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Authors: Jain, Shalu, and McPhee, Kevin E.

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Applications in Plant Sciences

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Primer Note

Isolation and characterization of novel EST-derived genic markers in *Pisum sativum* (Fabaceae)[1]

Shalu Jain[2] and Kevin E. McPhee[2,3]

[2]Department of Plant Sciences, North Dakota State University Fargo, North Dakota 58108-6050 USA

- *Premise of the study:* Novel markers were developed for pea (*Pisum sativum*) from pea expressed sequence tags (ESTs) having significant homology to *Medicago truncatula* gene sequences to investigate genetic diversity, linkage mapping, and cross-species transferability.
- *Methods and Results:* Seventy-seven EST-derived genic markers were developed through comparative mapping between *M. truncatula* and *P. sativum* in which 75 markers produced PCR products and 33 were polymorphic among 16 pea genotypes.
- *Conclusions:* The novel markers described here will be useful for future genetic studies of *P. sativum*; their amplification in lentil (*Lens culinaris*) demonstrates their potential for use in closely related species.

Key words: comparative mapping; expressed sequence tags; lentil; marker-assisted selection; pea; synteny.

Pea (*Pisum sativum* L.) is an important grain legume grown in temperate regions of the world because its seeds are a cheap and rich source of protein and contribute to the nutritional quality of human and animal diets. Marker-assisted selection (MAS) for agronomic traits such as yield, quality, and tolerance to abiotic and biotic stresses is not widely applied in pea due to unavailability of a reference pea genome and the limited number of molecular markers for tagging of agronomically important genes in pea improvement programs (Jain et al., 2012; Smykal et al., 2012). Pea expressed sequence tag (EST) sequences (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) are valuable tools for developing breeder-friendly markers from coding regions of genes and have been used in the past to develop a modest number of simple sequence repeat (SSR) markers in pea (Xu et al., 2012; Mishra et al., 2012; DeCaire et al., 2012; Zhuang et al., 2013). Genomic resources of the sequenced model legume *Medicago truncatula* Gaertn. (http://gbrowse.jcvi.org/cgi-bin/gbrowse/medicago/) also offer a wealth of information for developing EST-derived genic markers in closely related species using a comparative genomics approach (Smykal et al., 2012). Genic markers developed in this study using the conserved sequences between the two legumes are valuable because they can add density to gene-rich linkage maps of pea, establish macro- or microsynteny between *M. truncatula* and pea, and have higher chances of transferability between closely related species. This information can help in identifying markers that are tightly linked to the genes of interest or candidate gene/quantitative trait locus for agronomic traits.

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[3]Author for correspondence: kevin.mcphee@ndsu.edu

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Investigation of conserved regions in different studies has provided strong evidence for sequence correlations between *M. truncatula* and pea (Choi et al., 2004a; Aubert et al., 2006; Bordat et al., 2011). This information can be used to develop genic markers based on sequence homology between the related species. Choi et al. (2004b) developed EST-based intron-targeted primers after aligning *M. truncatula* ESTs with the homologous genomic sequences of *Arabidopsis* (DC.) Heynh. and used them to construct a genetic map of *M. truncatula*. The basic assumption for this strategy is that introns or noncoding regions contain more DNA polymorphism than exons or coding regions (Brauner et al., 2002). A similar strategy—one that allows amplification of genomic DNA fragments covering two or more exons and bracketing polymorphic intron regions between those exons—was used in this study to develop pea EST-derived genic markers. Markers developed in this study are also available as cross-species markers within the legume family.

METHODS AND RESULTS

Primers were designed from pea EST sequences having significant similarity (score ≥100; *E*-value ≤e^{-50}) using the BLASTn search with *M. truncatula* gene calls from the contig assembly (Mt3.0) of *M. truncatula*. Approximately 1200 *M. truncatula* gene calls were searched for presence of introns. One or more introns were present in 510 of the 1200 *M. truncatula* gene calls and were aligned with the available pea ESTs (n = 18576) in the database. Seventy-seven primers were designed from the pea ESTs having well-conserved sequences with *M. truncatula* gene calls spanning one or more introns. Primers were designed by importing sequences into Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) and selecting primers 18–24 bp long with annealing temperatures of 55–65°C. New primers were designed to amplify fragments from 150 to 1200 bp.

