PREMISE OF THE STUDY: Simple sequence repeat markers were developed based on expressed sequence tags (EST-SSR) and screened for polymorphism among 23 *Pisum sativum* individuals to assist development and refinement of pea linkage maps. In particular, the SSR markers were developed to assist in mapping of white mold disease resistance quantitative trait loci.

**METHODS AND RESULTS:** Primer pairs were designed for 46 SSRs identified in EST contiguous sequences assembled from a 454-pyrosequenced transcriptome of the pea cultivar, ‘LIFTER’. Thirty-seven SSR markers amplified PCR products, of which 11 (30%) SSR markers produced polymorphism in 23 individuals, including parents of recombinant inbred lines, with two to four alleles. The observed and expected heterozygosities ranged from 0 to 0.43 and from 0.31 to 0.83, respectively.

**CONCLUSIONS:** These EST-SSR markers for pea will be useful for refinement of pea linkage maps, and will likely be useful for comparative mapping of pea and as tools for marker-based pea breeding.

**Key words:** EST-SSR; Fabaceae; microsatellite; *Pisum sativum*; *Sclerotinia sclerotiorum*; transcriptome.

Pea (*Pisum sativum* L.) is one of the most important legumes grown and consumed worldwide. White mold caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary is a significant yield-limiting disease of pea in most areas that pea is cultivated. Despite the agricultural importance of pea, pea breeding is constrained by a large genome size (~4300 Mb), lack of genomic resources, and rich repetitive DNA (estimated at 75–97% of the pea genome) (Macas et al., 2007). Molecular markers have great potential to speed up the process of developing improved cultivars. Although several hundred simple sequence repeat (SSR) markers have been identified (Burstin et al., 2001; London et al., 2005; Gong et al., 2010), additional SSR markers with polymorphism are needed, particularly for the development of linkage maps for use in white mold–resistance mapping studies.

With the development of next-generation sequencing technologies, large amounts of expressed sequence tags (ESTs) have been generated for model species as well as economically important nonmodel plants. These ESTs offer an opportunity to discover novel genes and have also provided a resource to develop markers (Davey et al., 2011). Recently, we sequenced the transcriptome of pea infected by *S. sclerotiorum* using next-generation sequencing to understand this host–pathogen interaction. The transcriptome sequences from pea contain abundant SSRs, which we have used in this study to develop SSR markers. The SSR markers were screened against 23 pea cultivars and plant introductions (PIs), including parents of four recombinant inbred line (RIL) populations (Lifter and PI240515; Medora and PI169603; Bohatyr and Shawnee; Melrose and Radley) for white mold–resistance mapping studies. These new markers will be very useful for linkage mapping studies.

**METHODS AND RESULTS**

LIFTER, a cultivar susceptible to *S. sclerotiorum* (McPhee and Muehlbauer, 2002), was inoculated with *S. sclerotiorum* isolate WMA-1 (=ATCC MYA-4521) on the stem between the fourth and fifth detectable nodes. Seventy-two hours after inoculation, total RNA was extracted from 18 infected plants by cutting a 1 cm piece of pea stem containing the advancing lesion front toward the base of the plant using the TRizol Plus RNA Purification Kit (Invitrogen, Carlsbad, California, USA). Messenger RNA was purified from the total RNA with the Oligotex mRNA Mini Kit using the mRNA Spin-Column Protocol (QIAGEN, Valencia, California, USA) and converted into a normalized cDNA pool with the services of Evrogen (http://www.evrogen.com). Transcriptome sequencing of pea infected by *S. sclerotiorum* was conducted on a full plate of the Roche 454 GS FLX sequencer (454 Life Sciences, Branford, Connecticut, USA) at Washington State University. In total, 128,720 high-quality reads with an average length of 215 nucleotides were obtained and assembled into 10,158 contiguous sequences (contigs) with the program ABySS (Simpson et al., 2009). Pea and *S. sclerotiorum* contigs were parsed with a tBLASTx method (Zhang et al., 2012) against publicly available, closely related plant and fungal genome databases. The fungal genome database consisted of *S. sclerotiorum* (strain1980) and six closely related fungal (Ascomycete) species (*Botrytis cinerea* Pers., *Chaetomium globosum* Kunze, *Fusarium graminearum* Schwabe, *Magnaporthe grisea* (T. T. Hebert) M. E. Barr, *Neospora crassa* Shear & B. O. Dodge, and *Verticillium dahliae* Kleb.), and the plant genome database consisted of three sequenced legume (Fabaceae) genomes (*Glycine max* (L.) Merr., *Lotus japonicus* (Regel) K. Larsen, and *Medicago truncatula* Gaertn.). After parsing, 10,158 contigs were separated into 6299 pea ESTs, 2780 *S. sclerotiorum* ESTs, and 1079 unassigned ESTs. Among the pea ESTs, 118 potential SSR markers were screened against 23 pea cultivars and plant introductions (PIs), including parents of four recombinant inbred line (RIL) populations (Lifter and PI240515; Medora and PI169603; Bohatyr and Shawnee; Melrose and Radley) for white mold–resistance mapping studies. These new markers will be very useful for linkage mapping studies.
TABLE 1. Characteristics of 11 polymorphic and 26 monomorphic *Pisum sativum* EST-SSR markers.

