTs using blast and selected 274 ESTs as marker candidates. Oligonucleotide primers were designed from predicted exon sequences using the Lasergene PrimerSelect software package (DNAStar, Madison, WI) with the following general guidelines. In cases in which introns could be predicted by aligning an *M. truncatula* EST with a corresponding genomic sequence of Arabidopsis, primer pairs were designed to anneal in exon sequences and to amplify across intron regions. In cases in which an *M. truncatula* EST possessed similarity to sequences identified in other legumes (on the basis of blastn), sequence alignments were used to design oligonucleotide primers that would amplify DNA fragments from each of the corresponding legume genomes. The soybean database contributed most of the legume sequences for sequence comparison due to the relative abundance of soybean ESTs, and thus a majority of the EST primer pairs amplify sequences from the soybean genome (H.-K. Choi and D. Cook, unpublished results).

Identification of BAC clones for genetic marker development: RFLP probes previously mapped in crop legumes were used to identify homologous *M. truncatula* BAC clones on the basis of DNA hybridization. Soybean RFLP clones with high homology to genes in the NCBI database (May 1999) based on blastx were selected as probes for Southern blot analysis. High-density filters containing five times the coverage of the *M. truncatula* genome were obtained from the Clemson University Genome Center and hybridized with [^{32}P]dCTP-labeled probes essentially as described by Nam *et al.* (1999). Putative positive clones were retrieved from the BAC library, purified, and used for DNA isolation by means of the QIAGEN (La Jolla, CA) plasmid kit according to the manufacturer's instructions. Purified BAC DNA was digested with *Hin*dIII, resolved in a 0.6% agarose gel, and used for a second round of Southern blot analysis. Hybridization patterns were used to confirm the original hybridization result and to distinguish paralogous loci on the basis of the size of the hybridizing band and the correspondence between BAC fingerprints. The resulting BAC clones were end sequenced using oligonucleotide primers that are complementary to the BAC clone polylinker: SQ-BAC-L (5′-AACGCCAGGGTTTTCCCAGTCACGACG-3′) and SQ-BAC-R (5′-ACACAGGAAACAGCTATGACCATGATTACG-3′). Twenty-microliter sequencing reactions contained 500 ng of BAC DNA, 8 μl of ABI BigDye (Perkin-Elmer, Norwalk, CT), and 5 pmol of primer. Sequencing reactions were performed with a 2-min initial denaturation step at 97°, followed by 40 cycles at 97° for 6 sec and 60° for 5 min. On the basis of BAC end sequence information, oligonucleotide primer pairs were designed to PCR amplify the corresponding genomic DNA fragment from *M. truncatula* mapping parents, genotypes *A17* and *A20*.

Identification of polymorphic sequences and marker development: Parental genomic DNAs (Mt *A17* and Mt *A20*) were

amplified by the polymerase chain reaction using oligonucleotide primers designed from ESTs or BAC end sequences, as described above. Ten-microliter PCR reactions contained the following reagents: 20 ng of genomic DNA template, 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 5 pmol of each primer, and 0.5 unit of HotStarTaq DNA polymerase (QIAGEN). PCR thermocycling reactions were performed with a 15-min initial denaturation/activation step, followed by 35 cycles at 94° for 20 sec, 55° for 20 sec, and 72° for 2 min, with a final extension step of 5 min at 72°. PCR products were assessed by gel electrophoresis in 1% agarose, visualized by means of ethidium bromide staining. PCR reactions producing single bands were selected for sequencing using an ABI377 or ABI3730XL automated sequencer and the ABI PRISM BigDye terminator sequencing ready reaction kit (Perkin-Elmer). Sequencing reactions of 10-μl volume contained 10–50 ng of PCR amplicon, 4 μl of ABI BigDye reagent, and 5 pmol of primer. Sequencing thermocycling was performed with a 1-min initial denaturation step at 96°, followed by 35 cycles at 96° for 10 sec, 55° for 5 sec, and 60° for 4 min. DNA sequence alignments, produced with the Sequencher 3.1.1 program (Gene Codes, Ann Arbor, MI), were used to survey the parental alleles for polymorphic sites. Length and codominant polymorphisms could be assayed directly by means of agarose gel electrophoresis. Single-nucleotide polymorphisms (SNPs) were converted to cleaved amplified polymorphic sequences (CAPS) by identifying SNPs that confer differential restriction enzyme sites between the two parental alleles (Konieczny and Ausubel 1993; Hauser *et al.* 1998; Michaels and Amasino 1998). In cases in which a suitable restriction enzyme site was not identified, oligonucleotide primers with a single nucleotide mismatch were designed adjacent to the polymorphic position, such that a restriction site was created in the PCR product of one parent, but not the other (so-called derived CAPS markers, or dCAPS; *e.g.*, Neff *et al.* 1998).

Genotyping and data analysis: Plant genomic DNA was isolated using the DNeasy plant mini kit (QIAGEN) according to protocols provided by the manufacturer. Two parental lines of *M. truncatula*, Jemalong *A17* (the primary experimental genotype used in most investigations to date) and *A20*, were chosen previously (Penmetsa and Cook 2000) to facilitate genetic mapping and subsequent map-based cloning of genes defined by their mutant phenotype. The basic mapping population consisted of 93 F_2 progeny derived from a cross of *A17* and *A20*. In regions of specific interests, or where additional recombinants were desired to establish marker order, up to 120 individuals were genotyped.

For purposes of marker genotype analysis, the F_2 DNAs were analyzed in parallel with three control DNAs (*A17* maternal homozygous line, *A20* paternal homozygous line, and heterozygous DNA) in a structured 96-well microtiter plate format. Briefly, following PCR ~50–100 ng of product (1–2 μl) was transferred to a new 96-well plate containing 1–5 units of a predetermined restriction enzyme (Table 1) in a total volume of 8 μl. Digestion was carried out at the manufacturer-specified temperature for 2–4 hr. Cleaved DNA fragments were analyzed by agarose gel electrophoresis and genotypes were recorded as follows: homozygous maternal (*A17*) as "A," homozygous paternal (*A20*) as "B," heterozygous as "H," not A as "C," not B as "D," and missing data as "—."

For *M. sativa*, genetic marker candidates were first scored for polymorphisms in the parental plants (Mscw2 and Msq93) and their F_1 progeny (F_1/1). Markers that displayed easily scored polymorphisms (*e.g.*, length variation, dominant inheritance, or heteroduplex formation) were genotyped directly by means of agarose gel electrophoresis. In cases in which alleles could not be scored directly on agarose gels, the amplification products were sequenced to identify polymorphisms and to develop CAPS markers (as described above for *M. truncatula*). In cases in which CAPS markers could not be developed, alleles were scored in F_2 populations by direct sequencing of the PCR products. In such cases, a limited number of F_2 individuals were selected to provide fine discrimination within the desired genetic interval, aided by a color-coded genotype map of the diploid alfalfa population (Kiss *et al.* 1998). In a typical mapping experiment, 138 *M. sativa* F_2 individuals were analyzed. The F_2 mapping population was derived from a single F_1 plant (F_1/1), based on a cross between the diploid yellow-flowered *M. sativa* ssp. *quasifalcata* and the diploid purple-flowered *M. sativa* ssp. *coerulea* (described by Kiss *et al.* 1993).

Genetic distances were calculated by the "classical" maximum-likelihood method using MAPMAKER/EXP 3.0 (Lander *et al.* 1987; Lincoln *et al.* 1992). Linkage was determined by the "Group" command set at LOD 3.5 and a distance of 40 cM based on the Kosambi mapping function. The order of the markers was determined by the "Order" command (LOD 3.0, $\theta = 0.40$). Raw genotype data were checked using the color mapping method as described by Kiss *et al.* (1998). Color mapping provides a convenient means to visually inspect and curate genotypes for each individual of the population, thereby identifying potential genotyping errors and rare recombination events, and to propose linkage or nonlinkage.

Identification of BAC clones for FISH analysis: In cases in which BAC clones were not previously identified by means of DNA hybridization, we used the polymerase chain reaction to identify candidate BAC clones. BAC DNA pools were constructed either from the 5× coverage BAC library, as described by Nam *et al.* (1999), or from a more recently developed 20× coverage BAC library of *M. truncatula* (D. Kim and D. R. Cook, personal communication). Candidate BAC clones were purified and cultured overnight on Luria broth agar medium supplemented with 30 μg/ml of chloramphenicol. The identity of BAC clones was confirmed by PCR, with amplified products assessed for size and intensity by means of gel electrophoresis in 1% agarose.

FISH with BAC clones on prometaphase and pachytene chromosomes: Anthers of *M. truncatula A17* flower buds were used for producing mitotic prometa-phase (tapetum) and meiotic pachytene chromosome spreads. A detailed description of the chromosome preparation procedure and FISH is provided by Kulikova *et al.* (2001). BAC DNA used as probes was isolated according to the alkaline lysis method and labeled with either biotin-16-dUTP or digoxigenin-11-dUTP using a nick-translation mix (Roche). In some cases, BACs were labeled with a mixture of both dUTPs (in ratio 1:1) to produce yellow FISH signals after detection. Two to five probes were used simultaneously in each hybridization, including BACs that were mapped previously (Kulikova *et al.* 2001) and served as landmarks for individual chromosomes.

Biotin-labeled probes were detected with avidin-Texas red and amplified with biotin-conjugated goat-antiavidin and avidin-Texas red (Vector Laboratories, Burlingame, CA). Digoxigenin-labeled probes were detected with sheep-antidigoxigenin fluorescein-5-isothiocyanate (FITC; Roche) and amplified with rabbit-anti-sheep FITC (Jackson ImmunoResearch Laboratories, West Grove, PA). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in Vectashield antifade solution (Vector Laboratories) of 5 μg/ml. Some chromosome preparations were reused for FISH with a new set of probes according to the method of Heslop-Harrison *et al.* (1992). Images were captured for each fluorescent dye separately with a cooled CCD camera system (Photometrics, Tucson, AZ) on a Zeiss Axioplan 2 fluorescence microscope, pseudocolored, and merged by means of a CytoVision workstation (Applied Imaging). To separate individual

chromosomes, each chromosome was digitally excised and copied into a new image using Adobe Photoshop 6.0 (Adobe).

RESULTS

Development of genetic markers: With the goal of constructing a core genetic map of *M. truncatula* enriched with gene-based genetic markers, we focused on three distinct classes of sequences: (1) ESTs with high homology to genes known in Arabidopsis and/or other legume species, (2) *M. truncatula* BAC clones with high homology to mapped soybean RFLP probes, and (3) genes of predicted function. Table 1 provides a complete list of all marker information used in this study.

ESTs with similarity to Arabidopsis and legumes: To identify *M. truncatula* ESTs with high similarity to genes in other legumes or Arabidopsis, we used BLAST (Altschul *et al.* 1990) to search the NCBI nonredundant (nr) and EST (dbEST) databases for related sequences, using the following minimum criteria: tblastx against nr, $<e$-50; blastn against dbEST to identify ESTs from other legume species (principally the soybean EST data set), $<e$-45; and blastn against the Arabidopsis genome sequence, $<e$-30. Where possible, sequences were chosen to represent apparently low- or single-copy-number genes, using the Arabidopsis genome as a reference for gene copy number. In total, 141 EST-based genetic markers were developed on the basis of this approach.

BAC clones with homology to mapped RFLP probes mapped from soybean, alfalfa, or pea: In addition to providing a genetic context for the analysis of *M. truncatula* genes, we desired to produce a framework for comparison of the genetic maps of related crop legume species. To test the feasibility of this strategy for soybean (*Gylcine max*), a set of 256 publicly available soybean RFLP clones was purchased from BioGenetic Services and each clone was sequenced from both ends. The resulting soybean sequence information was deposited at NCBI as accession nos. AQ841751–AQ842207 and AQ842113–AQ842119. A total of 121 of the soybean RFLP clones, ~47% of the sequenced clones, contained a putative open reading frame based on BLASTX and TBLASTX searches of the NCBI database (as of May 1999). These putative protein-coding clones were used to screen a five-times version of the *M. truncatula* BAC library (Nam *et al.* 1999) on the basis of DNA hybridization. DNA was isolated from the candidate *M. truncatula* BAC clones, digested with restriction enzymes, and analyzed following agarose gel electrophoresis by Southern hybridization against the corresponding soybean RFLP clones. This analysis allowed us to verify the original hybridization result and to identify putatively paralogous loci on the basis of a hybridization fingerprint. In total, 79 of the 121 soybean RFLP probes analyzed in this manner hybridized strongly to the *M. truncatula* BAC library. Seventy-three percent of these 79 soybean RFLP probes identified only one BAC contig, which we interpret as a single locus in *M. truncatula*. On the basis of similar reasoning, the remaining soybean RFLP clones identified either two (13%) or three loci (14%). These results are likely to represent an underestimate of gene copy number in *M. truncatula*, as not all BAC clones identified by a given probe were subjected to Southern blot analysis. The corresponding BAC clones were end sequenced and the information was used to develop 60 genetic markers. On a more limited scale, RFLP clones previously mapped in alfalfa or pea were also used to screen the *M. truncatula* BAC library for homologous loci and to develop genetic markers on the basis of a similar strategy. In cases in which RFLP clones from other species were used to identify *M. truncatula* BAC clones and derived genetic markers, their species affiliation is listed in Table 1.

Markers developed from sequences of predicted function: As a counterpart to selecting genes on the basis of BLAST analysis or DNA hybridization, genetic markers were also developed from sequences selected on the basis of their presumed function. The largest class of this marker type represents the nucleotide binding site-leucine-rich repeat superfamily of resistance gene analogs (see Zhu *et al.* 2002 for a comprehensive analysis). An additional 12 genes were selected for mapping on the basis of their possible role in plant-microbe interactions, including symbiotic nitrogen fixation (*e.g.*, leghemoglobin, ENOD40, ENOD16, and rip1) and pathogenic associations (*e.g.*, homologs of plant chitinase proteins).

Identification of polymorphisms and genotyping: Polymorphic loci were identified following PCR amplification and sequencing of alleles from *M. truncatula* genotypes *A17* and *A20*, which served as parents of the mapping population used in this study (as selected by Penmetsa and Cook 2000). Seventeen length and 14 dominant polymorphisms were characterized and could be mapped by virtue of their inherent fragment size differences, or presence/absence criteria, between parental alleles. The remaining 257 polymorphisms were single-nucleotide differences between parental alleles. For the majority of SNPs, alleles were converted to CAPS markers. SNPs that could not be converted to CAPS markers were scored by direct sequencing of PCR products amplified from DNA of the segregating progeny.

