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

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 ≤−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 significant similarity with *M. truncatula* gene sequences to investigate genetic diversity, linkage mapping, and cross-species transferability.

Novel markers were developed for pea (*P. sativum*) from pea expressed sequence tags (ESTs) having significant homology to *Medicago truncatula* gene sequences. 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.
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) | $T_m$ (°C) | M. truncatula gene call number | Pea EST |
|---------|----------------------------------------------------------------------------------------|-------------------|------------|-------------------------------|---------|
| Mt5_001* | F: AGGAAAATCAGGAAATGCTGCTCCCC R: GCAAGAACATCTGGCCTCTCCCC | 510–540           | 62         | Medtr5g008110.1               | gbEX568712.1   |
| Mt5_002 | F: GGCGAGACGTCGTTGAGAACCC R: GAGGGGCAAGAATGATGGCTCCTCGG | 310–1200          | 60         | Medtr5g007580.1               | gbCD860473.1   |
| Mt5_003* | F: GTGGATGCTGGATTTGAGGGGT R: CCTACAGCTTCCCTCACAAGCAGCA | 350               | 62         | Medtr5g011160.1               | gbEX569130.1   |
| Mt5_004* | F: TTGTCATCTGCAACATTTAGGAGGC R: TGGGGGAGTTTTCAATCAGAGTGGGG | 810–1200          | 62         | Medtr5g011250.2               | gbCD861142.1   |
| Mt5_005* | F: TGAGCAGCAATGCGACGGCGC R: CCCATACGCTCCTGCTGCGG | 300               | 62         | Medtr5g012870.1               | gbGH720478.1   |
| Mt5_006* | F: GAACCCACACACCTCAGCAAGC | 380               | 62         | Medtr5g013110.1               | gbFG530896.1   |
| Mt5_007 | F: AATGCGAGCTACGACGAGAGTGGCGG R: ACCATAAGAATCTCTGCTCAGG | 505               | 58         | Medtr5g013750.2               | gbFG530508.1   |
| Mt5_008* | F: AGGAAACACAGGCAACCCGCAGG R: ATGGCAAGAATCAGCCACGG | 340–1200          | 62         | Medtr5g016230.1               | gbFG536800.1   |
| Mt5_010 | F: TGCTTGTGCTGGCTAGGAGGGT R: GCAGCAGCAATCAGTTGAGGAG | 320               | 62         | Medtr5g016380.1               | gbCD858783.1   |
| Mt5_012* | F: GGTGATACGAGATCTCTGCCG R: GGAGGGAATGCTGCTGCGG | 1200              | 62         | Medtr5g016490.1               | gbFG537114.1   |
| Mt5_013 | F: AGGTGCTGTGCTGATCTCTGCCG R: TGTTGCTGACAGGCTGAGGCCACG | 250               | 62         | Medtr5g018040.1               | gbFG536363.1   |
| Mt5_015 | F: TTGAGTACGAGGCAACGACTGGGGG | 330–450          | 62         | Medtr5g019760.1               | gbFG535260.1   |
| Mt5_017 | F: CCAAAGGATAGAGCTGACTTTGCAGC | 350               | 62         | Medtr5g021320.1               | gbCD859147.1   |
| Mt5_018* | F: TCCATACGACTGCAAGCAAAAACCCG R: GCCGCGGCTGTTGCGCAAGCG | 200               | 62         | Medtr5g021730.1               | gbFG530030.1   |
| Mt5_019 | F: CAGGTAGAGGTAGGTGTCTGCGG R: CTATAGATGATCTGACCTGCGG | 1200              | 62         | Medtr5g022640.1               | gbCD861082.1   |
| Mt5_020* | F: AATGGGAGGAATGCTGACTTTGCGG | 520               | 62         | Medtr5g024350.1               | gbFG530254.1   |
| Mt5_021 | F: GAGATGCTGTGACGACCCGGG C: CGAGTCTCTCTCTACAGTCTCTCTCTCCG | 510               | 62         | Medtr5g027470.1               | gbCD859365.1   |
| Mt5_022 | F: GGGTGGATAGGACCCGGAGAGTGGCGG R: TGGTTGATGAGGAGATGGTGAGGGG | 510               | 62         | Medtr5g032270.1               | gbFG534942.1   |
| Mt5_023 | F: AGGTGTTGAGGAGGGCTGCCC R: AATTGGATGGGAGGGTTCGCC | 150               | 62         | Medtr5g034530.1               | gbFG536062.1   |
| Mt5_024* | F: AAAACCTCATGCTCTGCTCCC R: TACCCATCAGCTCCTCCTCCTACATGGG | 300–420          | 62         | Medtr5g036270.1               | gbFG530106.1   |
| Mt5_025* | F: ACAGCGAGCAACGAGCTGGCTCAGG R: CGCTAGATGAGAGGAGGAGAGGGCGG | 720               | 62         | Medtr5g036610.1               | gbFG535769.1   |
| Mt5_026 | F: AACTGCTCTCAGGCTGACGACG R: ACCGCGACCAGTGGGACGACG | 390               | 62         | Medtr5g038320.1               | gbFG530798.1   |
| Mt5_027* | F: GCCATGCTGATTTTGGTCTTCCG | 600–1200         | 58         | Medtr5g038460.1               | gbFG536762.1   |
| Mt5_028 | F: GGTCTCCTCTCCCGCTCCAGG | 980               | 58         | Medtr5g039270.1               | gbFG535137.1   |
| Mt5_029 | F: TCCACGGGAGGCACCGAGCAGG | 280               | 60         | Medtr5g04680.1                | gbFG533184.1   |
| Mt5_030 | F: CATGGCTGACACCTCTCCAGG R: TTTTCTGTTCCTGACGGCGG | 490–550          | 60         | Medtr5g045820.1               | gbFG533235.1   |
| Mt5_031 | F: GCTTGGACACAGCTCAATCGG R: CCAAAGCAGACACACACACCA | 520               | 60         | Medtr5g046470.1               | gbEX569990.1   |
| Mt5_033* | F: AATGGGAGGAAATGCTGACGAG R: TTGGAGCTATGAGGAGAATTTGCGG | 420–430          | 62         | Medtr5g048930.1               | emblAM161971.1   |
| Mt5_034* | F: ACATGGATTCTGCACTGACGACGG | 480               | 60         | Medtr5g049600.1               | gbGH720878.1   |
| Mt5_036 | F: CATCGTCAAGCTCTCTGACAGCGG | 510               | 60         | Medtr5g05000.1                | gbFG530443.1   |
| Mt5_037* | F: TTCGAGCCAACAGTTTGGTCTAGTGGC | 550               | 62         | Medtr5g05120.1                | gbFG533265.1   |
| Mt5_038 | F: GATGTGCTGACAGCTTATGAGGGG | 510               | 61         | Medtr5g06790.1                | gbFG529092.1   |
| Mt5_039 | F: TGGAGAGAGGGAATGCTGACGAGG R: CCTCTGCTCAGTCAGGGCGG | 430               | 61         | Medtr5g067140.1               | gbFG533231.1   |
| Mt5_041 | F: TTATGGGTGTGTTGGAACACCGG R: CACCTGGGAAATGCTCTCCAGGC | 290               | 60         | Medtr5g068460.1               | gbFG531379.1   |