Genomic DNA of 16 pea genotypes including widely grown cultivars and plant introduction lines (i.e., Shawnee, Melrose, Medora, Lifter, Radley, PI 179449, Green Arrow, Frolic, A778-26-6, Sparkle, JI73, Bohatyr, ICI12043, PI 240515, PI 103709, PI 169603) was extracted from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Rogers and Bendich, 1985). PCR amplifications were performed in 25-μL reaction mixtures with 50 ng of template DNA, 0.2 μM of each forward and reverse primers, 200 μM

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TABLE 1. Specific primer sequences and characteristics of 75 EST-derived genic markers developed in *Pisum sativum*.

Locus		Primer sequences (5′–3′)	Product size (bp)	T_a (°C)	*M. truncatula* gene call number	Pea EST
Mt5_001*	F:	AGGAAAATCCAGAAGTGTGCTCCCC	510–540	62	Medtr5g008110.1	gb|EX568712.1|
	R:	GCAAGAACATTGGCGCTTCCCC				
Mt5_002	F:	GGCAGAGACGGTTGGAAAGCC	310–1200	60	Medtr5g007580.1	gb|CD860473.1|
	R:	GAGGGAGCAAAAGTGGAGCTATCGG				
Mt5_003*	F:	GTGGATGCCATGTTGGGAAGGT	350	62	Medtr5g011160.1	gb|EX569130.1|
	R:	CCTAACATGTCCTCAAACACCAGCA				
Mt5_004*	F:	TGTTCACTGTCACATTAGTGGAGGC	810–1200	62	Medtr5g011250.2	gb|CD861142.1|
	R:	TTGGGGCAGTTTCAAATCAGAGTGGG				
Mt5_005*	F:	TGGACCGAATGAGCGAGCCG	300	62	Medtr5g012870.1	gb|GH720478.1|
	R:	CCAATTACTTGGCTCCATCGTCGC				
Mt5_006*	F:	CAAGTTGAAGTGTGGTTTCAGAATCGG	380	62	Medtr5g013110.1	gb|FG530896.1|
	R:	GAACCACCACCTTCTGCCACGC				
Mt5_007	F:	AATCTGAAACTGACAGTGAAGAGTCGG	505	58	Medtr5g013750.2	gb|FG530508.1|
	R:	ACCATAAAGCATCTCTGCTGCCG				
Mt5_008*	F:	AAGAAACACAGCGCACCGGC	340–1200	62	Medtr5g016230.1	gb|FG536800.1|
	R:	ATGGCAGCCAAGCCCAATGCC				
Mt5_010	F:	TGCTTTGTTCAACACTTCTGGATGGT	320	62	Medtr5g016380.1	gb|CD858783.1|
	R:	GCAGCGCGAATCATGGTAATGGAG				
Mt5_012*	F:	GGTTGATCCGGAGATTCTCGACGC	1200	62	Medtr5g016490.1	gb|FG537114.1|
	R:	AGGAGTATTGGCCAGAACACGGG				
Mt5_013	F:	AGTCGTGTGTACTCATTCATCCGC	250	62	Medtr5g018040.1	gb|FG536363.1|
	R:	TGGTTACTTCAGAACGATGGAAACCG				
Mt5_015	F:	CGGTTGGAGAAGATGGTTCTGTTTGGG	330–450	62	Medtr5g019760.1	gb|FG535260.1|
	R:	CCATCCGCATAATAGCCCCACCC				
Mt5_017	F:	CCATGGCCCATTCAATTGCTGATGC	350	62	Medtr5g021320.1	gb|CD859147.1|
	R:	ACTGATATGGTGGATGAGTTGCTTGC				