In 60 EST markers, PCR primers were designed to anneal in conserved exon regions and to amplify across the more highly diverged intron regions. The closest Arabidopsis homolog was used to infer intron position and thereby aid primer design. This "intron-targeted" marker strategy assumes that polymorphisms will be more frequent in intron *vs.* exon regions. To test this assumption for the *M. truncatula* genotypes under analysis, we compiled intron and exon sequences for 47 of the intron-targeted markers. Pairwise alignments between the marker genomic sequences and the *M. truncatula* EST data at NCBI allowed us to distinguish exon from intron sequences and to calculate the relative di-

TABLE 1

Attributes of genetic markers

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
11B2R	AZ757871	BEST	AC144473.3	6	Resistance gene analog	*Bgl*II	CAPS	414	192 + 222	AATCTTTCTAGC CAATGTAAATC TTCAAT	TTTGCGAAAATTG GATCTAATGGG TTC
11N15L	AZ757877	BEST	NA	6	Resistance gene analog	NA	Dominant	400	0	AAGCTTGAATCCA CACTATTTGA CCC	GATGCAAAAACCAG GAAAAGAAGAAT
11N17R	AZ757879	BEST	AC144502.3	4	Resistance gene analog	*Mnl*I	CAPS	100 + 327	427	CTACTCCCTGCACC TAACCATTCACG	GCACAATTATTCAT CTCTTCCCAAA
11O9L	AZ757880	BEST	NA	3	Resistance gene analog	*Ssp*I	CAPS	197	42 + 155	CATGGCATCCAGAT CCCACAT	TATAGACTTAGCCC TCAAAGTATT TCCC
13B3R	AZ757888	BEST	AC130653.16 AC135312.7	3	Resistance gene analog	*Bst*NI	CAPS	75 + 365	440	CTCGTTGTAAAAAA GCGTTACCAAA CAGA	GTATTCATGTTACA CAAAATAAACGTC ATTGAG
1433P	AI974411	ESTi	AC144342.7	3	14-3-3-like protein	480	SNP	T/114/F G/123/F A/133/F	T/114/F T/123/F G/133/F	AAGGTTTTCTACCT TAAGATGAAGG GAG	GTTTAGCAAGATTG CAGGCACGA
15J11L	AZ757898	BEST	AZ757898	1	Resistance gene analog	*Dde*I	CAPS	190 + 72	262	GTGCCCCGCCCCT TTATT	TGCAAATAGGCCC CATCC
15L4R	AZ757901	BEST	NA	6	Resistance gene analog	*Xmn*I	CAPS	155 + 363	518	AAATTTTAGAGACC TGAGACATTGGG	GTTTTAGTTTAC CATGACTTTGCT CCTCT
18A5R	AZ757906	BEST	AC135396.17	6	Resistance gene analog	*Bsr*I	CAPS	350	123 + 227	AATGGAAGGCCA GAAGCATAAGT	GTTGAATTGGACAT TGAGTTTGGGA
18D24R	AZ757908	BEST	AC130808.13	3	Resistance gene analog	*Ssp*I	CAPS	211 + 82	293	CAATCCTGATCTAC TTAACCAAAATA ACAGC	GGATGAAAACAGA GAACCGTGAAA CAC
18L14L	AZ757909	BEST	AC135160.15 AC137667.8	4	Resistance gene analog	*Sty*I	CAPS	412	180 + 232	CGTAACATTCTCAT TATCGCTGCTAT	AAGTAATCCGGT GATTGATTTTT CTCC
19D7L	AZ757911	BEST	NA	5	Resistance gene analog	*Cla*I	CAPS	212 + 49	261	AATTTCTTCCATTT GTCTTGTTTT GAA	CAATCGTGGTCTCT GAACATATT TGG
19F14R	AZ757913	BEST	AC137508.6	3	Resistance gene analog	*Bfa*I	CAPS	157 + 318	463	AAGCTTACCTGA TACCATTTGT ATGTTGTA	AACCTATTGCCTTT GTATTTTGAGA TGG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
19L13L	AZ757914	BEST	AC133863.15	3	Resistance gene analog	*Mbo*I	CAPS	113 + 135	248	TGTGTACAACAA CAACAAGATA GAGGAACATT	CCATGGTTAAATG GAAGTAGTAAC TGCTCC
19O4L	AZ757915	BEST	AC135396.17	6	Resistance gene analog	*Hin*dIII	CAPS	297 + 138	435	GGGAATGATAATG GTATGTGATAT GAAAATG	CTATGCCAGACTGC CTCCAATGT
1E19L	AZ758056	BEST	NA	6	Resistance gene analog	*Alw*I	CAPS	325	136 + 145	ACCCGGTGCGAAT GTAATGA	GCAATGCTTGCCA GAACTCCA
1N1R	BH001061	BEST	AC136472.15	2	Converted AFLP marker	*Bsm*AI	CAPS	500	200 + 300	CATATTGTTAGAT TTGTGG	GTGAGCGTTAAGTT GGTAGAG
21L20L	AZ757924	BEST	NA	5	Resistance gene analog	*Bsr*I	CAPS	292	105 + 187	AGGTGGAAAAACC CAACGAGAATA	CTGCAAATAAACCC TCTAGAAAAGT CTG
23L16R	AZ757931	BEST	NA	4	Resistance gene analog	NA	Dominant	300	0	CAAATTCTCAACCC TTCTGCTCAAC TACT	GAACCAAATGAGCC GAACTTGAGCT
24D15R	AZ757932	BEST	NA	3	Resistance gene analog	*Hpa*II	CAPS	180 + 70	250	AATAATTGACGAGC TACCAGCATATG	TGGATTTGAATGT GATCTTTTGAT TAA
25A23L	AZ757939	BEST	AC144502.3	4	Resistance gene analog	*Nla*III	CAPS	76 + 304	76 + 59 + 244	TTTTATTTCGCGTT GTTTATTTGA TTC	ACGCGCAGCAGC CATCC
26E21L	AZ757942	BEST	NA	6	Resistance gene analog	*Dra*I	CAPS	120 + 297	417	AAGCTTCGTGCCAT TGGTGAATACA	AAGCTTCGTGCCAT TGGTGAATACA
26G3L	AZ757943	BEST	AC130653.16 AC135312.7	3	Resistance gene analog	*Rsa*I	CAPS	116 + 268	384	TTCTGACCAATCC GAAGAGCAGTGA	TGGGGTTAGATTT TAGTTACATGT TTGACACA
2M10L	AZ758065	BEST	NA	8	Resistance gene analog	*Dra*III	CAPS	282	152 + 130	TATTTGCTCCTAGT TTTGGGTCAAG	AAGTTAATGATTTT TCACTTGCAGAA TAAG
2M10R	AZ758066	BEST	NA	8	Resistance gene analog	*Nla*III	CAPS	78 + 183	78 + 61 + 122	ACAAAGGGTCTGG GCGATGA	CACCCTGATTTCCC GCAAGTGA
33I23R	AZ757960	BEST	AC121243.10	2	Resistance gene analog	*Bst*UI	CAPS	329	214 + 115	CTCTTCATTTATG AGTGTACTTGTC TTTCC	ATGAATAGCCGTGT TTTGGTGG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
34D20L	AZ757963	BEST	NA	5	Resistance gene analog	*Hpa*II	CAPS	50 + 200	250	CTGTTTTGCACTGA TATTGTTAGGA AGA	TGCTATACAGAAAA GAGGTGGTGAT TATA
36N1L	AZ757974	BEST	NA	6	Resistance gene analog	*Bgl*II	CAPS	277 + 129	406	GAAGCAGCCGGA CATTGGACACA	TGTTAGTTCAAATG GATCTTCTATG AGGTAT
38K1L	AZ757981	BEST	NA	3	Resistance gene analog	*Afl*II	CAPS	39 + 210	249	GGCTGGTATGAAA GAAGAGCAGAA	AGTTTAAATAAGGA CCAGGATGTTCG
3F12R	AZ758069	BEST	AC134823.12	6	Resistance gene analog	*Rma*I	CAPS	178 + 207	385	CCAGAATTCTTCGG TTGATGATGTT TTG	ATAAGGGGTGAAC TAATTGTGATG ACTCTTGA
3F15R	AZ758071	BEST	NA	3	Resistance gene analog	*BstU*I	CAPS	478	56 + 422	ATGTCACGAAAA TAAGCATACAAA TCCTTC	GTGATGTTGCTTC CAGATGAATG TGG
3N6L	AZ758072	BEST	NA	8	Resistance gene analog	*Xho*I	CAPS	154 + 73	227	AAGAGACTTAAGA ATTTTCGATGG GATGC	ACTTGGGTTGGTCT GATGGTGCTG
40H12L	AZ757985	BEST	NA	8	Resistance gene analog	*Bsr*I	CAPS	280	152 + 128	GAGCTGGAAGGTT TATATAAATAT CTGCCC	AAGAGAAATGAATT GTCTTATGTCT GTGTGTG
40L12R	AZ757988	BEST	NA	3	Resistance gene analog	*Nla*III	CAPS	302	96 + 206	ATGACATACTTCA AGAAATAAACC ACCAG	ATCCAAATCCCATC TCCAACAGG
41F23L	AZ757991	BEST	NA	5	Resistance gene analog	*Bsm*AI	CAPS	195	103 + 92	CCCCCGCATGTAA GGATGTT	TCGCAATTGCAAC TTGTCCAC
41O18L	AZ757993	BEST	NA	4	Resistance gene analog	*Alu*I	CAPS	268	167 + 101	AGATATATCAGAA AAAACTAACCC AACCTT	AATACCCTTCCC TTTCCTTCCC
42J16R	AZ757996	BEST	AC144341.10	3	Resistance gene analog	*Hin*cII	CAPS	227 + 242	469	ACCTCTTCTATAGA GATAACTTGTGT AGCAAG	GATAAACTGGCATT CCATGACTTTCA
43I21L	AZ757998	BEST	NA	4	Resistance gene analog	*Nla*IV	CAPS	147 + 232 + 45	45 + 379	GCTTTTGTTTTAA TGCATTTCTTA GTGTTTC	TGGAGCCTCATGT GTTTCAAACG
44D11L	AZ757999	BEST	AC138199.5	6	Resistance gene analog	*Ase*I	CAPS	481	170 + 311	TCTTACAAACTA CAATATCACAG AGGACTAAA	CCAAAATTATTAT CATTTGGTTAGC ATTA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
48N18L	AZ758017	BEST	NA	6	Resistance gene analog	*Bsm*AI	CAPS	510	130 + 380	TCTTTTCCTCCGATTTCTTGATTCTC	AGGCTTGCTTGCTGTTGGTTGTA
50O1R	AZ758020	BEST	AC126790.20	6	Resistance gene analog	NA	Dominant	300	0	CGACATCCCTTACATGTTGCCACTT	TTGCGACCATGGAATTTGTGAGGA
51J1R	AZ758021	BEST	NA	6	Resistance gene analog	*Hin*cII	CAPS	233 + 163	396	CGGTTGCAGGGGTCAAATGTAAT	CGGTTGCAGGGGTCAAATGTAAT
5J9L	AZ758081	BEST	NA	3	Resistance gene analog	*Hin*fI	CAPS	359	235 + 124	TCCTTTGGGAAGAATGGTAGAGG	CTCTGAAGAAGTATTTTCCTTCCTTGAC
6M23L	AZ757852	BEST	AC142223.4	3	Resistance gene analog	*Mse*I	CAPS	50 + 265	170 + 95 + 50	GTTAGTTTACCACTTTTGAGTAGTGTAAGCAC	TTAATGTTAGAGATTGAAGGTGAGAGAAAC
74O5R	AZ758032	BEST	AC144341.10	3	Resistance gene analog	*Cla*I	CAPS	228	131 + 97	AGTTCATTACTTGATTAGCACACTTGTACA	AGTCTAGAATGGAACACGTTGTTTCG
75D1L	AZ758033	BEST	AC124955.21	4	Resistance gene analog	*Nla*III	CAPS	220 + 43	263	AAGCTTCCAATGATAGATGATCGTAGA	TGTGGTATCATTAGTCCTTCTCATCAGG
78B21L	AZ758038	BEST	AC134824.15	6	Resistance gene analog	NA	Length	380	300	TTAGAAATTGAAAAGCGTGTATGACC	TTGCGATGGTGTCAGAAATGT
79H20R	AZ758047	BEST	NA	5	Resistance gene analog	NA	Dominant	380	0	GCTTCGTGAGGTGATAGATCTAGAGATGAC	TGTGCTCATGTTAATTGGTTTGGTGTAT
79P21R	AZ758049	BEST	NA	6	Resistance gene analog	*Dde*I	CAPS	211 + 298	211 + 87 + 200	GCTACTGCAACGTTCCCTCTCACAG	TCTAAATATGCGTGGTTGTTCTAAAGTGTT
7G13R	AZ757859	BEST	AC123574.20	6	Resistance gene analog	*Rsa*I	CAPS	356	292 + 64	AATTGCACATATTTCATTTGAGTTGAGAAG	TTTCTTCTTATAAAAAATGGTGAAGTATTGTG
7H15L	AZ757860	BEST	AC134049.14	5	Resistance gene analog	*Alu*I	CAPS	30 + 250	280	TTTCATCTTCTGCCTCATTTGGTC	GCCTATGGAGGTGAGGATTTGG
8C10R	AZ757865	BEST	AC126790.20	6	Resistance gene analog	BSPH	CAPS	387	243 + 168	TGCAGTCTCGAGTACAACAAATGAAAT	TCTCGGGTGGTGATGATGACTCTG
AA661025	AA661025	ESTi	NA	8	Resistance gene analog	*Eco*RV	CAPS	351 + 277	628	CAAACCATACTTAAGCAAACCTACAATGC	CTTCGGACCTTCAGCAAAACACAG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
AAS	AW171678	ESTe	AC129091.11	2	Acetolactate synthase precursor	400	SNP	C/257/F	T/257/F	GATGGTGATGGA AGTTTTATGA TGA	AACTTATCCTGCC ATCGCCCTCGG TTA
AAT	AA660344	ESTi	AC126783.10	5	Alanine amino-transferase	*Apo*I	CAPS	500 + 500	300 + 200 + 500	TGCTTCACGATGC CACCAA	TCCGACATTAGGAT CATCAAGTAGG
ACCO	AI974230	ESTi	AC121236.14 AC122163.16	2	1-ACC oxidase	*Mnl*I	CAPS	290	40 + 250	GAAGATGGCGCA AAAAGAAAGT	CGATGTGTCGTGT CATCTCGTTAAG TTCCCT
ACL	AA660349	ESTi	AC132565.8	2	ATP citrate lyase	*Bcl*I	CAPS	30 + 270 + 900	1200	AAGGTTAAGACCG TATTTATTCCA ACA	AGTCCAAATTCGT CCCCACTG
AGT	AW126002	ESTi	NA	7	Putative 4-α-glucano-transferase	1000	SNP	C/303/F G/304/R	T/303/F A/304/R	GATTTGGGCCTCA TTCCTTCTTGT GTGCA	CCTGAAGGGGGA AAATTGCCCAC ATTGA
AI974451	AI974451	ESTi	NA	8	Resistance gene analog	*Hph*I	CAPS	470	400 + 70	CGATGGGTTTTTC CAGTTTTCTAT TACA	GCAACAAGAGTCA AATTCAGTTCCA
AI974519	AI974519	ESTe	NA	2	Resistance gene analog	NA	Dominant	300	0	GCTGAATTAGTAG GCTGGGATGTC	CTATGGTTTGCC GAAGGATTTG
AIGP	AW125928	ESTi	NA	3	Auxin-independent growth promoter	460	SNP	G/99/R	C/99/R	CTGATAGGGCCAG GAGGCAGGGA AGA	GTTTTTTAGCATTT GGACGAATGGT TGGT
APX	AA660806	ESTi	AC121235.16	4	Ascorbate peroxidase	*Spe*I	CAPS	2000	650 + 1350	ATCTTCGCCATTTT CCTTCTTAG	GCTTTGCCAAACA CATCCCTC
APYR1	AA660474	ESTe	NA	7	Mt apyrase1	*Alu*I	CAPS	95 + 38	143	AACCCTTGCCTTTT GGCTGGATTTG ATGG	TCTGAAATTATCA TAGCCGAAATA GTGTG
ASN2	AW208061	ESTi	NA	5	Asparagine synthetase	400	SNP	G/103/F G/152/F	C/103/F A/152/F	TAAAAAAAGATGAA GGACGAATTGA GAAG	CAAAGATCATTCTG TAGTAGTATGC TTCCT
ASNEP	AW208187	ESTi	NA	1	Asparaginyl endopeptidase	700	SNP	A/239/F	T/239/F	GAAGAAAAAACAT GCTTCTGGATCA TATA	ATCTTTAACCAATT TATATTGCTGG TGCA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
ASPP	AW126301	ESTi	NA	7	Aspartic protease precursor	900	SNP	C/250/F	T/250/F	GGTGGTGAATTAG TTTTTGGTGGTG TTGA	ACATAAACCAACTT GTGAACAGAC GTCA
ATCP	AW207998	ESTi	NA	3	Aquaporin-like channel protein	900	SNP	G/290/R	A/290/R	AACCAATTGGTATT GCAGCTCAGA GCCA	TTCCTTGCCAAGA ACAAACCGAAT GTCA
ATP2	AI974613	ESTi	NA	1	ATP synthase β-chain, mitochondrial precursor	>1000	SNP	A/58/F T/273/R C/442/R	C/58/F:/ 273/R T/442/R	ATTGCTATGGATG CTACTGAAGGT GTTG	TGGTATGGTGCAA GCAGGTCAA
AW125982	AW125982	ESTe	AC134049.14	5	Resistance gene analog	*Mbo*II	CAPS	133 + 166	299	GCTTCAACCTCATT CCCAACAACTTC	CCTTACCAGGTCTG GCAACTTCTCT AATATC
AW256557	AW256557	ESTe	NA	1	Resistance gene analog	*AlwN*I	CAPS	334	300 + 34	GATATTTTCATTAC TCAGCAACTTT TTCACAG	TGCTTCATCCCAC TTATCATCAA TACC
AW256637	AW256637	ESTi	AC144658.4	4	Resistance gene analog	*Sca*I	CAPS	525	404 + 121	TTCACCTAATTTC CATCTATACCA TCCATGT	TATTTGTTAGCTTT AGTGATCGCT GCTACAC
AW256656	AW256656	ESTi	AC125480.20	4	Resistance gene analog	*Bsm*AI	CAPS	505	190 + 315	CCCAGACAACATT TCCTTACTATC GTCA	CCAAGTAGTAGGC AAAACCCAAC AAATT
AW257033	AW257033	ESTe	NA	8	Resistance gene analog	*Mbo*II	CAPS	314	260 + 54	TGCGTCATTAACC AAAGATGATGT TGTAA	CCAACAGTAACATC CCCAAAGACAA TATTC
AW257289	AW257289	ESTi	NA	4	Resistance gene analog	*Bsm*AI	CAPS	341 + 137	478	CTTCGGACCTTCA GCAAAACACAG	CGGGTGACAGAT TATTTGGTGA CATC
AW684911	AW684911	ESTe	AC137825.17	6	Resistance gene analog	NA	Dominant	350	0	TCTAAACCAAGTG GGGAGTATCGC	GTGTTATTATGCC AAGGAGTTTG ATGT
AW688464	AW688464	ESTe	NA	3	Resistance gene analog	NA	Dominant	350	0	TGATTTGAAGGCA TGGGTTTGTGT	AAAATTGAAGTCC ACTCTGTTATGT CTAC
AW696588	AW696588	ESTe	NA	6	Resistance gene analog	NA	Dominant	400	0	GATTCCCATATTTT CTGCCAACTATG	CATCATCAGGTCCC TCATCAAGAAG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
AW696680	AW696680	ESTe	AC144502.3	4	Resistance gene analog	NA	Dominant	350	0	GGGAAGACAACTC TGGCTACTGCT	GTCATATGTCAAA CTCCTCATAA TCACT
AW736136	AW736136	ESTe	NA	7	Resistance gene analog	NA	Dominant	380	0	ATCTTCCAACCCT CATCATCAAT AGA	ATAGCCCAAAGAA TAGATGTGAA GCT
AW736703	AW736703	ESTi	NA	3	Resistance gene analog	*Taq*I	CAPS	160 + 290	450	AGCAAGGTATTCA ACTTCTTTTCAT CTT	GCAAATATTTCTT ACCAGTTAGTT TGTGC
AW774053	AW774053	ESTe	NA	3	Resistance gene analog	*Hin*dIII	CAPS	365	250 + 115	CCGGGTAGTAGG GTCATCATTACA	CACAGCCATTTTA TTATCTCCTCTC AAC
AW774849	AW774849	ESTe	NA	6	Resistance gene analog	*Eco*RV	CAPS	258	82 + 176	TGAAAGTCACGG GGGAGCAC	AACGGTGGATTTT ATGATGGACTC
BADH	AW126256	ESTi	NA	4	Benzyl-alcohol dehydro-genase	700	SNP	A/226/F C/58/R	G/226/F T/59/R	GTGATGATTGGAC TGGCACAAGCTG	TACAGCCCGACCGA CTATTTTTCCT
BE187590	BE187590	ESTe	NA	3	Resistance gene analog	*Bam*HI	CAPS	370	80 + 290	ATGCGGATAGAAG GGCTGATGA	CAATTGTCCGGTCT GCTCTTCC
BE325283	BE325283	ESTe	NA	6	Resistance gene analog	*Bsr*I	CAPS	72 + 275	357	GTCCATCAATTTCA ATGTTCCTGTTT	TCGCATTCAATTCT CTTCCTTGC
BGAL	AW126229	ESTi	NA	1	β-Galacto-sidase	600	SNP	C/130/F A/362/F	A/130/F G/362/F	AGACTTGTCTTGG CAGAAATGGTCT TACA	AATATGATACCATT TCTGTGTTGGTT CTCCA
BiPA	AW125959	ESTe	NA	4	BiP isoformA	400	SNP	T/284/F	C/284/F	GAGGAGTCTCACA AAGGATTGC	GGTTTTTCATGTTG TAGACATAGGT TTCA
CAF	AA660318	ESTe	AC136449.9	8	Caffeoyl-CoA *O*-methyl-transferase	*Bsi*EI	CAPS	195	30 + 165	AATTACAACCAGA AATTAAGTATTC GACC	TGGAAATTGGTGTC TACACTGGCTAC
CAK	AA660544	ESTi	AC135795.3	7	Calcium-dependent protein kinase	*Apo*I	CAPS	345 + 200 + 210 + 110	545 + 210 + 110	TTCAACCCCTCTG CGAACC	CATCTATAGCAATT GCTGTTGTCATCT