http://www.bioone.org/lo/i/apps
| Locus        | Primer sequences (5'-3') | Product size (bp) | T<sub>r</sub> (°C) | M. truncatula gene call number | Pea EST          |
|--------------|--------------------------|-------------------|-------------------|-------------------------------|------------------|
| Mt5_042*     | F: AACCTGCTCTGTTGGCAGATGGGC<br> R: AACCTGCTCTGCTGGAGGACCTCCCG  | 320                | 62                | Medtr5g068500.1              | gbiFG530312.1    |
| Mt5_043*     | F: TCCAGAAGACACCAACACACTTGCA<br> R: TCCAGAAGACACCAACACACTTGCA   | 400                | 58                | Medtr5g069000.1              | gbiFG534946.1    |
| Mt5_044      | F: TGCTGGAGAAAATCGAGCTCCGGG<br> R: AAACCTGGGATGAGAGGTAACCCG   | 660                | 62                | Medtr5g069480.1              | gbiFG535471.1    |
| Mt5_045      | F: TGTTTTTGCTAGGTACCCTAGTTGAGGGCC<br> R: CCATTGTCGGGTTTGTGGAGGCC | 395                | 62                | Medtr5g071720.1              | gbiFG530120.1    |
| Mt5_046*     | F: TCAGTTTTTCAGGAAATAGAGGCC<br> R: AGCTCTCAACAAAGCCTTGGCC     | 380                | 62                | Medtr5g072140.1              | gbiFG536413.1    |
| Mt5_047      | F: GCACCTGAAGCAGTGCGAGGCC<br> R: TGTTTTTGCTAGGTACCCTAGTTGAGGGCC | 490                | 62                | Medtr5g072570.1              | gbiFG530301.1    |
| Mt5_052*     | F: CTGACATGCCTGACTGACCTGCC<br> R: GAAATGTCTGATGGAGTGGCC         | 450                | 58                | Medtr5g070790.1              | gbiFG535146.1    |
| Mt5_053*     | F: GCCATCAAACAGCTATTGACCTGCC<br> R: CGGTAGTCGTCTGATGGAGTGGCC       | 550                | 58                | Medtr5g070790.1              | gbiFG535776.1    |
| Mt5_054      | F: TGCATACCATGATGGAGGACCTCCGG<br> R: CTCATACTGCCATGATGGAGGACCTCCGG | 540                | 58                | Medtr5g070790.1              | gbiCD858894.1    |
| Mt5_055*     | F: GCCATCAAACAGCTATTGACCTGCC<br> R: CGGTAGTCGTCTGATGGAGTGGCC       | 450                | 58                | Medtr5g070790.1              | gbiCD858894.1    |
| Mt5_057      | F: ACCACCAAGGACCTACCG<br> R: ACATGCTTTTCCTGAGCTCAGCCCGG | 290                | 60                | Medtr5g079650.1              | gbiGH719720.1    |
| Mt5_058*     | F: GCATACCATTTCCGAGGAGATCCTGGCC<br> R: CGATTGCAACACCTCCGGCC         | 550                | 58                | Medtr5g080340.1              | gbiCD858878.1    |
| Mt5_059*     | F: TGCAACCTGCTATATAGCTTGGCC<br> R: CGGTAGTCGTCTGATGGAGTGGCC       | 700                | 58                | Medtr5g080730.1              | gbiFG531745.1    |
| Mt5_060      | F: CCATCTCTCCCTCCACCGGG<br> R: GTAACCACGCAGCTTGGCC         | 490                | 62                | Medtr5g080900.1              | gbiFG533819.1    |
| Mt5_061      | F: AAGAGCTGCTGTGGATCTGACAGGGG<br> R: TTCAAGATCTCCTGATGGAGCC         | 495                | 62                | Medtr5g081470.1              | gbiFG538061.1    |
