Development and characterization of EST-SSR markers for *Vitex negundo* var. *heterophylla* (Lamiaceae)

Lele Liu, Jingwen Wang, Meiqi Yin, Xiao Guo, Yunfei Cai, Ning Du, Xiaona Yu, and Weihua Guo

PREMISE OF THE STUDY: *Vitex negundo* var. *heterophylla* (Lamiaceae) is a dominant shrub in the warm temperate zone of northern China. Expressed sequence tag–simple sequence repeat (EST-SSR) markers were developed to investigate its genetic diversity and structure.

METHODS AND RESULTS: We detected 12,075 SSRs in *V. negundo* var. *heterophylla* using transcriptome sequencing. Primer pairs for 100 SSR loci were designed and amplified in three populations of *V. negundo* var. *heterophylla*. Sixty loci were amplified, of which 14 were polymorphic. The number of alleles per locus ranged from two to 15, and levels of observed and expected heterozygosity ranged from 0.241 to 0.828 and from 0.426 to 0.873, respectively. All primer pairs amplified PCR products from *V. rotundifolia* but only four of them amplified products from *Leonurus japonicus*.

CONCLUSIONS: The identified EST-SSR markers will be useful for future molecular and reproductive ecology studies of *V. negundo* var. *heterophylla* and *V. rotundifolia*.

**KEY WORDS** expressed sequence tag–simple sequence repeat (EST-SSR) markers; Lamiaceae; transcriptome sequencing; *Vitex negundo* var. *heterophylla*; *V. rotundifolia*.

**METHODS AND RESULTS**

Two *V. negundo* var. *heterophylla* individuals were sampled for transcriptome sequencing from Fohui Mountain in Jinan, Shandong Province, China (Appendix 1). RNA was extracted from collected leaves using the RNAPrep Pure Plant kit (Tiangen, Beijing, China), and mRNA was isolated from total RNA using the NEBNext Ultra RNA Library Prep Kit (New England Biolabs, Ipswich, Massachusetts, USA). After ultrasonic fragmentation, mRNA was converted to double-stranded cDNA using the same kit. Purification and size selection were conducted using AMPure XP beads (Beckman Coulter, Brea, California, USA). Finally, DNA fragments of approximately 400 bp in length were sequenced using an Illumina HiSeq instrument (Illumina, San Diego, California, USA). The raw data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (accession no. PRJNA491662).

The raw sequences were filtered by removing adapters and low-quality reads (quality score < 30), resulting in 45,568 and 43,082 million clean reads, from the two libraries, respectively. These reads were de novo assembled using Trinity (Grabherr et al., 2011) into 45,568 and 43,082 million clean reads, from the two libraries, respectively. These reads were de novo assembled using Trinity (Grabherr et al., 2011) into 52,072 unigenes, with an N50 length of 1414 bp. The putative functions of EST-SSR sequences were determined by BLASTX against the NCBI non-redundant protein (nr) database. We detected 12,075 SSR loci from these unigenes using MISA (Thiel et al., 2003), consisting of 4242 mononucleotide SSRs and 7833 di-, tri-, tetra-, penta-, and hexanucleotide SSRs. Primers were designed using Primer3 (Untergasser et al., 2012).

Fresh leaves were collected from three populations of *V. negundo* var. *heterophylla* in Shandong Province, China (Appendix 1), and genomic DNA was extracted from dried leaf tissue using the
Table 1. Characteristics of 14 polymorphic EST-SSR markers developed for Vitex negundo var. heterophylla.