Mt5_018*	F:	TCCACATAATCGCCAGCAAATCCC	200	62	Medtr5g021730.1	gb|FG530030.1|
	R:	GCCGACGTTGTTGCCACCG				
Mt5_019	F:	TGCCCATTGGTTTTCCCTGCGG	1200	62	Medtr5g022640.1	gb|CD861082.1|
	R:	CATGATCGAAGATGATTGCGCCG				
Mt5_020*	F:	CACCCGAAGAGACTGCGAAAGCG	520	62	Medtr5g024350.1	gb|FG530254.1|
	R:	TCTGGACTGTGCTTTTTGTACTGCC				
Mt5_021	F:	GGAGATGCATTGGAGCCGGG	510	62	Medtr5g027470.1	gb|CD859365.1|
	R:	CGAGTTCCTTTCCAATGAGTTTCTCCC				
Mt5_022	F:	GGCGATGAAATAGTGGAAGAGAGTCCG	510	62	Medtr5g032270.1	gb|FG534942.1|
	R:	TGGTGTTTGGAAGTCACAGTGAACCC				
Mt5_023	F:	AGTGGATGAAGCGGGCTGCC	150	62	Medtr5g034530.1	gb|FG536062.1|
	R:	CACCCTTTTCACCAGCACGGC				
Mt5_024*	F:	AAAGCTCCTGGTTCTGTCCGC	300–420	62	Medtr5g036270.1	gb|FG530106.1|
	R:	TCACCTCACATCCTTCTCCAATGGG				
Mt5_025*	F:	ACACAGGAAGACGCAGTTCTGCC	720	62	Medtr5g036610.1	gb|FG535769.1|
	R:	CGTGATGCTTTGTAAAGAAGGGCGC				
Mt5_026	F:	ACTCTTAGTGCTGGATTGGAGGGC	390	62	Medtr5g038320.1	gb|FG530798.1|
	R:	ACGACTTTCTCAAAGCCATCCGC				
Mt5_027*	F:	GCCATTGCTAGATTTTGGGTTGCC	600–1200	58	Medtr5g038460.1	gb|FG536762.1|
	R:	TGAGCAGCAAATGCCTCAGCCC				
Mt5_028	F:	GGCTCCATCTCCCGCATCCG	980	58	Medtr5g039270.1	gb|FG535137.1|
	R:	CTCCTCAAAGGTACATCAGCTCGC				
Mt5_029	F:	TCCACAACCCCAACAACAACAACA	280	60	Medtr5g044680.1	gb|FG533184.1|
	R:	ACTTTGTGTCCATGCTTTTGCACC				
Mt5_030	F:	CATGGTGCACACCTCCACGC	490–550	60	Medtr5g045820.1	gb|FG533235.1|
	R:	TTTCCGTGCTTCAGCAGCCG				
Mt5_031	F:	TTCTTTCCGGAGGGAACACGC	520	60	Medtr5g046470.1	gb|EX569990.1|
	R:	GGCTTGAGTCAGCACACTTGCG				
Mt5_033*	F:	AGTTGGGTGAGGAATTGCAGGC	420–430	62	Medtr5g048930.1	emb|AM161971.1|
	R:	TGGAGCTTATAGTGAGAATTTGCCGC				
Mt5_034*	F:	ACATGAATCTTGACATCGTCACCAGG	480	60	Medtr5g049600.1	gb|GH720878.1|
	R:	ACTGTCATAGATACTCTTTGCAAGCGC				
Mt5_036	F:	TCATGAGTCTTTGTCAGAGGCCCG	510	60	Medtr5g065000.1	gb|FG530443.1|
	R:	TCTAGCCGCAACTTTTCTGAATTTGCC				
Mt5_037*	F:	AGTCCTGATCTTGTCTTAGGTGTGCC	550	62	Medtr5g065120.1	gb|FG533265.1|
	R:	GCAAAGCCTTCTTCCATTACTGAGGG				
Mt5_038	F:	GATGTTGCAACTGGTTATGGTGTGGG	510	61	Medtr5g066790.1	gb|FG529092.1|
	R:	ACTGAGGAACTAACCCGATTGGCC				
Mt5_039	F:	TGCAGACGATGTGTTACCACCGG	430	61	Medtr5g067140.1	gb|FG533231.1|
	R:	CCATGCCAGTTCTCAGTCGTGGA				
Mt5_041	F:	TTATGGGCTGTGGAAGACACCGG	290	62	Medtr5g068460.1	gb|FG531379.1|
	R:	GCCTTGTGATAATGCATCCTCAGCC				