| Mt5_064      | F: GCCGACAGCGTCTGTGGACTTGTG<br> R: CGGTAGTCGTCTGATGGAGTGGCC       | 610–1200           | 58                | Medtr5g082870.1              | gbiGH720629.1    |
| Mt5_065*     | F: GGATCTGCTGAGTTTTGGGAGCTCC<br> R: CTCATTGCCGTTTCTCTCTCC   | 150–350            | 58                | Medtr5g083280.1              | gbiGH719482.1    |
| Mt5_066*     | F: AACAAACCAAGACGGCTTGGCC<br> R: TTGGTCTACGCTGACGTCTTGGCC       | 8200               | 58                | Medtr5g083430.1              | gbiEX571173.1    |
| Mt5_067      | F: GGCTGCCGCGTGCCTATTGGG<br> R: GGATTTGCAAGCAGCTTGGGAGCC    | 520                | 55                | Medtr5g084140.1              | gbiFG534893.1    |
| Mt5_068      | F: GTGTCATGTTGTGTTATGACCGCC<br> R: CTGACATCCCTGCTGCTAGGGG    | 290                | 55                | Medtr5g084410.1              | gbiFG535302.1    |
| Mt5_069*     | F: AACGGGACAGCTGCTGCTGCTG<br> R: TAGGACTTTCTACAAAAAGCCCGG   | 320                | 58                | Medtr5g084550.1              | gbiFG529821.1    |
| Mt5_070*     | F: CTGCTGCTGCTGCTGCTGCTG<br> R: GGATTTGCAAGCAGCTTGGGAGCC    | 700                | 55                | Medtr5g084740.1              | gbiGH720486.1    |
| Mt5_071*     | F: CCCCTGCTGCTGCTGCTGCTG<br> R: TTGGTCTACGCTGACGTCTTGGCC       | 400                | 58                | Medtr5g084890.1              | gbiCD860585.1    |
| Mt5_072*     | F: TCTGACATCCGCTGAGCTTGGGC<br> R: GCCACCAAACAAACAGATTTGAGGGCG | 200                | 58                | Medtr5g085020.1              | gbiCD860768.1    |
| Mt5_073      | F: AGACCTGCAAATGATTTGGAAGGG<br> R: AGTGACAAATGATTTGGAAGGG    | 1200               | 55                | Medtr5g085470.1              | gbiFG533738.1    |
| Mt5_074      | F: TGGCAGACGACGACGACGACG<br> R: CGCTGAGAGCAGAGAGAGAGAGAGACG | 800                | 55                | Medtr5g085560.1              | gbiFG537000.1    |
| Mt5_075      | F: CAGAGCATGACGACGAGACGAGAGAGG<br> R: ACCGGCAATCACCCTACCCCGG | 750–800            | 58                | Medtr5g085630.1              | gbiFG534721.1    |
| Ps4_001      | F: TTCCTGACATCCGCTGACG<br> R: ACCGGCAATCACCCTACCCCGG | 363                | 59                | Medtr8g008440                | FG531483        |
| Ps4_003      | F: TTGGTCTACGCTGACGTCTG<br> R: GGATTTGCAAGCAGCTTGGGAGCC    | 568                | 51                | Medtr8g008880                | FG537838        |
| Ps4_004      | F: TTCCTGACATCCGCTGACG<br> R: ACCGGCAATCACCCTACCCCGG | 684                | 58                | Medtr8g011640                | FG530764        |
| Ps4_005      | F: TTCTGACATCCGCTGACG<br> R: ACCGGCAATCACCCTACCCCGG | 628                | 58                | Medtr8g011640                | FG530764        |
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.