(*continued*)

TABLE 1
(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
CALTL	AW126242	ESTi	NA	1	Calreticulin	440	SNP	C/108/F A/199/F C/287/F A/340/F	T/108/F T/199/F T/287/F G/340/F	GTGGAAGGCACCA TTGATTGACAAC	TCTTCTTCTCAGC CTCTTCAAATGC
CDC16	AI974470	ESTe	AC121241.15	8	Cell division control protein 16	*Alw*I	CAPS	260	210 + 50	CCTCCCGCTTCAC TTCACTTT	GGTAATGGTGGCC GAGGAATA
CDC2	AW171750	ESTi	AC144481.3	1	Putative cdc2 kinase	820	SNP	T/310/F	C/310/F	CAACTTTGCAAGG GTGTTGCTTTCT	ACTAACACCTGGCC ACACATCTTCA
cgO008F		ESTi		2	Gibberelin 3 β-hydroxy-lase, Vr AZ254216	400	SNP	T/113/F T/248/F	C/113/F C/248/F	TCGCTCGTATCTC TTCCTTCTTCC	AGTTTGGCCATTAG TACCGTCACC
cgP137F	AW257467	ESTe	NA	1	Unknown protein	400	SNP	T/354/F	C/354/F	TGTTAACCTCAGT TACTGCAACAGA	AGGATGCAATCAAG CATATATCTTGA
chit1	Y10373	GS	AC121239.12	3	Mt Chitinase I	NA	Length	98	95	GGTAAGGTAATGC TCTATCTTAATC	CTTACGGATGAAA GGTATTGTTTCC
CNGC4	AW126067	ESTi	NA	8	Cyclic nucleotide-regulated ion channel	380	SNP	T/197/F G/201/F A/282/F	A/197/F T/201/F G/282/F	AGAGATGAGAAT CAAGAGGAGGG ATGCA	CATGATGAAGAGC ATTTCGTCCAC TGGA
CoA-O	AI974546	ESTi	AC119408.5	8	Acyl-CoA oxidase (ACX1)	*Apo*I	CAPS	10 + 410 + 60 + 210	10 + 410 + 270	TTTGGGGGAAAT AATGGAAGTCT	CTCGGGCAATGTT GAAAAATC
CP450	AW171693	ESTe	NA	4	Cytochrome P450	310	SNP	A/134/F G/152/F T/158/F	G/134/F T/152/F G/158/F	AGTGTGAGATCAAT GGTTATGTGATC	CATCATCACCTTT CAATATTTGTCC
CPCB2	AW191283	ESTi	NA	2	Putative coatomer protein complex, β2	600	SNP	T/420/F	A/420/F	AGAAAGAGTGAAG TCTGTGGATCTA CATC	GGATGAACAGCCA CACACCTAATGT AATC
CPOX2	AW127442	ESTe	NA	8	Cationic per-oxidase 2	300	SNP	A/100/R	T/100/R	GATAATGGCCTTG TTATGAATTAC TACA	GCTCAGACAAGCT TCTTCTTGTGGA
CrS	AI737624	ESTi	AC122724.13	6	Cystathionine-gamma-synthase precursor	NA	Length	750	950	CAAATGGTGCTTT GGAGATTGAT	TTAAAAAAGTAGA CTGAAGTGTTG ACCA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
CTP	AW126130	ESTe	NA	2	Putative carboxyl-terminal peptidase	300	SNP	G/80/F	A/80/F	GTTTCGACCCGGACCACCATAATAGAAGTA	TGGAAATGACCATGTTCTAGGATACTGGC
CYS	AA660257	ESTe	AC139601.4	7	Cysteine synthase	*Apo*I	dCAPS	30 + 210	30 + 55 + 155	CGAAGCATATTTTTATTTACAGCRTCTC	ACCTTAATATTGAATTGCATGTGAAAGT
CYSK	AW207985	ESTi	NA	5	Cysteine synthase	400	SNP	G/305/F	A/305/F	GGAATTGCTAAAGATGTTACAGAATTGA	AATGAGGACACTCTGTCCAGGTGTGA
CYSP	AW125930	ESTi	NA	1	Cysteine protease	500	SNP	C/233/F	T/233/F	AAAGGACTATGCTTACACCGGAAGAGA	CACATACATTTCGGCCTCTGCAGATCT
CysPr1	AI974595	ESTe	NA	3	Cysteine proteinase-like protein	*Acl*I	CAPS	230	60 + 170	GAGAATTCAAAGAAGAAATTAAGACAAAGA	GAAGAATTCATGGGGAGCAAAGT
CysPr2	AI974635	ESTe	NA	1	Thiolprotease	*Spe*I	CAPS	240	30 + 210	CCAAAAACTTGCTTCTATACTCTTCATTC	GACAAAACCCACCCAGACAAATCAACTAG
CYSS	AW127154	ESTi	NA	5	*O*-acetyl-L-serine (thiol)-lyase	640	SNP	T/232/F T/254/F G/394/F	A/232/F C/254/F A/394/F	CTGATGCAGAAGAGAAGGGGCTTATCA	CCAATTCAGCTCCAAAAGCTAATAGAATGA
DENP	AI974308	ESTe	NA	4	Dentin phosphoryn [*Homo sapiens*]	NA	Length	195	222	AGAATTGGACTTCTTCTCACTCACG	CGGATGAAAAGCCTGAAGATAAGTC
DK003R	AQ841082	BEST	NA	5	Pea-PTO-like kinase	NA	Length	350	900	TCTGCGGTCATGAGGTGGTT	GATATATAGGTGATTTGGTTTCTACTAA
DK006R	AQ841074	BEST	NA	5	Pea-PTO-like kinase	NA	Length	450	420	GAACATAACCCCGAAGTGGAT	GAGTTTGGGAACAAAATTAGTATGAT
DK009R	AQ841079	BEST	NA	5	Ms-syntaxin-CG13	*Dra*I	CAPS	450	280 + 170	TAGCATCATCTTTCCCATACAA	GGGCAGGCAGCACCAGATA
DK012L	AQ841080	BEST	AC140772.7 AC137509.9	5	Mt-rRNA gene	*Dra*I	CAPS	200 + 30	230	AGCTTCTTTCCATTATTCCTTCC	GGAAAGTTAGGGGTGCTAAAACTTGCTTTTTAA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK013L	AQ841055	BEST	AC140772.7 AC137509.9	5	Mt-rRNA gene	*Rsa*I	CAPS	110 + 300	410	AAGCTTATCTATGC CATCTCGTT	CGAAAATGGTTAC TTTTAAGATGC
DK015R	AQ841060	BEST	NA	5	Ms-U224-1	NA	Length	100	90	ATTTCACGTGTATG CAAGATTATATG	CATCATATCTTCA CCCAAAGTCTTA
DK018R	AQ841066	BEST	NA	5	Ms-U492	NA	Length	230	220	TGATACATGTGG GAGCCTTGAAG	ACTGCATTTGGTT CGGATGTATTCG
DK020R	AQ841084	BEST	NA	2	Mt TL4 probe	NA	Length	140	150	TCCATATTCAAGC CACCAATTCCCAT	ATACAAAATGTTAC ACTAAAACACG ATA
DK024R	AQ841087	BEST	AC139670.10	4	Mt-TL4 PCR product	*Bgl*II	CAPS	440	130 + 310	GCCGCGCCATCT TTATTGA	GACGATTTTACCC TTTATCTAAGC
DK039R	AQ841114	BEST	NA	5	Pea-PTO-like kinase	*Dra*I	CAPS	370 + 80	450	CAATTACTAGATC TATTTTATTTT CAAGC	ACCACAAGCAGAGG GAGGATAGT
DK043R	AQ841097	BEST	NA	4	Mt-expansin I	*Rsa*I	CAPS	40 + 310	350	GGGACTAATTAAA AGAGAGAAAAAG AAAA	CGCGTGAGCCTCTT GAGCTTGATGC
DK045R	AQ841099	BEST	NA	2	Mt-chitinase III	*Hin*fI	CAPS	60 + 270	330	TGGCAATATCCAC CAAATCAAA	CGAACCCACGACC ACAAGG
DK049R	AQ841103	BEST	NA	1	Mt-chitinase III	NA	Length	400	370	GTGATTCTCATGT GCTCTGATGC	CGTTGGATAAACCC TAGACAAGATATT
DK103L	AQ841172	BEST	NA	4	Mt Histone H3	*Bgl*II	CAPS	430	80 + 350	GAAACTGCGCTGC TCTGGAATCTC	TCGATCCCAAAA CGAAAACTCC
DK128L	AQ841726	BEST	AC123571.5	5	Mt EST-AA660812	*Bsm*AI	dCAPS	330	300 + 30	AAAGTCGTTCCCC TCATTGTT	ACATATTTGAGGA GCGTTATTCTTTG CTAGTC
DK132L	AQ841734	BEST	NA	3	Mt EST-AA660521	*Xmn*I	dCAPS	320	290 + 30	TGGACCTAAGACT TCAAAGATTC AGA	CCTATTAAGCATAT TTGCAGCATGAA CAATTT
DK139L	AQ841733	BEST	NA	5	Unknown	*Eco*RV	CAPS	260	130 + 130	CTAGCAAAACTCA GAAAACCAAGAA	CGTTTTAGAGGAAA TATGATGAGCA
DK140L	AQ841738	BEST	AQ841738	1	Unknown	*Alw*NI	CAPS	139	29 + 110	ACCATGGCCAATG CGAGTTA	AAATGAATCTGAG CTTGGTAACGCC AGTAT