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Table 1. Continued.

Locus		Primer sequences (5′–3′)	Product size (bp)	T_a (°C)	*M. truncatula* gene call number	Pea EST
Mt5_042*	F:	AACTTGCTCTGGTGCATGGGC	320	62	Medtr5g068500.1	gb|FG530312.1|
	R:	AACTTCCCTGGCTCGAGCACTCCG				
Mt5_043*	F:	TCCAAGAACACCACAACCACTGCA	400	58	Medtr5g069000.1	gb|FG534946.1|
	R:	TCCAGATCCTCCTGTTACAGCCAGA				
Mt5_044	F:	TGGCTGAGAAAACTGACCCTGGG	660	62	Medtr5g069480.1	gb|FG535471.1|
	R:	AAACCTGGCATGAAGAGAGTAACCG				
Mt5_045	F:	TGGTTCTCATGTCTGGTGGGCC	395	62	Medtr5g071720.1	gb|FG530120.1|
	R:	CCCTATTGCCGGGTTTGGACGC				
Mt5_046*	F:	TCAGTTCTTCTATGCAATAGAGCGGC	380	62	Medtr5g072140.1	gb|FG536413.1|
	R:	AGCCTCAAACAAAGCCCTTGCC				
Mt5_047	F:	GCACTTGAATCCGCGGAACGC	490	62	Medtr5g072570.1	gb|FG530391.1|
	R:	TGTGCTTTGCTCCTTGTGGCC				
Mt5_048*	F:	CATTTGCGGTCTGGCCCCG	630	62	Medtr5g072790.1	gb|FG536675.1|
	R:	TGTTTTTGTTGCAGTCCATGAATTGGC				
Mt5_049	F:	ACAAGATCAGCACCATTGAGGGCC	210	62	Medtr5g072900.1	gb|FG535776.1|
	R:	TCGCTTCAATAATCTGTGCAACCCC				
Mt5_050*	F:	CGGACAGAAGGAAGAAAGCAGAGGC	800	60	Medtr5g073680.1	gb|EX568722.1|
	R:	GAGAAGTTCAGAGCAAGACCAAGCC				
Mt5_051*	F:	ACTGAGCTGCCTCCAACTCACCC	480–500	58	Medtr5g073770.1	gb|EX568722.1|
	R:	GTGCTATCCTTGTATGACTCCTCTCCC				
Mt5_052	F:	CTGGATATAGTGCCGCATCGCC	355	58	Medtr5g075640.1	gb|CD860246.1|
	R:	GAAGTGCACTAAAACCTTCCAAAGGC				
Mt5_053*	F:	GCTTTTGATGTTGATGATGTGGACCC	550	58	Medtr5g077400.1	gb|EX569929.1|
	R:	CCTTAGCTCTTCGAGTGCGTCGG				
Mt5_054	F:	CTCAAATGACTGACATCTTCGAGGGC	540	58	Medtr5g077950.1	gb|FG531468.1|
	R:	CCACGTCGAAGGCTTTCACTTGC				
Mt5_055*	F:	CACCTTGTGCTGTAATAACCAAAAGCC	450	58	Medtr5g079090.1	gb|CD858894.1|
	R:	CTGTCAAGTTTCTAAGGGTTCTCTCCG				
Mt5_057	F:	AACCCCGAAAGGCACATCGG	290	60	Medtr5g079650.1	gb|GH719720.1|
	R:	ACATTTCGAAGTTTTTCCGCCCGG				
Mt5_058*	F:	GGATACTATTTTCGAGGGATCTGTGGC	550	58	Medtr5g080340.1	gb|CD858878.1|
	R:	CGATTTGCAACGCCTGGCCG				
Mt5_059*	F:	TGGCAGCCTCTATACTACGCGC	700	58	Medtr5g080730.1	gb|FG531745.1|
	R:	CGGTAGTCCTCGAGTTTGTGCCC				