| Locus   | Primer sequences (5′–3′) | Repeat motif | Size (bp) | \(T_s\) (°C) | GenBank accession no. | Putative function [organism] | E-value |
|---------|--------------------------|--------------|-----------|-------------|-----------------------|-----------------------------|---------|
| Psat61* | F: CCGGTTCGTTCCGCTGAGGG | (GGGTTT)_4   | 81        | 60          | JR344273              | unknown                     | 2.03E-04 |
|         | R: ACGGACGCACGCAACACCA   | (TTT)_5       | 135       | 58          | JR344282              | chalcone reductase [Medicago sativa] | 7.44E-12 |
| Psat900*| F: GCCGTACCCATCTACCAAGGCC | (GCTTT)_6    | 248       | 58          |JR344282              | unknown                     | 3.98E-05 |
|         | R: ACGCCACGGCCCTTCCAGAAGC | (CT)_3       | 103       | 56          | JR344267              | unknown                     | 5.32E-26 |
| Psat921*| F: TCACTTCTCACAAGCCGCTG  | (ATATCG)_4   | 81        | 58          |JR344277              | predicted protein [Medicago truncatula] | 5.59E-15 |
|         | R: TGCGGCTGAGGGCGTGAGG   | (GAAATC)_5   | 171       | 58          |JR344287              | predicted protein [Glycine max] | 1.28E-27 |
| Psat5404*| F: ACTTCAGATGACATCTTCCTCAC | (GTGTT)_5     | 123       | 56          | JR344268              | predicted protein [Medicago truncatula] | 3.11E-39 |
|         | R: TGAATCCCATGCATTCACAAATGCGGAC | (ATG)_6       | 124       | 58          | JR344254              | unknown                     | 1.54E-96 |
| Psat5545*| F: TGGGTGCTCTGAGGAATTGAGCTGG | (GGGTTC)_4     | 123       | 58          | JR344269              | unknown                     | 1.57E-25 |
|         | R: AGAGGCCTGCTGAGGAAAGAGT | (AG)_5       | 135       | 58          | JR344269              | unknown                     | 5.18E-21 |
| Psat5712*| F: TGATGATGCTGCTGATATTGCTCGT | (TTTT)_4     | 170       | 58          | JR344274              | unknown                     | 1.82E-05 |
|         | R: AGATGAGCCGAGAAGGTCAGACG | (GAA)_5     | 209       | 54          | JR344275              | basic helix-loop-helix protein [Ricinus communis] | 3.43E-04 |
| Psat7598*| F: ACTAACAGAGTTGAAATTCGCGGAG | (GGTCT)_5     | 152       | 58          | JR344257              | predicted protein [Medicago truncatula] | 6.40E-11 |
|         | R: CAACTGATACAAAGAAGAAGACGACG | (GA)_5       | 122       | 58          | JR344276              | mannose-P-dolichol utilization defect 1 protein [Arabidopsis thaliana] | 3.31E-39 |
| Psat7818*| F: TGAGATTGCTTTGTTGTTGTTGGT | (GTATT)_5     | 224       | 58          | JR344258              | predicted protein [Arabidopsis thaliana] | 1.77E-70 |
|         | R: AAAAAGAAGATTTTGGGAGGCACG | (GAA)_5     | 171       | 58          | JR344260              | predicted protein [Glycine max] | 2.99E-08 |
| Psat9662*| F: AGGAGCCGCTGCTGAGGAAAGAGT | (AG)_5     | 135       | 58          | JR344269              | unknown                     | 2.03E-04 |
|         | R: GCCGGCTGAGGGCGTGAGG   | (GAAATC)_5   | 171       | 58          | JR344269              | unknown                     | 8.16E-22 |
| Psat10014*| F: ATATAAGCGATGGATGATGACG | (GT)_6       | 124       | 58          | JR344254              | unknown                     | 5.76E-28 |
|         | R: GTCGACCGCTACGCAAGGACG | (GTG)_5     | 123       | 56          | JR344265              | proteinase inhibitor \(I_1\) [Medicago truncatula] | 1.57E-25 |
| Psat368 | F: AATGCCGAACGAGGACGAGAAGATG | (TTT)_4     | 124       | 58          | JR344265              | unknown                     | 2.03E-04 |
|         | R: GTCGACCGCTACGCAAGGACG | (GTG)_5     | 123       | 56          | JR344265              | predicted protein [Medicago truncatula] | 7.44E-12 |
| Psat373 | F: CGCTCTGATGCTCTCAAGCCA | (TGG)_5     | 123       | 56          | JR344278              | predicted protein [Medicago truncatula] | 1.54E-96 |
|         | R: TACGCTGATATCTCAACGAGCC | (CTG)_3     | 123       | 56          | JR344278              | predicted protein [Glycine max] | 1.54E-96 |
products with expected sizes were successfully amplifi ed for 37 primer sets, for Biotechnology Information (taxid: 3888) with a cutoff parameter of 1e −20. desribed in this study and those previously published, all 37 ESTs were exe-
morphic in parents of at least one RIL population for white mold–resistance.