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK201R	AQ917159	BEST	NA	3	Gm RFLP-A352, AQ841755, AQ842022	*Dra*III	dCAPS	110	30 + 80	GGACGCGAGAAA TGGCAAAC	AGACTATTGGTGA TTTCATTGCACG AAGT
DK202R	AQ917161	BEST	NA	3	Gm RFLP-A352, AQ841755, AQ842022	NA	Length	285	300	CTGTATTTATCTC CTTTGCGAGAAT GTA	CAAAATGATAATT ATGCACGTAAAA GTAAG
DK224R	AQ917190	BEST	AC139355.5	7	Gm RFLP-A235, AQ841798, AQ842064	*Eco*RI	dCAPS	80 + 30	110	CGAAACAATAAT CACAAAACAAA TCAG	ATCTTGTTTATAT GTGTTTGTTGAA GACAGAATT
DK225L	AQ917191	BEST	NA	7	Gm RFLP-A235, AQ841798, AQ842064	*Nco*I	CAPS	270	60 + 210	TGTCCTTGCTTCT TATCCTTCCTTCA	AGCAGCACAACAAC TTACAACAACTC
DK229L	AQ917196	BEST	NA	6	Gm RFLP-A235, AQ841798, AQ842064	*Sty*I	CAPS	250	200 + 50	TGGCAAGTGGAG GAGAAGACG	ACCAGCCAGAAATC GAAACAGAA
DK236R	AQ917202	BEST	AC122726.17	8	Gm RFLP-A381, AQ841805, AQ842052	*Mwo*I	dCAPS	125	25 + 100	GCAATTTAAATGT AATCCATTGAA CCA	GTATTGTATTGTG AAGGGCATTGCT AGTA
DK238R	AQ917205	BEST	NA	2	Gm RFLP-A315, AQ841810, AQ842017	*Sac*I	CAPS	50 + 250	300	GCAATTTAAATGT AATCCATTGA ACCA	TTATGCTTCTGATT CTAACTAACCCCA
DK242R	AQ917211	BEST	NA	5	Gm RFLP-A947, AQ841815, AQ841993	*Hinc*II	CAPS	230 + 100	330	CGTATGTTTAAT CCGTTAGTCCG TCTT	GCTTGCTTAGATAT TTGGCACTTCA
DK258L	AQ917077	BEST	NA	3	Gm RFLP-K007, AQ841948, AQ842189	*Xba*I	CAPS	470	100 + 360	GTATTCAGGGATT GAGTAAGAAA AAGGA	ACAAAATCCGTGG ATGTATAAAAG TGTA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK264L	AQ917083	BEST	AC127167.16	4	Gm RFLP-A688, AQ841912, AQ842149	*Bsr*I	CAPS	290 + 110	400	GGGGAGTGTTG AGATATGCGT AAT	TGATCGAGGAA CCAAAATAAAG AAA
DK265R	AQ917086	BEST	AC126783.10	5	Gm RFLP-B139, AQ841929	*Hinc*II	CAPS	50 + 140	190	GTGTGTCTAATA GAAATGAATG ACGAAAAA	ATTACATTTATTTC CACTGCCTAATC AAC
DK273L	AQ917094	BEST	NA	3	Gm RFLP-K300, AQ841961, AQ842203	*Bcg*I	CAPS	30 + 100 + 220	350	TATGCCTGGTCTGT TCTTTCTTTACG	GCCCGTCCACCGC TTTTA
DK274L	AQ917096	BEST	NA	7	Gm RFLP-K390, AQ841966, AQ842113	NA	Dominant	380	0	TGCATAAGCTCA AAAATAAGTC AATCC	AGTAGATAAGCCCA CATAAGCTCAA AATA
DK277R	AQ917103	BEST	NA	2	Gm RFLP-A748, AQ841917, AQ842154	*Mnl*I	dCAPS	35 + 165	190	CTCAAATTCTCTA GTTTCAACATGG TATCA	GGGCTGTAGTATT TATACCTGAGTT AGTGAG
DK287R	AQ917123	BEST	NA	7	Gm RFLP-K390, AQ841966, AQ842113	*Dde*I	CAPS	270	135 + 135	AGCCGCCCTCTT GAACCTCC	TAGCTGCAACAAA GAAACCAAAACC
DK293R	AQ917133	BEST	AC121246.19	2	Gm RFLP-A748, AQ841917, AQ842154	*Dra*I	CAPS	80 + 210	290	ACTTACAAGGTTA GCGTCATTCTCC ATC	GCTATCCCACCTTA AAATTTCTTC ACAA
DK296L	AQ917136	BEST	NA	7	Gm RFLP-K390, AQ841966, AQ842113	NA	Length	380	670	GAAAGGATGAGAA GCGGGGATAC	TCGTCGATGAAA AAGTACCAATA GAA
DK297L	AQ917138	BEST	AC124609.12	2	Gm RFLP-A656, AQ841906, AQ842143	*Xba*I	CAPS	350	310	GGGAAACACATG AGCGAAGGAGT	GCATAGCAAAACC ACAATCTAACCA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK298R	AQ917141	BEST	AC126783.10	5	Gm RFLP-B139, AQ841929	*Bsu*36I	CAPS	430	215 + 215	ATAAGATAAGGGC CAACATAAGTA GAAAA	GAAACTTGAGAGT GAAGAAAGTGA TAGAAC
DK302L	AQ917144	BEST	NA	7	Gm RFLP-A681, AQ841814, AQ842066	*Ase*I	CAPS	30 + 200	230	GCATGGAAATAGT TTGGGTTAGTAG TTAGT	CTGATAAATGCATA TTTTCAACATAT GAATTAA
DK313L	AQ917231	BEST	AC122728.16	3	Gm RFLP-A059, AQ841836, AQ842008	NA	Length	265	240	GCCAAACATAGG CTAAGTGTGAA AAA	TGACACATAAATT GTTAGCATCTGA AGG
DK321L	AQ917245	BEST	AC122730.17	6	Gm RFLP-A233, AQ841844, AQ842071	*Msl*I	CAPS	120 + 250	370	GAGCGAGCTCAG GATAGACTTTA GAA	TCCCACCTCCAATT TGTAGACGAT
DK322L	AQ917247	BEST	NA	7	Gm RFLP-A023, AQ841847, AQ842074	*Dde*I	CAPS	90 + 175	90 + 30 + 145	GGACCGAACTGGG TCAACAAT	GCACCGAGATCCAC CAACAACTT
DK326R	AQ917254	BEST	NA	2	Gm RFLP-A064, AQ841851, AQ842078	*Dde*I	CAPS	130 + 220	80 + 130 + 140	CCAGCATGTAAA CAATTGAAAG GCA	GTTGAACGGCTTA AATATCGCACTA
DK332R	AQ917264	BEST	NA	2	Gm RFLP-A095, AQ841860, AQ842087	*Dra*I	CAPS	90 + 120 + 140	90 + 260	GGAAAATTATAAG CCAAACAACAG TAAAG	GATGATAACAATCG GGGAAAATAATG
DK340R	AQ917278	BEST	NA	1	Gm RFLP-A636, AQ841903, AQ842140	*Swa*I	dCAPS	230	205 + 25	GAGAGAGAGAAG AAATAGTTTG TTTTGCTT	GTCTTTTTTTTAAG GAGTTTTTCTAG AGATTTAAA
DK347L	AQ917286	BEST	AC138449.8	2	Gm RFLP-A063, AQ841754, AQ842009	*Bsi*HKAI	CAPS	390	40 + 350	AGATTTCATACCA GACGGAGGAT AGTTC	TTTAGGTGATGG TGGCGTTGTTC

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK348L	AQ917288	BEST	AC121242.15	7	Gm RFLP-A572, AQ841760, AQ841986	*Xmn*I	CAPS	125	25 + 100	TTGAGAGCTCGGG TCACATTTC	CTAATGTGTAAACC CTAATGTTTCAA CAGA
Dk351L	AQ917294	BEST	NA	8	Gm RFLP-A110, AQ841767, AQ841975	*Bfa*I	CAPS	50 + 280 + 140	330 + 140	TGCTTGGGCTTGA GCTTTTAGAA	CTGTTTGGGTATTA GTTTTTGTTGGG
DK353L	AQ917298	BEST	NA	4	Gm RFLP-A110, AQ841767, AQ841975	*Sph*I	CAPS	90 + 240	330	CCATGCCATGGA AGGGTGTTT	GCAAGAACCAGATA CCCTTGACATTT
DK355L	AQ917302	BEST	AC122165.24	5	Gm RFLP-A135, AQ841786, AQ842014	*Msl*I	CAPS	25 + 175	200	AACTAACTCTAA GATGCCACAT TATAGGCT	CAAAACATTCATCC GCCTATMCCAC CTCA
DK358L	AQ917308	BEST	NA	2	Gm RFLP-A363, AQ842067, AQ842107	*Eco*NI	CAPS	70 + 230	300	GTTTGCGCCACT TAAGGTTATCT CATT	TGTCACCATGTGG CACATTCATT
DK360R	AQ917313	BEST	AC131239.16	3	Gm RFLP-A363, AQ842067, AQ842107	*Mbo*I	CAPS	45 + 305	350	AAGATAGTGCGCT GGTGTGTCAT	TTAGCCCATTTGTA TATTTGGTCTTTT
DK363L	AQ917316	BEST	NA	4	Gm RFLP-A006, AQ841823, AQ842006	NA	Dominant	380	0	TTTGTTTTGTATG TATATGAATGG AATAACTTG	GTTTAGGTTATGC CTTGGGAATGA
DK368L	AQ917324	BEST	AC122171.12	1	Gm RFLP-A450, AQ841842, AQ842069	NA	Dominant	420	0	AGCTTGTGCACTT TTCCGTTTTA	CCTCTCTTAAGCTG CTTTATTTTTGT CTAT
DK369R	AQ917327	BEST	NA	1	Gm RFLP-A450, AQ841842, AQ842069	*Nla*IV	CAPS	150 + 160	310	GGAACGTGGAGTT GTTGATGGTAT TAT	GATGTAAAAACCTT TACACTTGATTG ATTG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK377R	AQ917335	BEST	NA	1	Gm RFLP-A487, AQ841888, AQ842123	*Bfa*I	CAPS	360 + 55 + 85	325 + 35 + 55 + 85	AGCAGCTAGCAC GTGTCCTTTGA	CCGACTAATTATCA ATATTGTCAAC TACATCT
DK379L	AQ917338	BEST	AC119416.14	4	Gm RFLP-A487, AQ841888, AQ842123	*Cla*I	CAPS	50 + 40 + 80 + 70 + 170	50 + 40 + 80 + 240	AGCTTGTTGAGGT GGAAGGAAGTC	GTGTGTATGAGTGT CGTAAGCCCCT
DK381L	AQ917341	BEST	AC119416.14	4	Gm RFLP-A487, AQ841888, AQ842123	*Hin*fI	CAPS	250 + 80	40 + 210 + 80	TGTTACAAAAAGA GTTGGTTGTCG TTC	GTGCACTTTTCAAT TTGTCCATCATA
DK382L	AQ917343	BEST	AC122162.19	3	Gm RFLP-A487, AQ841888, AQ842123	*Bcl*I	CAPS	250	220 + 30	GGATGGAGAGGGA CAGGAGGA	CTCTAAAAATAGC GAATGACTGAC TGTGAT
DK407L	AQ917366	BEST	AC123898	2	Gm RFLP-A086, AQ841762, AQ842011	*Alu*I	CAPS	320	250 + 70	TTAATTTTATCAA CCCACCATATTA GTCAA	CCAGTGCTGGAAAA GACAATCAATC
DK412L	AQ917373	BEST	NA	8	Gm RFLP-A538, AQ841896, AQ842132	*Bsm*I	CAPS	390	170 + 220	GCTGCATTCCTT CAAAACTTCA TCA	CGGCGCCATTCTT CACCTTAT
DK413L	AQ917375	BEST	NA	4	Gm RFLP-A538, AQ841896, AQ842132	*Spe*I	dCAPS	170 + 30	200	TGATTGACCCCTG CTTTGATGCT	GTCAGGTTTGTTGT TGTTTTTTCTTGC ACTA
DK417L	AQ917383	BEST	AC121232.16	3	Gm RFLP-A685, AQ841911, AQ842148	*Bbv*I	CAPS	410	180 + 230	ACTCGTCGCCTA ACAATATCAAC CAG	GAATTCCATATCC AACACCTTTAG ACTTA
DK419R	AQ917388	BEST	AC121232.16	3	Gm RFLP-A685, AQ841911, AQ842148	NA	Dominant	420	0	CATCAGAAGTTGC CAAGCTATCA GAG	GCTTTGGTGCCGTT GTCAGAAGTA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK427R	AQ917398	BEST	AC137986.8	7	Gm RFLP-B046, AQ841833	*Bsm*AI	CAPS	60 + 440	500	CCAAACAAGGAA AAGTGTTGGTG TCA	ATGAGAAACTTTT GAAATTTAGGAT ACGATAG
DK439L	AQ917416	BEST	NA	7	Gm RFLP-K070, AQ841952, AQ842193	*Bcg*I	dCAPS	60 + 32 + 8	100	GCATTTTCTCTT GAACAAATTAT AGTAGTCG	CTCAGAGCAATAA CACAGATCGAT TTATTT
DK445R	AQ917427	BEST	NA	2	Gm RFLP-K102, AQ841953, AQ842194	*Pml*I	CAPS	410	350 + 60	AAAGGCGAGCGA CAGTAGCAG GAC	AGCACGCGACGCA CAAATAACT
DK447R	AQ917430	BEST	AC135160.1 AC137667.8	4	Gm RFLP-K365, AQ841964, AQ842206	*Bgl*I	CAPS	100 + 270	370	GTGGACGGCGAC CACTTTGA	TGAGCAAATTTC GATTTACCC CTT
DK453L	AQ917438	BEST	NA	5	Gm RFLP-K494, AQ841971, AQ842118	*Xmn*I	CAPS	150 + 320	470	GAGATCCGAAACA ACGTCCAAAAAT	GGCACTACCCAACT CAGCAAACT
DK455L	AQ917442	BEST	NA	8	Gm RFLP-A060, AQ841826	*Bsp*HI	CAPS	330	220 + 90	AAGGTTGTGTTG CAGGCGGTTTT AGT	CACATACAGAGTT TCCAGGATTAC CATT
DK460R	AQ917453	BEST	NA	7	GM probe	*Pvu*II	CAPS	40 + 360	40 + 160 + 200	GTACCCGCACGC GACTTTTT	CCATGGAGCTACAT TCACAACACTTC
Dk473L	AQ917474	BEST	AC122172.19	3	Gm RFLP-A635, AQ841902, AQ842139	*Bcl*I	CAPS	130 + 230	360	AACTGGTTAACTC GCTAATTGCTA CATA	CAATCCTAAACCTC CCAAAAAGC
DK490L	AQ917504	BEST	NA	2	Gm RFLP-A611, AQ841776, AQ841988	*Hin*fI	CAPS	350	100 + 250	TGGTTCCAAATTC CACTCAAAAGC	AAAAATTGTGTTGT GGTTTAGTGGTA GAC
DK497R	AQ917515	BEST	AC123547.14	2	Gm RFLP-A073, AQ841751, AQ841973	*Mse*I	CAPS	20 + 60 + 400	20 + 60 + 110 + 290	GGGAAAAAGCCAA AGGGAATGAAG	AAATGTGAAGGGTG GTGGATAGGAT GATA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
DK500R	AQ917521	BEST	AC141414.2	3	Gm RFLP-A597, AQ841900, AQ842136	*Apo*I	CAPS	40 + 280	320	TGCAACAAAGCT TAAGAAATAG GAGAT	GGTAAGCCATCA CACTTTTTCA CAA
DK501R	AQ917523	BEST	AC141414.2	3	Gm RFLP-A597, AQ841900, AQ842136	*Apo*I	CAPS	200 + 220	420	TATTTGGGATG GAAGCTATGT TGATTGG	TGCTTTAAAGGA GAAGGTAGAT GATGAT
DK505R	AQ917527	BEST	NA	8	Gm RFLP-B212, AQ841789, AQ842050	*Ase*I	CAPS	200 + 180	380	GCCGCCGCTCCC AAACTT	CAATTCCCTCCG GCGTCACTT
DK511L	AQ917538	BEST	AC126779.10	5	Gm RFLP-A702, AQ841800, AQ841991	NA	Length	400	360	TAAATCCGAGCT TCAAACCAACT CAC	TCCAAACCATACCC TTAATTACTGA GCAT
DMY	AA660709	ESTe	NA	6	Putative protein	*Dra*I	CAPS	130 + 243	130 + 66 + 177	TCAAAGTCTCTTT TGCCGAACA	ACAATATAACAAAT TTTGAGGTCTA TGC
DNABP	AI737524	ESTi	NA	4	SAR DNA-binding protein	*Afl*II	CAPS	280 + 580 + 260	860 + 260	CCCTATGAGCTTG GGTTTGTCT	CTCATGGCATACG TGTTCAGC
DSI	AA660979	ESTi	NA	1	Disulfide isomerase P5 precursor	*Ssp*I	CAPS	510	100 + 410	CCAAGACATCTTT GGTTTCATCC	ACTGCAGAATCAC TTGCCGAGTT
DSIP	AI974248	ESTi	NA	3	Protein disulfide-isomerase precursor	960	SNP	T/104/F G/137/F	A/104/F T/137/F	TCCTTCTCAGATC TTCGCTGAGGAA TCA	CTTAATGGTTGG GAATCCCTTA ACATCA
EIF5A	AI974513	ESTi	AC122160.14	8	Eukaryotic initiation factor 5A3	*Dde*I	CAPS	980	360 + 620	CGCGCAGAGAAAG CATCAA	CACAATTGTGGGA CGAAGGAAC
ENOD16	X99466	GS	AC136953.4	8	MtENOD16	*Mse*I	dCAP	100	75	GTGTCCCGATGA CACTATTTAAG GATTC	AAGGACACTCTTC AAGCACACTTT AGATA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
ENOD40	X80262	GS	NA	5	Early nodulin 40	NA	Length	136	177	AACCAATGCCACT TTTCACTTTGC CTCC	AGACTCTTGCGAG TGCTACCATTT GACC
ENOD8	AF064775	ESTi	NA	1	Enod 8	*Sca*I	CAPS	900	400 + 500	CCATGCCCATTCC TACTTTTCA	GTGGATTCCACG GACTTTACTT ACT
ENOL	AA660534	ESTi	NA	7	Enolase	*Sph*I	CAPS	450 + 1050	1500	TTCCATCAAGGCC CGTCAGA	TTGCACCAACCC CATTCATT
EPS	AA661012	ESTi	NA	4	3-Phospho-shikimate 1-carboxy-vinyl trans-ferase	*Bgl*II	CAPS	1620	1050 + 570	GCTGTTGTGGAA GGCAGTGG	ACGACATACGGA ACAGAAATCAGT
EST158	AA660289	ESTe	NA	2	Vacuolar sort-ing recep-tor-like protein	*Bgl*I	dCAPS	180	25 + 155	TTACAAACCACAC CATAATTGCCA AATTG	TTGGTGACAACTG ACACGAATGAAA CTAC
EST400	AA660514	ESTi	NA	3	Unknown	*Sac*I	CAPS	500 + 500	1000	GGTGGCTGTCCC ACTGATTATGT	AAATGCTTGTGTT ATGCGGAGAG
EST671	AA660779	ESTe	NA	8	Hypothetical 71.3-kD protein	*Bgl*I	dCAPS	240	20 + 200	GGTGTTATCTATG AAAGAGGCCT CATTGG	TGTCCTTGGGTAT GTAGAAAAGCCT TCAC
EST718	AA660824	ESTe	NA	4	Hypothetical 15.4-kD protein	*Sca*I	CAPS	180 + 90	270	CGGCGGCATGCT TAGTGG	TCTTGGAATGCCT TTGAATGAATA
EST758	AA660863	ESTe	NA	1	Hypothetical protein	*Spe*I	CAPS	230	80 + 150	TCACTTCCCCTAA ATACGCTTCT	CTTAATTTCAGCT GCCATTTCAAC
EST763	AA660868	ESTi	NA	1	Hypothetical protein	*Hin*fI	CAPS	130 + 25	155	CACTCTAAAAAGG CCCAGAAGGTT TGACT	CTTATGACCAATA GTCTGTTCCA CTC
EST948	AA661051	ESTi	AC145021.5	4	Hypothetical 33.4-kD protein	*Hae*II	dCAPS	25 + 190	215	GCAGGGGTTTCG CTCCAGTG	AACTTAATGAAT GATTGGAAGG TTTAGCG
EXRN	AI974855	ESTi	NA	2	Putative exo-ribonucle-ase	910	SNP	C/160/R	G/160/R	GTTGGTGGTGGA AGTGATGGATCT CTGGA	AACCTAGATATGTT CGGGTAAGATA CTTGA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
FAL	AA661005	ESTi	AC122727.13	5	Fructose-1,6-bisphosphate aldolase	*Bcl*I	CAPS	450	110 + 340	TTATCGCCAATGCCGCCTACA	ATGATAAGTATGCATGTTCAGAGTCA
FENR	AW127593	ESTi	AC138010.9	5	Ferredoxin-NADP reductase precursor	670	SNP	T/28/F	C/28/F	ATGCTTATGCCAAAAGATCCAAATGC	CTCACAGCAAAGTCGAGCCTGAAGT
FIS-1	AI974522	ESTi	AC122726.17	8	fis1 protein	*Bbs*I	CAPS	1280 + 90	140 + 1140 + 90	TCAGTGATTGAGGGTTTTTCTACG	CTGTTTCATCAACTTCAGCAACTTT
GH1	AI974251	ESTe	NA	8	GH1 protein	*Bsm*I	CAPS	210	25 + 185	GAGCAATCAGACAATCCGAGGTA	GGTCTGTTTAATCTTTCTGCCAATGCATT
GH3	AW125947	ESTi	NA	8	GH3-like protein	530	SNP	C/337/F	T/337/F	CCTGTCTCGCAATGCAAACGTTGAATA	TCTCTAAAATAGGAACTTTTGTAATAGC
GLNA	AW125915	ESTi	AC139882.3	3	Glutamate-ammonia ligase	540	SNP	T/184/F T/314/F A/163/R C/165/R	C/184/F G/314/F G/163/R A/165/R	GAATGGTGCTGGTGCTCACACA	TGGTGGTGTCTGCAATCATGGAAG
GLO3	AA660821	ESTe	AC137670.5	8	Putative protein	*Hinc*II	dCAPS	210 + 30	240	TTTACTTTTGATTCATGAATTAAGCGTGTCAA	GTGATAAGAATTTGGAAGTTAGAACCTGAT
GLUT	AI974518	ESTi	NA	8	Putative glucosyl transferase	>1000	SNP	A/209/F T/213/F	G/209/F A/213/F	TACAAGGCAGGGAATCTTAAATCTGCA	TTATTCTCCAGACACCAGCAGTTCCA
GSb	Y10268	GS	AC139882.3	3	Glutamine synthetase	*Hin*dIIi	CAPS	130 + 50	180	CTATGAGAGAAGATGGTGGCTATGAAGTCATCTTG	GGAGAGAACAATATTATTATTTGCTTACC
HRIP	AW126332	ESTi	NA	1	Nicotiana HR lesion-inducing ORF	600	SNP	T/67/F G/68/F	C/67/F A/68/F	GGAAAAATTTATCCTCCAAATTGGGGGTA	AAAAATAGCAGTGCACCAAAAAGTGCTG
HYPTE116	AI737489	ESTe	NA	4	Unknown protein	*Bsr*I	CAPS	250 + 120	370	AACACAGAGGTAGCGTTTGGTTTAT	TCGGGTCAAGATCTCGTTCAA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
HYPTE3	AI974791	ESTi	NA	4	Hypothetical protein	350	SNP	C/83/R A/174/R	T/83/R G/174/R	TCGTCTCATGGTG GAATCGTGAT GGT	TTCCTCCTTTAAA CAAGCAAATT GGA
ISOFR	AW125903	ESTi	AC137669.4	5	Isoflavone reductase	370	SNP	A/152/R	T/152/R	GAAGGAGTTCCT TACACTTACCT TTGT	TGTTCCTCTGAA ACATAAGTTTT CTCAAGA
JUNBP	AW208151	ESTi	AC140026.6	3	JUN kinase activation domain binding protein	1150	SNP	C/49/F	T/49/F	TCTTCGTCATCAT CTTCGGCGATAG CACA	GGATGTGAATGA TACCATCCCA CGACA
KCoAT	AI974864	ESTi	NA	1	3-Ketoacyl-CoA thiolase	*Ear*I	CAPS	400	130 + 270	TGCTACTGCGGG TGGTAGATTTA	CTCCAGCACCAT CACTCACCT
LB1	X57732	GS	NA	5	Leghemoglobin	*Vsp*I	CAPS	210	170 + 40	TTTTAAAGAATAT AATGGCTTGT GGAGG	GGAGCGAAAATGT TACCTAAAATT AAG
MAAP	AI974800	ESTi	NA	8	Membrane alanylamino-peptidase	990	SNP	T/196/F T/205/F	A/196/F C/205/F	TACCTAAGACTG CACATGCTATG TAT	CATCACCAACAC GCTTTACAGTG CGGCT
MDH2	AI974363	ESTi	AC122171.13 AC122161.11	1	Malate dehydrogenase	*Dra*I	CAPS	70 + 100 + 1080	70 + 1180	CTTCCATTTTCGA TTCCTTTCATT	GCATGCCTCGACA ACATCAGT
MPP	AA660658	ESTi	NA	4	Mitochondrial processing peptidase	*Bst*BI	CAPS	110 + 330	440	TCCCCGAAACAAT CCTCATCTG	GCAAATGTGTAGC CCCAAAAGTTA
MRS	AA660381	ESTi	AC142222.7 AC144806.6	8	Methionyl-tRNA synthetase	*Dde*I	CAPS	210 + 856 + 55	210 + 720 + 136 + 55	GTCTGTGGTGGGA TCATGGAGT	TTTTGACCGGTTC CAAGTAGAGTAG
Ms/L27	AW574258	ESTi	NA	6	Translationally controlled tumor protein	410	SNP	T/56/F	A/56/F	ATGTTGGTTTAC CAGGATCTCC TTA	ATCATCATGCATGC TCTCACCAACA
Ms/L83	AW584613	ESTi	NA	7	Aldo/keto-reductase family, Ms AJ410092	1000	SNP	C/177/F A/368/F C/441/F	T/177/F G/368/F T/441/F	ATCCCCGCTTCC AAGCTGAGAACT	AAAGGGATTTATAC TTCAGGTTGAGA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
Ms/U131	AJ388687	ESTi	NA	4	Hypothetical protein, Ms AJ410096	410	SNP	C/177/R G/192/R T/195/R C/245/R	A/177/R A/192/R C/195/R T/245/R	ATGCTATTGGGAC TCAACACTCTGA	GGAATTGCACTATA CAGATGATAGGA
Ms/U141	AW587077	ESTi	NA	8	Hypothetical protein, Ms AJ410097	750	SNP	A/152/F C/284/F G/293/F C/331/F	G/152/F T/284/F T/293/F G/331/F	TTGATCAGCCACA GAAATATAAA CCA	GCCTCCCACAAAG TAACAAGTTTC
Ms/U336	X60386	GS	AC135796.11	8	Phytohemag-glutinin, Ms AJ410117	560	SNP	C/86/R A/242/R C/266/R A/517/R	T/86/R C/242/R G/266/R G/517/R	AGACGTGGCTAAC TTCGAAACACT	GAGCTTGAAACAT TAGCATTGTTG TTA
Ms/U515		ESTi		3	3-PGA dehy-drogen-ase, Ms AJ41-0128	850	SNP	C/155/F T/439/F	G/155/F C/439/F	GTTAAGGGAACCA TGACAACCACA	CATTCATTGTCA TACCAAGCAA CCA
MtEIL1	AQ841199	BEST	NA	3	Ethylene insensitive	*Eco*RI	CAPS	310	255 + 55	GACATGTATCGGA TTCTCACGAGC	CACCTGCGAGGTA TTCAAACTGTAA
MtEIL2	BH153075	BEST	AC124972.18	5	Ethylene insensitive	*FOK*I	CAPS	160 + 145	305	GGAGCATCCATAG CCACTGTTG	TATCTTTTATTTC GGTATTCATCT CCA
MTU04	AA660721	ESTi	NA	5	Hypothetical protein	*Nsi*I	CAPS	310 + 855	1165	ATGGGAAGAGGAT TGCTGTGATA	AAGCGAACATTTT TGGCATCTAC
MTU07	AI737610	ESTe	NA	4	Unknown protein	*Hin*fI	dCAPS	27 + 153	180	CAGACACCCAAAG AATTACCAGAA	GATGACCAGAGCC TAATACTATTT ATGACT
MTU10	AI974637	ESTe	NA	8	Unknown protein	NA	Length	200	250	CATCAATTTGTCA GTACTTCGGT CAG	TGGGTTCAAGAAG TGGAAGTAAAT AAT
NAM	AI974744	ESTe	NA	6	NAM (no apical meristem)-like protein	*Mse*I	CAPS	130 + 7 + 120 + 33 + 96	130 + 7 + 153 + 96	ATTCAGTGGCTC GATTGGTT CTA	TAACCTAAGTACAC CATGTAACTAAT TTTC
NCAS	AA660915	ESTi	AC139709.8	1	Neuronal calcium sensor 1	*Aci*I	CAPS	440	80 + 360	TTCCCAAGCCCA AATCCTAAT	CATCACCAGGCCAT CATCATAAGT