Mt5_060	F:	CCATCTCCTCCTTCACCGCGG	490	62	Medtr5g080900.1	gb|FG533819.1|
	R:	GATAACCACGCGCTTCAGCCC				
Mt5_061	F:	AAGAAGCTGTGTTGGACTCTCAGAGGG	495	62	Medtr5g081470.1	gb|FG538061.1|
	R:	CTTACGAGTCCTTGATTTGTCACCCCC				
Mt5_064	F:	GCCACAGCAGCTCGTGATTCTGT	610–1200	58	Medtr5g082780.1	gb|GH720629.1|
	R:	TGCTGTTCTTGCATCTCTTCTTCCC				
Mt5_065*	F:	GGATCGTCAGGTTTGGGGTCCG	150–350	58	Medtr5g083280.1	gb|GH719482.1|
	R:	CCACCCAAACATCAACAGCAACGG				
Mt5_066*	F:	ACAACACCCGAACGCTGTGCC	8200	58	Medtr5g083430.1	gb|EX571173.1|
	R:	CCTCGGCTGTCCACTCCTCCC				
Mt5_067	F:	GCGCTCCCTTGACATTTCGCG	520	55	Medtr5g084140.1	gb|FG534893.1|
	R:	GAGATTTTGCACCAGTGTTATTCAGCC				
Mt5_068	F:	GTTGTCATTGTTGTTATGCCACGCC	290	55	Medtr5g084410.1	gb|FG533023.1|
	R:	CTGAACTCCATGCTGCTGTAGGG				
Mt5_069*	F:	AAGCCTCAGCTCTCAACATTTAAGGC	320	58	Medtr5g084550.1	gb|FG529821.1|
	R:	TAGCATCTTCATCAAACCCGCCG				
Mt5_070*	F:	CGCTCTCCGTTGCGATACCGG	700	58	Medtr5g084740.1	gb|GH720486.1|
	R:	AGTTCAACATTGCTGCACCGGG				
Mt5_071*	F:	CCGGCTCATTGATGATATGGTGGC	400	58	Medtr5g084890.1	gb|CD860585.1|
	R:	TGTTTGTGCTGGTTTCTCCACCC				
Mt5_072*	F:	TCTCACATCTGGCATGGCTGGC	200	58	Medtr5g085020.1	gb|CD860768.1|
	R:	GCCACCCAACACAAGTAATGGCG				
Mt5_073	F:	ACACGTGGAATGGATGTTGAAGGG	1200	55	Medtr5g085470.1	gb|FG533378.1|
	R:	AAGAACCACCCTCAGCCTTTCGC				
Mt5_074	F:	TGCACAGCAGTTGCCAGAGGA	800	55	Medtr5g085560.1	gb|FG537000.1|
	R:	CTTGTTTCCACTCATCCGAGCTTCA				
Mt5_075*	F:	CAGAGAACAAGCAAGAAAAAGGGGC	750–800	58	Medtr5g085630.1	gb|FG534721.1|
	R:	ACCGGTCATCCACCTCCCGC				
Ps4_001	F:	TTGGCCAAACTGCTTGTCAAACTTGG	363	59	Medtr8g008440	FG531483
	R:	GCCTTGGGGGCATCATTAACATCATCC				
Ps4_003	F:	TCCCGGTACATGGAGCTCTAGTTG	568	51	Medtr8g008880	FG537838
	R:	AGGCTGCAAGAGAAATTCTTGGTCC				
Ps4_004	F:	TTCGGGTTACAACCCTTTCACGG	684	55	Medtr8g011640	FG530764
	R:	GCGCGCCATGACTATAGCAGC				
Ps4_005	F:	TTCTGGATTTGACCAAGAAGCGGC	628	58	Medtr8g011640	FG530764
	R:	TGAAAATCTCCGAACCGGGAACAC				

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Table 1. Continued.