### 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 1. Continued.

| Locus  | Primer sequences (5'-3') | Product size (bp) | *T*<sub>c</sub> (°C) | *M. truncatula* gene call number | Pea EST |
|--------|-------------------------|-------------------|---------------------|----------------------------------|---------|
| Ps4_006 | F: TGCCCAACTCCTGCTGCCG  
R: TGCCGCTAACCTGCTTACG | 220 | 61 | Medtr5g015460 | FG538362 |
| Ps4_007 | F: GACAACTCAACCATGATGCGCC  
R: TCAGTGATGACCTGAGACAGC | 275 | 59 | Medtr8g021260 | FG530143 |
| Ps4_009 | F: AGGGTCGGCAGCTGAACGGG  
R: AGGGTCGACGTACTCCCGC | 601 | 59 | Medtr8g024670 | FG533947 |
| Ps4_010 | F: GCACACGAAGATGTTGAGGAGATGCG  
R: GTGACAACGGAGAGAGGACG | 210 | 58 | Medtr8g026430 | FG529623 |
| Ps4_012 | F: AGGGTCGGCAGCTGAACGGG  
R: ATCCAATGCCGACCCGC | 425 | 56 | Medtr8g027050 | EX570946 |
| Mt8_002* | F: GGTGTCTTCAAGATCATTGCGCCG  
R: GCTTGCAACTGATATCTGAGCC | 300 | 61 | Medtr8g008860 | FG537838 |

*Polyorphic EST-derived genic markers.

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

| Locus  | *A* | *H*<sub>e</sub> | *H*<sub>o</sub> | 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        | 0.3538 |
| Mt5_12 | 2   | 0.2604        | 0.0000        | 0.2265 |
| Mt5_15 | 2   | 0.0377        | 0.0385        | 0.0376 |
| 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   | 0.3955        | 0.0000        | 0.3411 |
| 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*<sub>e</sub> = expected heterozygosity; *H*<sub>o</sub> = observed heterozygosity; PIC = polymorphic information content.
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