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
NPAC	AI737554	ESTi	NA	3	Putative nascent polypeptide-associated protein	*Ssp*I	CAPS	340 + 130 + 270 + 500	470 + 270 + 500	TGGCTCCAGGTC CAGTTATTGA	TCGGCTCTTCTTCTC GCTTCT
NRT2	AW225622	ESTe	NA	4	Putative high-affinity nitrate transporter	360	SNP	T/116/F C/248/F	A/116/F A/248/F	GGAAGCTCCATG CATGGAGTA	ATTGCACCCATTG CAAGCCTTGAGA
NTRBI	AI974835	ESTi	AC119410.4	8	Retinoblastoma-related protein	*Eco*RV	CAPS	428 + 132	560	CACGACTCTGCA CGCTTTGTTA	ACCCTTGTTGCGAG TCATTTG
NUM1	AA660969	ESTi	NA	4	Homolog of mammalian nucleolin	*Taq*I	CAPS	48 + 29 + 243	48 + 272	GATGCTGCTCCTGT TGTTGTTTC	AAAACAAAGTAAA AACAATATCTTT AAAAATC
OXG	AW126122	ESTi	NA	6	Oxygenase	720	SNP	G/311/F C/367/F T/411/F	A/311/F T/367/F C/411/F	AGGTGTAGCAAGA TATAACCAATT CAGGA	TTTGGTGGTGCATC CCAAACAGAGA AAG
PAE	AA660802	ESTi	NA	8	Pectinacetylesterase precursor	*Fok*I	CAPS	610 + 190	340 + 270 + 190	CTAAAAGCAGCAG AAGGGGTTAC	GATCCGGTCAAGGC AAGTAGTT
PCT	AI974454	ESTe	AC131248.5	4	Cholinephosphate cytidylyltransferase	*Rsa*I	CAPS	240	150 + 90	TTGGCAAAAACGA TAAACCTGT	CACGGCACATCTGG AATAACTT
PESR1	AA660526	ESTe	AC122166.14	7	Pectinesterase	*Aci*I	CAPS	237	176 + 61	CATCTGAACAAACC CATCTCCA	GCTGTTAATTCGGC GTTTGA
PFK	AA660630	ESTi	NA	2	Ppi-dependent phosphofructokinase β-subunit	*Ssp*I	CAPS	500	80 + 420	TCCCACTGCAAAT CATGTCAAAAC	ACACAAGTGGATAT TGATGGTTAG ACTAC
PGDH	AW126358	ESTi	NA	7	Phosphogluconate dehydrogenase	490	SNP	A/189/F	C/189/F	GAGTTGAAGCTG CAAAGGTCTT TAAATCA	TGTATGAGCACC GAAGTAGTCTC GTTGA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
PGKI	AA660202	ESTi	NA	2	Phospho-glycerate kinase	*Dra*I	CAPS	550	210 + 340	GATGACTGTATTG GCGAGGAAGT	GTTCGACACGGC TCCAACTA
PNDKN1	AW127113	ESTi	NA	8	Nucleoside diphos-phate kinase I	>1000	SNP	G/337/F	A/337/F	GGCCGAACAAACT TTCATCATGA TCA	CCAGGCTCGGATT GAGCAGGGTT TGT
PPDK	AI737496	ESTe	NA	8	Pyruvate phosphate dikinase	NA	Length	250	230	CAGCAGGGCATAG TCAAATAAGG	AATCCACATAAGTT CACCGTAAGAGT
PPGM	AA660893	ESTi	AC124215.13	7	Phospho-glycerate mutase	*Bbv*I	CAPS	165 + 210	375	AAAGAAAGATGG GAAGCACTG ATT	ACCAAGCGCGTTA TGACCAA
ppPF	AI974685	ESTe	NA	1	Ppi-dependent phospho-fructokinase sub	*Bst*NI	CAPS	130	30 + 100	TCTCGCCACCAA CAACAACTAC	AAAAATTGTTCAT GAACACTCACT TGAAGCCA
PROF	AW126318	ESTe	AC122730.15	6	Profucosidase	320	SNP	T/197/F	C/197/F	AGAAGTCAAAAAT GGTCTACCAG TGA	CAAATCTTCCAA TATCCAAACAA GTAGGA
PRTS	AI737609	ESTe	AC141863.6	3	20S proteo-some β-subunit	*Bsa*JI	CAPS	200 + 50	250	CATAGCTACTTGA TCTGAAAACTTG ACA	TGGTGAACTTCAC TACCATTACA ACC
PTSB	AW127108	ESTi	NA	5	Proteasome β-subunit	370	SNP	A/80/F	T/80/F	ACTAAACAACACG CTAATTGGTCT CCA	ATGCCTAGCAGA CAAAACCTTC TGCA
QORlik		BEST		4	Mt-apy2	*Rsa*I	CAPS	119 + 290	409	GATGGTCTGGCAA CTGT	AGGGAGGACTTTT CTTAG
RBBP	AA660276	ESTi	AC121243.10	2	WD-40 repeat protein MSI4	*Bgl*II	CAPS	220 + 860	1080	CAAGAGGACGCA AACCTAAACC	CACAATTCGCAAT CACCAAAGTAT
REP	AA660953	ESTi	NA	8	Poly(A)$^+$ RNA export protein	*Hae*III	CAPS	30 + 690	30 + 105 + 585	CTCCATTTCCCGT TCGTTCG	CACCGGTTGCCCT CCAGAC
rip1	U16727	GS	NA	5	Peroxidase precursor	*Ssp*I	CAPS	81 + 320 + 37 + 134 + 59	81 + 320 + 171 + 53	GCAATGCGTTGC TAGGGATTAATG ATGTGACC	AGTTTATAAAGAG TAACACACATC TCACC