Locus		Primer sequences (5′–3′)	Product size (bp)	T_a (°C)	*M. truncatula* gene call number	Pea EST
Ps4_006	F:	TGTCCCAAACCTACTTCGCTCCG	220	61	Medtr8g015460	FG538362
	R:	TGGCCGTCAACTTTCTATTCACCGC				
Ps4_007	F:	GAACCCAATCAAGTGTTGTTGTACGC	275	59	Medtr8g021260	FG530143
	R:	TCATCACGTACAATCACTGACATACGC				
Ps4_009	F:	AGGTGGCAGGCTCGAATCGG	601	59	Medtr8g024670	FG533947
	R:	AGGTGTCGACGTACTCCCGC				
Ps4_010	F:	GCACACGATGATGTGGATGGAGAATGC	210	58	Medtr8g026430	FG529623
	R:	TGTCACAGCCGAAGTGAGCCC				
Ps4_012	F:	AGCTGGATGGGTTTGATGCCCG	425	56	Medtr8g027050	EX570946
	R:	ATCCAATCGCCCAGGCCGC				
Mt8_002*	F:	GTGCTTCTACAAGATCATATTGGCGGC	300	61	Medtr8g008860	FG537838
	R:	GCTTGCAACTGATACTCTTGGACCG				

Note: T_a = annealing temperature.
*Polymorphic EST-derived genic markers.

dNTPs, 2.5 mM $MgCl_2$, 1× PCR buffer, and 0.5 U *Taq* DNA polymerase in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems, Carlsbad, California, USA). The PCR profile included an initial denaturation at 95°C for 3 min followed by 35 cycles of 95°C for 1 min, 51–62°C for 50 s (according to the primer's annealing temperature), 72°C for 1 min, and a final extension at 72°C for 10 min. Length polymorphism was viewed with ethidium bromide in 8% polyacrylamide gels run in a Mega-Gel high-throughput electrophoresis system for 5 h at 250 V (C.B.S. Scientific, San Diego, California, USA). If length polymorphism was not detected, PCR products were digested with restriction enzymes (New England BioLabs, Ipswich, Massachusetts, USA) to generate cleaved amplified polymorphic sequence (CAPS) markers and separated on 2% agarose to detect polymorphism. Amplified fragments were run with a 25/100-bp DNA ladder (Bioneer, Alameda, California, USA) and analyzed for fragment size using AlphaView Stand Alone analysis software version 3.4 (ProteinSimple, Santa Clara, California, USA). Each EST-derived genic marker was considered polymorphic when the PCR band pattern of one of the 16 pea genotypes was different from the others with regard to size or CAPS polymorphism (Appendix S1). Different polymorphic fragments for a particular locus were considered as different alleles. Seventy-five primer pairs resulted in successful PCR amplification in which 66% (42 primer pairs) were monomorphic and 44% (33 primer pairs) were polymorphic among the 16 pea genotypes, which are parents of several pea mapping populations being used to map different disease resistance loci. The segregation analysis using these polymorphic markers has been conducted in a large number of mapping populations developed from crossing of these genotypes as parents (data not shown). All the primers generated a clear fragment pattern, with PCR products ranging in size from 150 to 1200 bp with two to three alleles per marker. Table 1 summarizes the forward and reverse primer sequence, size range of the original fragment (bp), annealing temperature, *M. truncatula* gene call, and the equivalent pea EST GenBank accession number. These EST-derived genic markers are codominant, highly reproducible, and easy to score. PCR products among the 16 pea genotypes were analyzed for allele number, observed heterozygosity (H_o), expected heterozygosity (H_e) or gene diversity, and polymorphic information content (PIC) using PowerMarker version 3.25 (Liu and Muse, 2005) (Table 2). H_o and H_e values ranged from 0.0000 to 0.0625 and from 0.0377 to 0.6391, respectively. The PIC ranged from 0.0370 to 0.5659 with an average of 0.2708. Twenty-four EST-derived genic markers were tested in two lentil (*Lens culinaris* Medik.) genotypes, and PCR amplification of 12 markers determined the transferability of these markers in related genera (Appendix S2). This lends support from other studies on transferability of cross-species markers based on conserved sequences (Phan et al., 2006). Cross-species transferability of EST-derived genic markers is due to the conserved nature of primers picked up from coding sequences. More detailed polymorphism analysis and linkage analysis using mapping populations will establish connections between the genetic and genomic information of the closely related species.