We identifi ed 37 SSRs, with 11 being polymorphic in 23 P. sativum individuals. These novel EST-SSR markers will be valuable tools for marker-assisted breeding, development of pea linkage maps, and comparative mapping of pea.

**CONCLUSIONS**

In this study we demonstrate that next-generation sequencing is an eff ective tool to rapidly develop EST-derived SSR markers. We identifi ed 37 P. sativum EST-SSRs, with 11 being polymorphic in 23 P. sativum individuals. These novel EST-SSR markers will be valuable tools for marker-assisted breeding, development of pea linkage maps, and comparative mapping of pea.

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APPENDIX 1. Information on 23 *Pisum sativum* germplasm lines used in this study; germplasm lines were obtained from the collection of Dr. Kevin McPhee’s pea breeding program. Information presented: country of origin, name, registration number.

| Country | Name         | Registration Number | Note |
|---------|--------------|---------------------|------|
| USA     | LIFTER, PI 628276* | N/A                |      |
|         | MEDORA       | N/A                |      |
|         | MELROSE, PI 618628* | N/A                |      |
|         | NDP080111, N/A | PI160936, N/A      |      |
|         | PI240515, N/A | PS03101269, N/A    |      |
|         | PS05ND0327, N/A | N/A                |      |
|         | PS05ND0330, N/A | N/A                |      |
|         | PS05ND0434, N/A | N/A                |      |
|         | PS07ND0190, N/A | N/A                |      |
|         | SHAWNEE, PI 619079* | N/A                |      |
|         | SPECTER, PI 641005 | STIRLING, PI 634571, WINDHAM, PI 647868 |      |
| Canada  | AGASSIZ, 6093 | CDC GOLDEN, 5602.  |      |
|         | CDC STRIKER, 5550 | DS ADMIRAL, 5166. MAJORET, N/A |      |
| Europe  | BOHATYR, N/A | COOPER, N/A         |      |

*Note: N/A = not available.

*Pea cultivars, Plant Introductions (PIs), or breeding material not located in the Germplasm Resources Information Network (GRIN) are available from Dr. Kevin McPhee upon request. Voucher specimens have not been deposited due to their availability either within GRIN or the pea breeding community; additionally, some germplasm lines are the property of Dr. McPhee and North Dakota State University.

*Pea cultivars available from GRIN (http://www.ars-grin.gov/).