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
RL13	AA661027	ESTe	NA	2	Ribosomal protein L13	*Mse*I	CAPS	35 + 10 + 30 + 65 + 40	35 + 105 + 40	GCCAAGCAGGTATC TATTCTTCATCT	CGGTACACAACAT AACCCTAAAATCA
RL3	AI974458	ESTi	NA	1	Ribosomal protein L3	*Pac*I	CAPS	250 + 100 + 230	250 + 60 + 40 + 230	GACACGGTTCTTTG GGATTTCTC	CCTGGCTTTTCGAC TTCTCTGAC
RLPO	AI974311	ESTi	NA	3	60S acidic ribosomal protein PO	*Bsp*HI	CAPS	320 + 740	1060	TCTTGGCCCTTTAA TTTCCTCTC	AACCTTGTATTTA GCAACTTCTTCA CTG
RNAH	AI974503	ESTi	AC123899.13	7	ATP-dependent RNA helicases	*Apa*I	CAPS	940	280 + 660	GCTTCCACCAGCT GATACACG	TTAGCCCTAGCAA GAATGTCACTG
SAMS	AI974327	ESTi	NA	2	S-adenosyl-methionine synthase 2	*Bst*BI	CAPS	1050	525 + 525	CATAGCAAAGCGGG TTCAATCT	GTCAGCATCAAGAC CAACATCATC
SAT	AW126397	ESTi	NA	1	Sulfate-adenylyl transferase	810	SNP	::/260/R	CA/260/R	GTATCATGATGGA CTTGATCATTT TCGTC	AGCCTTTGCATGC CACTGCACCTCA
SCP	AA660552	ESTe	NA	1	Serine carboxy-peptidase II	*Hinc*II	dCAPS	100	30 + 70	CACCAGCAGGAGA ATCAAGGAAC	TCGATTCGTACCC AATTTGTTTTTC AATTGTT
SDP1	AI974323	ESTi	NA	8	Seed protein precursor	*Ssp*I	CAPS	76 + 829 + 62	76 + 726 + 103 + 62	TGGCTCTAAATCA GGGGAAGAATA	TGTGACGGTTGAA TATCTGAATG TTT
SQEX	AA660711	ESTi	NA	8	Squalene mono-oxygenase	*Eco*RV	CAPS	810 + 260	190 + 620 + 260	TGCCGCTATAA AAAGTAAACAA AGAA	CAATTCACCCACA ATTCTATCAGG
SUSY	AW126351	ESTi	AC135798.18	8	Sucrose synthase	500	SNP	T/103/F C/136/F	C/103/F T/136/F	TCGCAATGAACCA CACAGATTTCA	GTCCAACCTTGC CATGGTGAAG ATA
TBB2	AW191276	ESTi	AC144474.1	4	Tubulin β-2 chain	450	SNP	C/147/R	T/147/R	TGTGGGATTCCAA GAACATGATGTG	TTCATACTCATCCT CCTCTGCAGTA
TCMO	AW127521	ESTi	AC141923.7	5	*Trans*-cinnamate4-mono-oxygenase	880	SNP	A/130/R A/162/R C/220/R, etc.	G/130/R T/162/R A/220/R, etc.	GTCTACGGCGAA CATTGGCGTAA AATGC	CAATTGCAGCAACA TTGATGTTCTC AACA

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
TE001	AI737538	ESTi	AC130963.13	6	CCCH-type zinc-finger protein	*Xmn*I	CAPS	500 + 500	1000	CGGCGCCGGAGATTACACTG	AATCACAAACCCACCCAACATCTG
TE011	AI737478	ESTi	NA	4	SCARECROW gene regulator	*Acl*I	CAPS	1140 + 410	1140 + 220 + 190	GGAGAGAAACCGGACTGAAGAAACA	CAAGAAGAAGCCCTAGTCCTCCATT
TE013	AI737484	ESTi	AC130963.13	6	Same as TE001	*Xmn*I	CAPS	320 + 500	820	TCCGCATGTACGAGTTCAAGATAAG	AAATCACAAACCCACCCAACATC
TE016	AI737494	ESTi	AC138016.8	4	Cyanogenic β-gluco-sidase	*Sal*I	CAPS	1300	400 + 900	TCCCCAGGCCTTACAAGATGATTAT	AAACACTCCCACGTCGCACTAAG
TE019	AI737500	ESTi	AC137666.7	5	Unknown protein	*Afl*II	CAPS	150 + 950	1100	GCATGTGACCGATGAGGAAACC	TTTTAGAATCAACAATGCAACCAGAAG
TGDH	AA660742	ESTi	NA	4	dTDP-glucose 4-6-dehy-dratase	*Mnl*I	CAPS	340	60 + 280	CGGTGGCTTCATCGGTTCT	GACGTGTATTGTAATCAGCAGGAGTA
TGFRIP	AW225617	ESTe	AC124957.12	2	Putative TGF-β receptor interacting protein	320	SNP	T/254/F	C/254/F	ATTCTGATGAAAGGCCACGAGAGGCCA	CAAGCTTATCACCAACAGAGAAATCGA
tRALS	AW126282	ESTi	AC125476.9	4	Cytosolic tRNA-Ala synthetase	330	SNP	A/52/R	G/52/R	GGTCTGCGAGCTGTTTTTGGAGAAG	GCAATTCCCTCCTCAGCTAAAAGTG
TRPT	AA660362	ESTe	AC122170.16	3	Triosephos-phate translocator	*Dde*I	CAPS	290	60 + 230	AACCACAATCTTTTCTCCCATCTT	AACATTCAAAGCCCACCAAGTT
TUP	AA660945	ESTi	AC136288.12	1	Translationally controlled tumor protein	*Bcl*I	CAPS	620	230 + 390	GAATGGGATGCTATGGGAAGTG	TGGATCAGTGGCACCATCTTTAT
UDPGD	AI974577	ESTi	AC119411.3	7	UDP-glucose 6-dehydro-genase	*Mnl*I	CAPS	1350	230 + 1120	CAAAAGCGTTTCATCACTCATCTCT	ATCGTCAAGGCCAGGTTCATAG

(*continued*)

TABLE 1

(Continued)

Marker name	Template sequence accession no.	Type	Sequenced BAC accession no.	Linkage group	Putative function or probe	Restriction enzyme or fragment size (bp)	Method	A17 restriction fragment pattern of CAPS or SNP position	A20 restriction fragment pattern of CAPS or SNP position	Forward primer sequence	Reverse primer sequence
UNK16	AI974672	ESTe	NA	4	Hypothetical protein	*Fsp*I	CAPS	330	150 + 180	CCTTCCAATATCC CTCCCACAT	GAAGAAAATGATG AAAAGCCAAAAG
UNK21	AI974840	ESTi	NA	7	Hypothetical protein	*Ear*I	CAPS	90 + 830	920	TCGCCTCCATGT CCACCTC	CGGCCTTGCTAAA TCAGTCAG
UNK27	AW225606	ESTe	NA	4	dTDP-glucose 4-6-dehydra-tase	320	SNP	T/47/F C/80/F:/ 220/F A/259/F	C/47/F T/80/F T/220/F C/259/F	GGCTTCATCGGTTC TCATCTCTGCGA	TGTAATCAGCAGG AGTACAAATTGC AGCCA
UNK29	AW171637	ESTi	NA	1	Unknown protein	>1000	SNP	C/328/R	T/328/R	CTAACTAATTCTG ATCTTCGAGAA GAGGC	TGGAGTGATTATAT TTGTCGGCTGAA ATA
UNK3	AI974271	ESTi	NA	4	VAMP-associated protein	*Mbo*II	CAPS	470	80 + 390	CACCGGAAATTCA ACAGCAAC	GACCTAGGCAACA CAACTCCATTA
UNK7	AI974400	ESTi	NA	5	Putative protein	*Xho*I	CAPS	1250 + 170	1420	AAAAAGCAGCAAG AGAAATGTCAAT	GAGAATCTTTCTC CATCGTATCTTA CTT
VBP1	AI974413	ESTi	AC122169.12	7	TGA-type basic leucine zipper protein	*Drd*I	CAPS	950	110 + 840	CTGGAGAGCAGAC CCATTCAAT	GCGAAAGCCTCCAA TCCAC
WPK4	AA660716	ESTe	NA	5	Serine/ threonine kinase	*Spe*I	dCAPS	220	190 + 30	AGCAACTACAAAT AGTTATGCAAA AGACTA	TGCTGTATGAGCT GCACTTGTCTG
zwilik	BH153068	BEST	NA	1	Zwille-like gene	*Xmn*I	CAPS	475	350 + 125	ATTTGAGTGTACC CATTGAGAT	TTTTGAAGATTTAT TTGTAGAGTA

ESTe, exon-derived markers; ESTi, exon-derived/intron-spanning markers; BEST, BAC end-sequence-tagged markers. Markers derived based on genetic markers in other legume species are indicated by the prefixes Gm (*G. max*), Ms (*M. sativa*), and Vr (*V. radiata*) under the "Putative function or probe" column. Where possible, GenBank accession numbers are also given for the corresponding legume homolog. NA, not available. In the case of SNP markers, the nomenclature indicates the nature of the base change and its position in the amplicon relative to the forward or reverse primer. Thus, "A/259/F" refers to adenine at position 259 relative to the forward primer.

TABLE 2

Intron analysis for EST markers

Total no. of loci analyzed	47
Total length analyzed (bp)	
Exon	10,693
Intron	12,599
Intron size (bp)	
Minimum/maximum	78 ~ 747
Mean size	161
Total no. of polymorphisms	
Exon	21
Intron	89
Average no. of nucleotides/polymorphisms	
Exon	509
Intron	142
Polymorphism ratio of exon/intron	1:3.6
No. of mutations in exons	
Synonomous	17
Nonsynonomous	4

vergence of each (Table 2). On the basis of this limited survey, the average intron size in *M. truncatula* was 161 bp, with a range of 78–747 bp, and the GT-AG rule for intron junctions was strictly conserved. As expected, polymorphisms were more frequent in intron sequences (on average, 1 SNP every 142 bp) than in the adjacent coding regions (on average, 1 SNP every 509 bp), with 80% of exon SNPs predicted to represent synonymous changes. In the case of 40 marker genes, we analyzed the correspondence between 64 empirically determined *M. truncatula* introns and the number and position of introns in the Arabidopsis homologs. We identified only a single discrepancy, namely a first intron in marker gene ASN2, present in Medicago but absent from the Arabidopsis homolog (At3g47340). The same first intron was present in six additional legume species (*i.e.*, *M. sativa*, *Pisum sativum*, *Phaseolus vulgaris*, *Vigna radiata*, *Lotus japonicus*, and *G. max*) from which the ASN2 PCR product was sequenced (data not shown), indicating that the intron is ancestral to this group of Papilionoid legumes.

Genetic map construction: The genetic map shown in Figure 1 was derived from the analysis of 274 codominant and 14 dominant PCR-based genetic markers. In total, 93 F_2 individuals from a cross between *M. truncatula* ecotypes *A17* and *A20* were genotyped. A skeleton version of this map was used previously to develop an integrated cytogenetic and genetic map of *M. truncatula* genotype *A17* (Kulikova *et al.* 2001), and thus the eight genetic linkage groups correspond to the individual chromosomes, with chromosome numbering derived from the corresponding linkage groups in *M. sativa* (Kiss *et al.* 1993; Kalo *et al.* 2000), as determined below. By convention, the cytogenetically determined short chromosome arms define the top of each linkage group. The 288 genetic markers span 513 cM with an average distance between markers of 1.8 cM (Table 3). Although the estimated correlation between the physical and genetic distance is 970 kbp/cM, in practice this value varies according to the specific regions under analysis, with previous analyses of five distinct euchromatic chromosomal regions yielding values ranging from 200 to 1100 kb/cM (Ané *et al.* 2002; Gualtieri *et al.* 2002; Schnabel *et al.* 2003).

A total of 177 codominant markers with complete genotype information were designated as "framework" markers (Figure 1). The majority of framework markers segregated as expected for codominant (1:2:1) alleles; however, 32% (56/177) of the markers exhibited distorted segregation, with the expected frequency of heterozygous individuals but overrepresentation of one homozygous state and underrepresentation of the other. In all cases, distorted marker segregation identified regions of multiple markers with abnormal ratios of alleles. In addition to linkage groups 4 and 8, which are discussed in greater detail below, three markers (*i.e.*, ppPF, NCAS, and TUP) on the short arm of chromosome 1 exhibited an excess of *A17* homozygotes; 11 contiguous markers on the long arm of chromosome 3 (*i.e.*, GSb through DK273L) exhibited an excess of *A20* homozygotes; and two markers on the long arm of chromosome 7 (*i.e.*, VBP1 and ENOL) exhibited an excess of *A17* homozygotes.

In the initial analysis, six well-defined linkage groups could be identified. These linkage groups were characterized by normal Mendelian segregation of marker loci (with the exception of the regions noted above), as shown by example for linkage group 2 (Figure 2a). The integrity of each of these six linkage groups (*i.e.*, linkage groups 1, 2, 3, 5, 6, and 7) was confirmed previously (Kulikova *et al.* 2001) by FISH studies in which multiple BAC clone probes from each linage group could be assigned to a single pachytene chromosome.

In contrast to the situation for the six linkage groups mentioned above, the 55 additional marker loci resolved unexpectedly into four linkage blocks. A majority of these loci exhibited distorted segregation ratios, with an excess of *A20* homozygotes and an underrepresentation of *A17* homozygotes, as shown in Figure 2, b and c. Two lines of genetic evidence suggest that these 55 genetic markers belong to two linkage groups. First, we mapped 26 of these loci on the genetic linkage map of the closely related *M. sativa*, where they resolved into two well-defined linkage groups (Ms LG4 and Ms LG8, respectively), as described below. Second, selected marker loci from within the distorted regions were genotyped in the *M. truncatula* segregating population derived from genotypes *A17* and DZA315 used by Thoquet *et al.* (2002) for construction of an AFLP- and RAPD-rich genetic map. In each case, the markers mapped to *M. truncatula* linkage groups that had been previously determined to correspond to the counterparts of *M. sativa* linkage groups 4 and 8 (G. Kiss, personal communication).

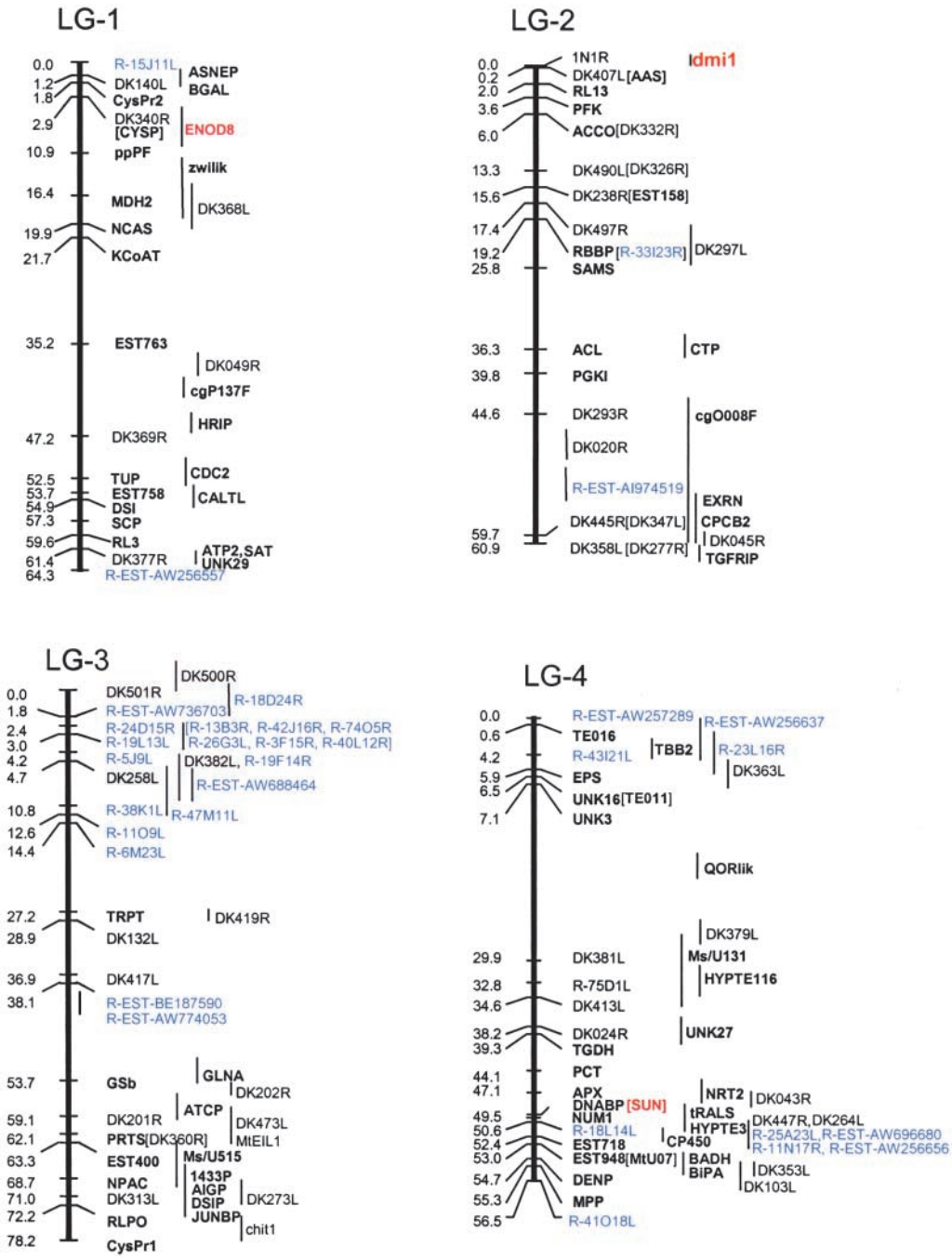

FIGURE 1.—Core genetic map of *M. truncatula*. A total of 288 molecular markers and 5 phenotypic markers were positioned on eight linkage groups, as follows. A total of 177 codominant markers are classified as "framework" markers, designated by horizontal lines connecting to the linkage group diagram. Framework markers shown in brackets could not be separated genetically from the adjacent, leftward framework marker. Markers that were genetically inseparable from more than one framework marker (*e.g.*, those with incomplete genotype information or dominant markers) are placed to the right of a vertical bar; the position of the vertical bar designates the extent of the genetic interval containing the marker. EST-based markers are shown in boldface type; nodulation-related markers are shown in red; BAC end-sequence-tagged (BEST) markers are preceded by the initials "DK" [with the exception of BEST resistance gene analog (RGA) markers]. RGA markers are shown in blue, with the prefix "R-" signifying BEST markers and "R-EST" signifying EST-based markers.