CONCLUSIONS

The current study identifies and characterizes new EST-derived genic markers based on comparative mapping between pea and *M. truncatula*. Thirty-three polymorphic and 42 monomorphic primer sequences were described in this study. These EST-derived genic markers were mined from conserved *M. truncatula* gene sequences; therefore, they can be used to anchor genomic regions between pea and *M. truncatula* and possibly among other members of the legume family. These markers show polymorphism among 16 pea genotypes that include parents of several pea mapping populations being used to map different disease resistance loci. These molecular markers will be useful to develop gene-rich linkage maps and to tag genes for agronomically important traits. In addition, amplification of these markers in lentil demonstrates the transferability of these markers across related species.

Table 2. Results of 33 polymorphic EST-derived genic loci screened in 16 genotypes of *Pisum sativum.*

Locus	*A*	H_e	H_o	PIC
Mt5_01	2	0.4800	0.0000	0.3648
Mt5_03	3	0.3507	0.0000	0.3222
Mt5_04	3	0.6391	0.0000	0.5659
Mt5_05	2	0.2041	0.0000	0.1833
Mt5_06	2	0.4032	0.0000	0.3219
Mt5_08	2	0.4234	0.0000	0.3338
Mt5_12	2	0.2604	0.0000	0.2265
Mt5_18	2	0.0377	0.0385	0.0370
Mt5_20	2	0.4527	0.0000	0.3502
Mt5_24	3	0.3225	0.0000	0.2896
Mt5_25	2	0.4872	0.0400	0.3685
Mt5_27	2	0.3200	0.0000	0.2688
Mt5_33	2	0.1528	0.0000	0.1411
Mt5_34	2	0.2188	0.0000	0.1948
Mt5_37	2	0.3750	0.0000	0.3047
Mt5_42	2	0.0605	0.0625	0.0587
Mt5_43	2	0.4992	0.0000	0.3746
Mt5_46	2	0.4800	0.0000	0.3648
Mt5_48	2	0.3935	0.0000	0.3161
Mt5_50	2	0.4970	0.0000	0.3735
Mt5_51	3	0.5408	0.0000	0.4529
Mt5_53	2	0.2604	0.0000	0.2265
Mt5_55	2	0.0740	0.0000	0.0712
Mt5_58	2	0.0740	0.0000	0.0712
Mt5_59	3	0.5910	0.0385	0.5252
Mt5_65	2	0.4800	0.0000	0.3648
Mt5_66	2	0.3107	0.0000	0.2624
Mt5_69	2	0.0740	0.0000	0.0712
Mt5_70	2	0.1420	0.0000	0.1319
Mt5_71	2	0.4970	0.0000	0.3735
Mt5_72	2	0.1420	0.0000	0.1319
Mt5_75	2	0.4734	0.0000	0.3613
Mt8_002	2	0.1420	0.0000	0.1319

Note: *A* = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; PIC = polymorphic information content.

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