To test the assumption that these markers correspond to loci on chromosomes 4 and 8, respectively, of *M. truncatula* genotype *A17*, we used FISH to determine the physical location of 16 of these markers in pachytene chromosome spreads (Figure 3, a–e). As a prelude to this analysis, each genetic marker was converted to a corresponding BAC clone contig by hybridizing PCR fragments to high-density filters of the *M. truncatula* BAC library (NAM *et al.* 1999) or by PCR analysis of a BAC library DNA multiplex. A total of 16 BAC clones were used as probes for FISH analysis, as highlighted in Figure 3, b and c. Initially, we observed that BAC clones 34J06 and 43B05 gave signals on different chromosomes. One of these chromosomes, containing 34J06, could be identified as chromosome 4 according to our knowledge of centromere position and location of a diagnostic repeat, *Mt*R1, in the pericentromeric heterochromatin of the short arm (data not shown). A first series of hybridizations was performed with five BACs, four of which, namely 10F20, 1P05, 5K15, and 41H08, were mapped in a previous study (KULIKOVA *et al.* 2001). In a second series of hybridizations, a new set of probes, including 15B23, 06B09, 66M02, 34J06, 47M03, 70L14, and 11C13, was used. All of these BAC clones mapped to chromosome 4. The individual hybridization patterns are shown in Figure 3, a–c, while a

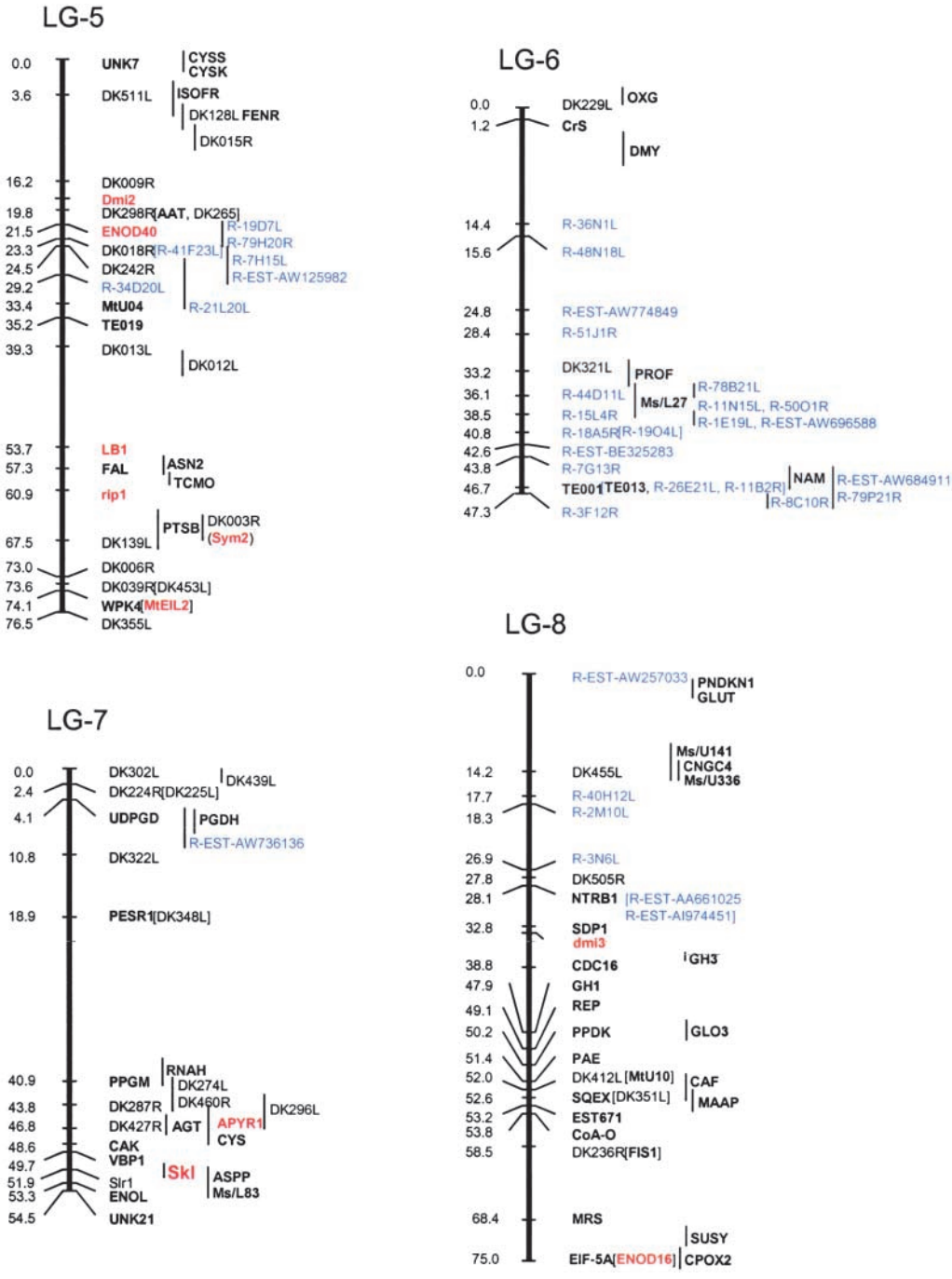

FIGURE 1.—*Continued.*

composite diagram integrating genetic and cytogenetic data for linkage group 4 is shown in Figure 3f. On the basis of a similar set of analyses, five BAC clones, 43B05, 22O13, 69K21, 50M17, and 10M16, were positioned on chromosome 8. The individual hybridizations are shown in Figure 3, c–e, with a composite summary of the genetic and cytogenetic data for linkage group 8 shown in Figure 3g.

Comparative linkage analysis between *M. truncatula* and *M. sativa*: Constructing a comparative map between *M. truncatula* and *M. sativa* was facilitated by the high level of nucleotide conservation between these two species, which allowed the direct application of genetic markers in either direction. Of 81 markers analyzed, 68 were successfully mapped. For the remaining 13 markers, 4 primer pairs failed to amplify *M. sativa* DNA, 2 markers lacked polymorphism, and 7 markers generated uninterpretable sequence (probably mixtures of multiple loci). As shown in Figure 4, the marker alignment between the two Medicago maps reveals an extremely high level of synteny between *M. truncatula* and *M. sativa*, including the distorted regions of *M. truncatula* linkage groups 4 and 8, described above.

Despite the overall high level of similarity, several

TABLE 3

Distribution of marker types by linkage group

	No. of markers					Distance (cM)	
LG	Total	EST	BEST	RGA	Phenotypic	Total	Average
1	31	22	7	2	0	64.3	2.01
2	32	14	15	2	1(*dmi1*)	60.9	1.97
3	47	15	14	18	0	78.2	1.78
4	47	25	11	10	1(*sunn*)	56.5	1.23
5	39	16	15	7	1(*dmi2*)	76.5	1.96
6	31	8	2	21	0	47.3	1.48
7	28	15	11	1	1(*skl*)	54.5	2.02
8	38	26	5	6	1(*dmi3*)	75.0	2.08
Total	293	141	80	67	5	513.2	1.78

EST, expressed sequence tag marker; BEST, BAC end-sequence-tagged marker; RGA, resistance gene analog markers. Phenotypic markers represent nodulation mutations mapped on the basis of the segregation of nodulation phenotypes in F_2 progeny of genotype *A17* mutant lines crossed with *A20* wild type.

differences were noted. One apparent difference was the position of a 5S rDNA locus. In *M. truncatula*, a 5S rDNA locus mapped to LG5, while in *M. sativa* a 5S rDNA locus was mapped to LG4. However, cytogenetic analysis indicates the presence of three 5S rDNA loci in *M. truncatula* genotype *A17* on LG2, LG5, and LG6 (Kulikova *et al.* 2001), while the number of 45S rDNA loci has been observed to vary between genotypes of *M. truncatula* (T. Bisseling and O. Kulikova, personal communication). The position and number of 5S rDNA loci has also been observed to vary between ecotypes of *A. thaliana* (Fransz *et al.* 1998), so it should not be surprising to find such a difference between species of the same genus.

We noted two additional differences that are likely to be more substantive than those of the rDNA loci, described above. The PCT primers listed in Table 1 identified a single locus on *M. truncatula* linkage group 4. However, Southern blot analysis of *M. truncatula* genomic DNA using the PCT PCR fragment as probe identified four putative paralogous sequences that hybridized to the PCT marker. One of these loci was polymorphic and mapped to linkage group 2 (Figure 4), while the other three fragments were not polymorphic for the enzymes used. In *M. sativa*, only one hybridizing locus was evident, corresponding to a polymorphic, single locus at the syntenic position on linkage group 2 (Figure 4). In a second case, the NUM1 gene was mapped to LG4 in *M. truncatula* by means of NUM1-specific primer pairs. Using the same primers in *M. sativa*, an ~2-kbp nonpolymorphic fragment was amplified. The gel-purified fragment was used as a probe to map NUM loci in both *M. truncatula* and *M. sativa* by means of RFLP. The hybridization pattern of *M. truncatula* identified two loci, Mt-NUM1 on LG4 and Mt-NUM2 on LG8. The location of the Mt-NUM1 locus on LG4 corresponded to the locus mapped by CAPS. By contrast, the hybridization pattern of the NUM1 probe in *M. sativa* was complex, generating >30 bands. The deduced genotypes generated at least five polymorphic loci, of which one (Ms-NUM1) mapped to LG4 and the other (Ms-NUM2) mapped to LG8. The middle repetitive-like hybridization patterns of PCT in *M. truncatula* and of NUM1 in alfalfa suggest that PCT and NUM sequences may have evolved differently in these two closely related plant species.

DISCUSSION

In this study, we positioned 288 sequence-based markers on the genetic map of *M. truncatula*, covering 513 cM. Each linkage group contained an average of 36 markers, with a range of 27–47 (Table 3). Thoquet *et al.* (2002) recently published a genetic map of *M. truncatula* that spans 1125 cM and is composed of 289, predominantly RAPD and AFLP, genetic markers. The difference in total genetic distance covered by the two mapping efforts may derive from inherent differences in mapping parents [*A17* and DZA315 in the case of Thoquet *et al.* (2002) and *A17* and *A20* in the present study] and also from the marker types used. Thus, in contrast to mapping expressed genes as codominant markers, which was the focus of the current study, the AFLP and RAPD strategy used by Thoquet *et al.* (2002) maps anonymous loci that typically exhibit dominant inheritance. Although it is likely that the two strategies surveyed different regions of the genome, both efforts produced eight well-resolved linkage groups that could be readily aligned with the eight genetically defined linkage groups of diploid *M. sativa* (Kalo *et al.* 2000; Thoquet *et al.* 2002). Efforts to link the two genetic maps of *M. truncatula* based on simple sequence repeat markers derived from ESTs and sequenced BAC clones are currently underway.

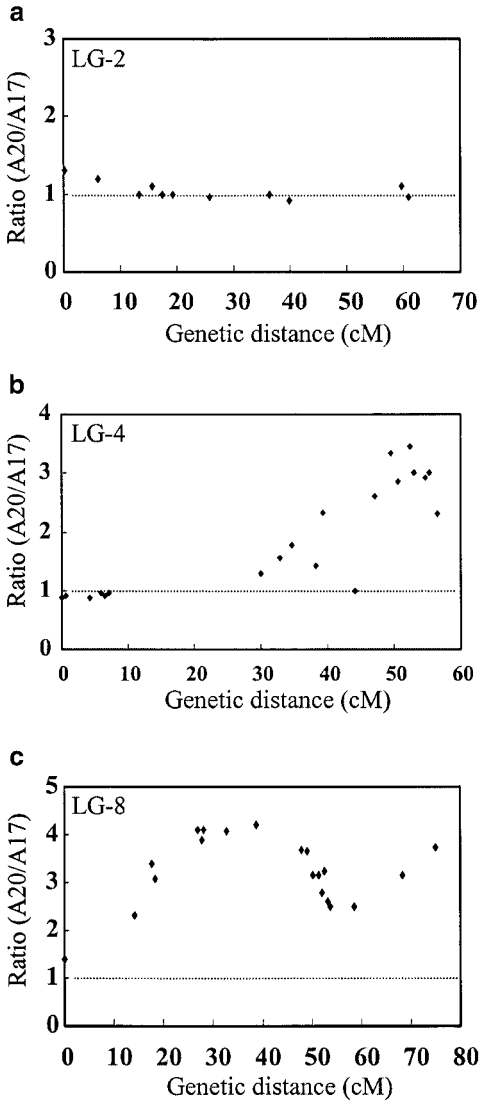

FIGURE 2.—Segregation ratios for markers on linkage groups (a) 2, (b) 4, and (c) 8. The primary feature of segragation distortions on linkage groups 4 and 8 is an overrepresentation of genotype *A20* homozygotes and a corresponding underrepresentation of genotype *A17* homozogotes. Values on the horizontal axis correspond to the genetic position of markers in the respective Figure 1 linkage groups.

Because the genetic markers used in this study are primarily expressed sequences or BAC clones that contain predicted genes, their position in the genome can be considered to provide a rough definition of the "gene space" of *M. truncatula*. On the basis of cytogenetic analysis (KULIKOVA *et al.* 2001), the structure of *M. truncatula* chromosomes is apparently relatively simple, with condensed heterochromatic DNA in centromeric and pericentromeric islands, flanked by mostly euchromatic arms. In the process of constructing the cytogenetic map for this species, >60 of the EST-containing BAC clones genetically mapped in this study also have been mapped to euchromatic regions of the *M. truncatula* genome by means of FISH (KULIKOVA *et al.* 2001; O. KULIKOVA and T. BISSELING, personal communication). These results indicate a high level of correspondence between euchromatin and transcribed genes, reminiscent of the relationship observed in *A. thaliana* where >96% of the transcribed genes are contained within euchromatic regions of the genome (ARABIDOPSIS GENOME INITIATIVE 2000). Consistent with this hypothesis, the average predicted gene density for the 92 genetically mapped and sequenced, EST-containing BAC clones is ~1 gene/6 kbp (B. A. ROE and D. KIM, personal communication). Thus, the correspondence of sequenced BAC clones with genetically mapped loci expands the total number of ESTs and predicted genes on the genetic map to ~1800. The accession numbers for these sequenced BAC clones are given in Table 1.

In addition to the mapping of ESTs or BAC clones selected strictly on the basis of homology criteria, the genetic positions of five phenotypic markers associated with nodulation, *dmi1*, *dmi2*, *dmi3*, *sun*, and *skl*, are shown in Figure 1. Map positions were determined by virtue of the fact that the genetic markers developed in this study were used to map the respective loci in F_2 populations of mutant *A17* × wild-type *A20*. With the exception of the *skl* locus (PENMETSA and COOK 1997), located on the long arm of chromosome 7, the map locations of the other loci have been previously reported (ANÉ *et al.* 2002; ENDRE *et al.* 2002; SCHNABEL *et al.* 2003) and the information is included here for purposes of integration. Interestingly, recent evidence from physical map data and complete sequencing of a 500-kb BAC contig indicates that *dmi1* is immediately adjacent to the telomere (ANÉ *et al.* 2004), and thus this locus defines a genetic and physical terminus of this linkage group. In addition to genes implicated in nodulation based on phenotypic criteria, we also mapped several genes whose expression patterns are correlated with nodule development or function. Several of these genes, including ENOD40 (YANG *et al.* 1993; CRESPI *et al.* 1994), the Rhizobium-induced peroxidase (*rip1*; COOK *et al.* 1995), and the leghemoglobin gene LB1 (GALLUSCI *et al.* 1991), map to LG5, which also contains *dmi2* (ENDRE *et al.* 2002) and the syntenic counterpart of the *Sym2* region of *P. sativum* (GUALTIERI *et al.* 2002; LIMPENS *et al.* 2003). Despite the apparent abundance of nodulation-associated genes on linkage group 5, several nodulation genes (nodule expressed transcripts and phenotypically mutant loci) are distributed elsewhere in the genome, including a cluster of ENOD8-like genes on linkage group 1 (DICKSTEIN *et al.* 2002), ENOD16 on linkage group 8, a cluster of apyrase genes on linkage group 7 (COHN *et al.* 2001), and *sunn*, *skl*, *dmi1*, and *dmi3* on linkage groups 4, 7, 2, and 8, respectively.

In addition to genes implicated in symbiosis, >100 resistance gene analogs have been previously mapped to 67 separate loci (ZHU *et al.* 2002). The majority of markers for toll/interleukin receptor (TIR) and non-

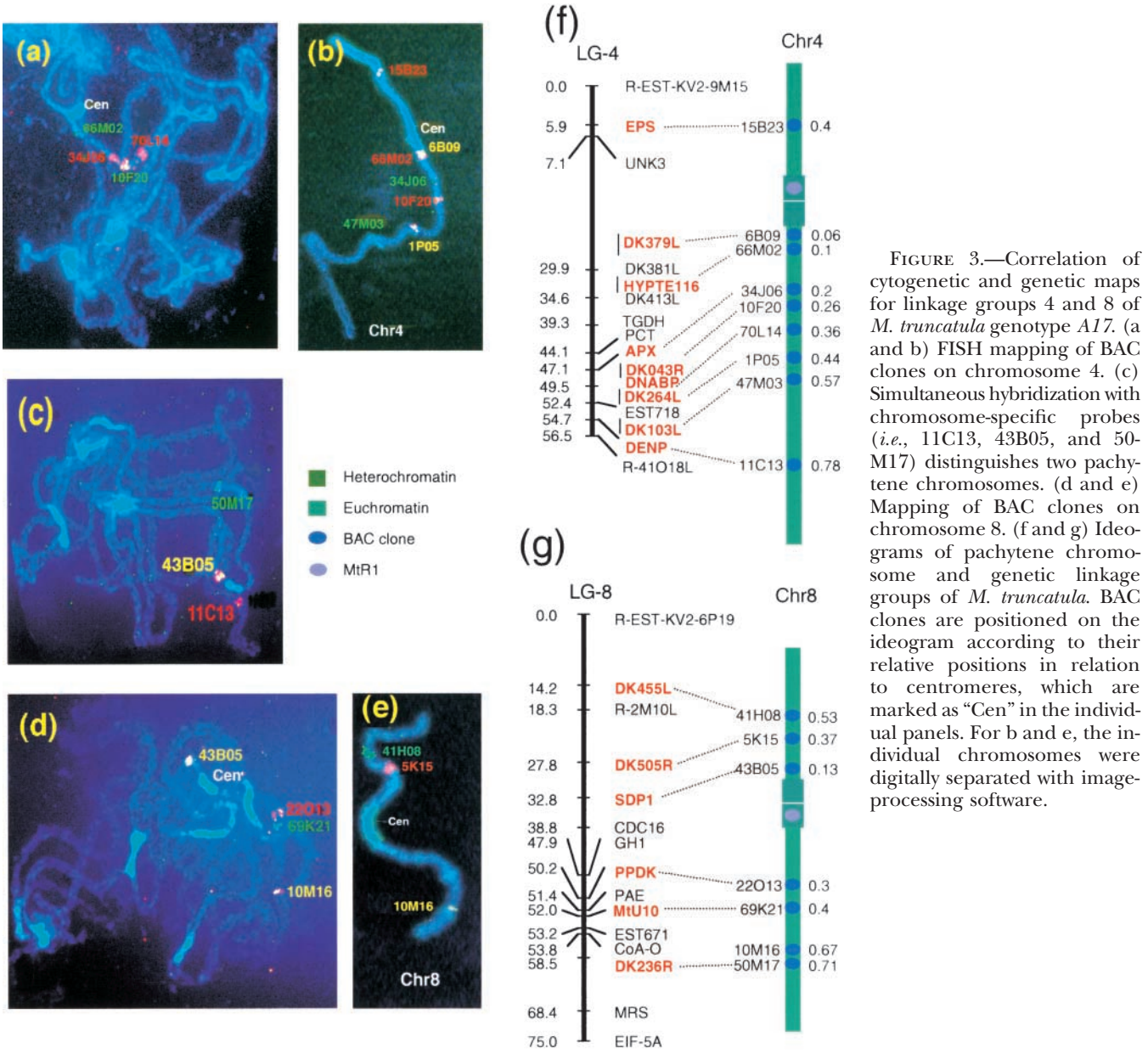

FIGURE 3.—Correlation of cytogenetic and genetic maps for linkage groups 4 and 8 of *M. truncatula* genotype *A17*. (a and b) FISH mapping of BAC clones on chromosome 4. (c) Simultaneous hybridization with chromosome-specific probes (*i.e.*, 11C13, 43B05, and 50-M17) distinguishes two pachytene chromosomes. (d and e) Mapping of BAC clones on chromosome 8. (f and g) Ideograms of pachytene chromosome and genetic linkage groups of *M. truncatula*. BAC clones are positioned on the ideogram according to their relative positions in relation to centromeres, which are marked as "Cen" in the individual panels. For b and e, the individual chromosomes were digitally separated with image-processing software.

TIR NBS-LRR resistance gene analogs are clustered, with major clusters identified on the short arm of linkage group 3 and throughout linkage group 6. Interestingly, in the absence of the resistance gene analog markers, linkage group 6 contains only 10 genetic markers and fails to coalesce as a distinct linkage group. Thus, linkage group 6 is threefold underrepresented in the number of non-RGA genes compared to the seven other linkage groups, while containing 33% of all mapped RGA loci. Chromosome 6 is also unusual in the respect that it is the shortest and most heterochromatic of all *M. truncatula* chromosomes (KULIKOVA *et al.* 2001).

The genetic map of *M. truncatula* was difficult to interpret for linkage groups 4 and 8. In each of these cases significant deviation from Mendelian segregation was observed, with *A20* homozygotes significantly overrepresented in the populations (Figure 2, b and c). On the basis of a combination of comparative genetic mapping in *M. sativa* and analysis of an alternate mapping population of *M. truncatula* (THOQUET *et al.* 2002), we were able to resolve the genetic relationships between these two linkage groups. FISH analysis with genetically mapped BAC clones was used to verify the predicted marker order and linkage group assignments, while color mapping was used to determine that the recombination map was consistent with the interpretations from these analyses. The value of 32% distorted marker segregation observed in this study is similar to the 25% distorted segregation reported by THOQUET *et al.* (2002). Moreover, in both studies a cluster of markers with dis-

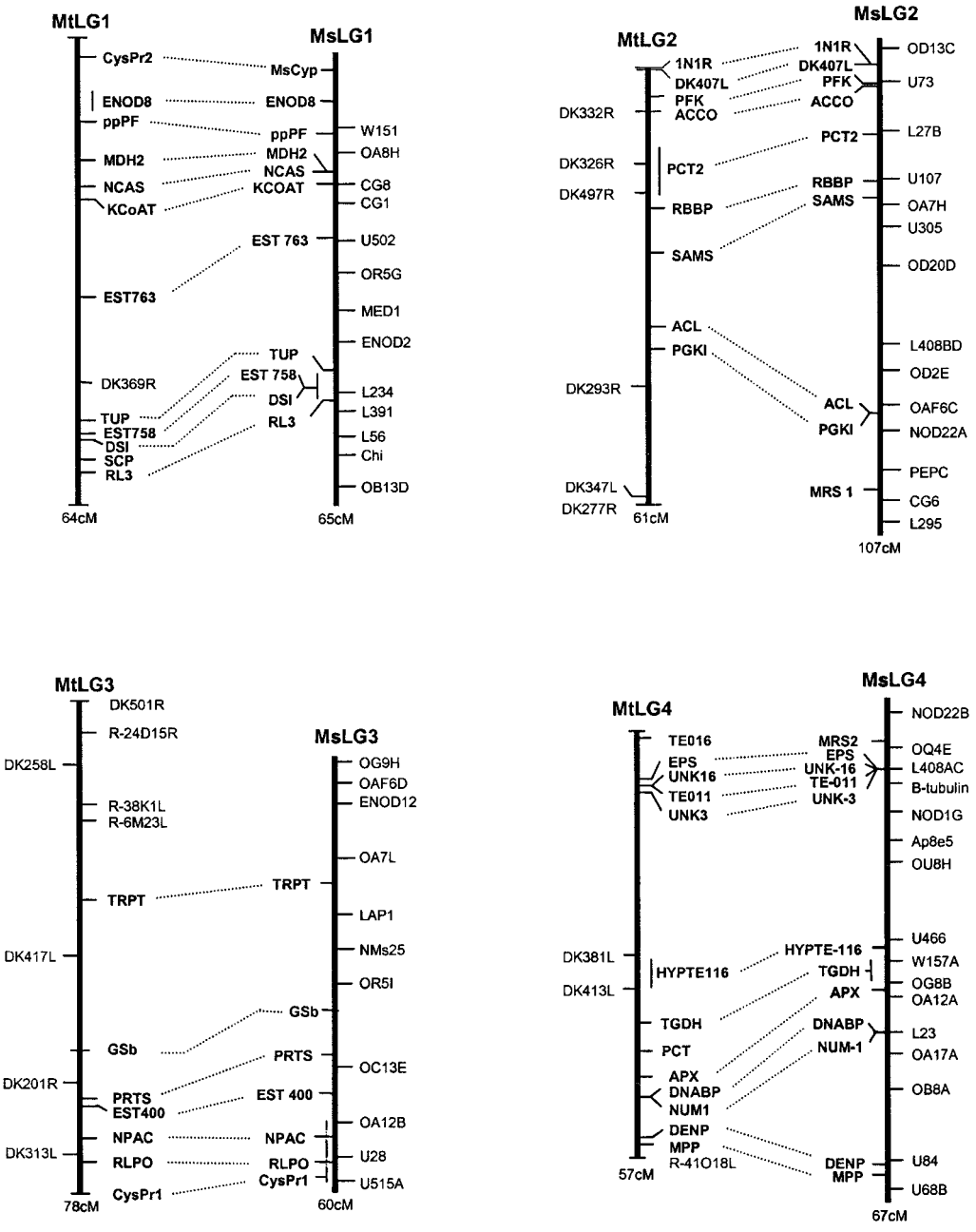

FIGURE 4.—Comparative genetic map of *M. truncatula* and *M. sativa*. The relative separation of genetic markers on each linkage group is correlated with genetic distance, although centimorgan scales have been omitted for simplicity. Genetic markers mapped between the two genomes are designated by boldface type.

torted segregation was observed on chromosome 3, including the common marker locus "GSb," suggesting a possible contribution from the common parental background of genotype *A17*. By contrast, the distorted marker segregation for linkage groups 4 and 8 observed in this study was not evident in the THOQUET *et al.* (2002) analysis, suggesting a possible incompatibility between *A17* and *A20* alleles in these genome regions. In the case of *M. sativa*, which is an outcrossing species, segregation distortion is typified by an overabundance of the heterozygous genotype (KALO *et al.* 2000). This contrasts with the overabundance of paternal (homozygous *A20* or *A17*) genotypes, described in this study.

The ultimate goal of constructing this genetic map was to describe structural/genetic features of the genome of *M. truncatula*. We anticipate that an EST-based genetic map will also have utility for the many map-based cloning projects currently underway in *M. truncatula*. Finally, a sequence-based genetic map of *M. truncatula* should have utility for comparison of genome structure between legume species and thus for the characterization of traits with potential application to agriculture in legumes. We have documented a high degree of conservation in gene content and order between the genomes of diploid *M. sativa* (alfalfa) and *M. truncatula*, suggesting that the current genetic map and ongoing genome sequencing of *M. truncatula* will have significant utility for defining genome organization in cultivated alfalfa (BROUWER and OSBORN 1999). Moreover, we anticipate that many of the gene-based genetic markers

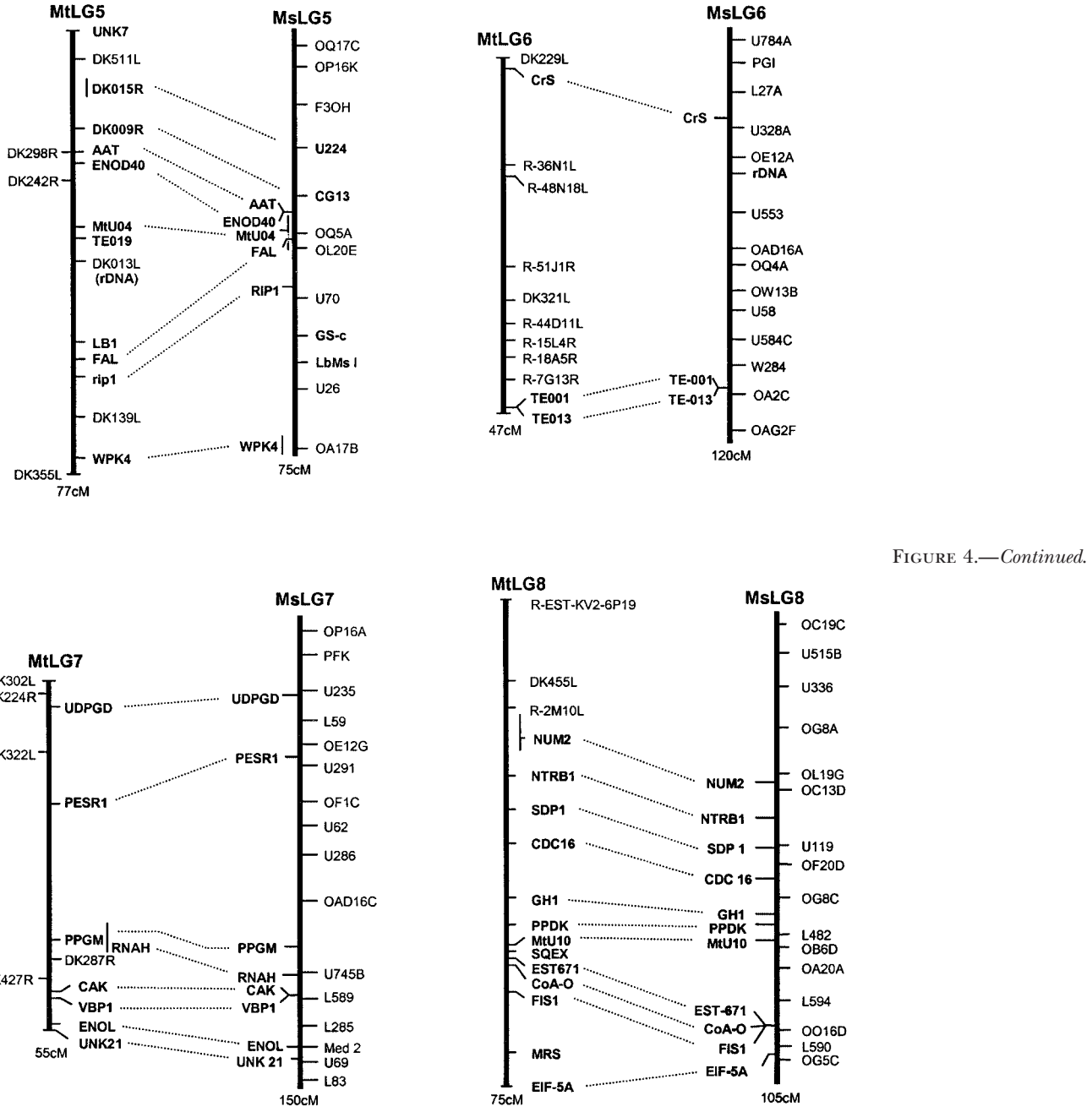

FIGURE 4.—*Continued.*

developed in this study will have applications for comparative mapping to other related legume species.

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