| Locus | Primer sequences (5′-3′) | Repeat motif | T<sub>a</sub> (°C) | Expected allele size (bp) | Allele size range (bp) | Putative function [Organism] | E-value | GenBank accession no. |
|-------|-------------------------|-------------|----------------|--------------------------|-----------------------|----------------------------|--------|---------------------|
| V02   | F: AGCAGGGAGGAGGGAGAAC  | (TGG)<sub>11</sub> | 58             | 172                      | 156–187               | No hit                     | —      | MH825839            |
|       | R: ACCACACCACTCCATAGGA  |             |                |                          |                       |                           |        |                     |
| V07   | F: CCTCTGTCGCCATGCTCTAT | (AG)<sub>8</sub> | 56             | 125                      | 107–133               | Serine hydroxymethyltransferase, mitochondrial [Erythranthe guttata] | 8.90E-114 | MH825840          |
|       | R: AAAGGTCCTGCAAATGGAG  |             |                |                          |                       |                           |        |                     |
| V15   | F: CAACAGAGGGGCTTCAAGT  | (AT)<sub>6</sub> | 56             | 220                      | 206–237               | No hit                     | —      | MH825841            |
|       | R: GGGAGGTGCTGAAATGGAG  |             |                |                          |                       |                           |        |                     |
| V25   | F: ACAAGGACCATCTAGCTGT  | (GT)<sub>10</sub> | 58             | 236                      | 207–253               | No hit                     | —      | MH825842            |
|       | R: GATGAGGCGGCATTCAGCA  |             |                |                          |                       |                           |        |                     |
| V30   | F: GCTGGGGAGGAGCTAGTGT  | (TGC)<sub>6</sub> | 56             | 191                      | 189–205               | ABC transporter G family member 11 [Sesamum indicum] | 6.20E-172 | MH825843      |
|       | R: CCAGACAGTTCGTTGCTAC  |             |                |                          |                       |                           |        |                     |
| V49   | F: CGTCCTGGCTCTTGGTCTAC | (AG)<sub>14</sub> | 56             | 215                      | 200–242               | No hit                     | —      | MH825844            |
|       | R: CCTCAGAGTTGGAGCTCT   |             |                |                          |                       |                           |        |                     |
| V55   | F: GCAAGCTTCCCTTCCCTCTCT | (CTC)<sub>10</sub> | 56             | 198                      | 192–206               | Probable protein 3-acyltransferase 12, isoform X3 [Sesamum indicum] | 6.00E-99 | MH825845          |
|       | R: ACGAGGAATGGTAGGTGCG  |             |                |                          |                       |                           |        |                     |
| V59   | F: AGCTGATTGCACATCTTGCA| (GAT)<sub>7</sub> | 56             | 238                      | 224–237               | Intracellular protein transport protein USO1-like [Sesamum indicum] | 8.80E-78 | MH825846          |
|       | R: CGATGACTCTACGCTTGGCTT|             |                |                          |                       |                           |        |                     |
| V70   | F: GTTGGCCGCTAGCTAGTT  | (GCT)<sub>6</sub> | 56             | 144                      | 133–153               | Hypothetical protein MIMG18_v1000263039 [Erythranthe guttata] | 1.00E-146 | MH825847       |
|       | R: GCACAGGCGCTTCCATTAGC|             |                |                          |                       |                           |        |                     |
| V76   | F: TGACCTGCTGATCACTAC  | (AG)<sub>11</sub> | 56             | 121                      | 94–128                | Uncharacterized protein At4g26450 [Sesamum indicum] | 5.50E-52 | MH825848          |
|       | R: GGTCACAGCTTTGGATTGCC |             |                |                          |                       |                           |        |                     |
| V95   | F: CGATGATACGACGGCAACT  | (GCC)<sub>2</sub> | 56             | 253                      | 242–266               | Zinc finger protein 8 [Erythranthe guttata] | 6.10E-13 | MH825849          |
|       | R: GGTGCTGCTGACGCATTGT |             |                |                          |                       |                           |        |                     |
| V97   | F: GTCAAGCTACGCGGCAATA  | (CA)<sub>12</sub> | 56             | 229                      | 211–232               | Uncharacterized protein LOC105175071 [Sesamum indicum] | 2.90E-40 | MH825850          |
|       | R: GCAGGCTGCTACGCGGCAATA |             |                |                          |                       |                           |        |                     |
| V99   | F: AGCAGGACTGCGCAATGAA  | (GTG)<sub>7</sub> | 56             | 161                      | 155–173               | Transcription factor bHLH63 [Sesamum indicum] | 5.50E-52 | MH825851          |
|       | R: GATACGAGCGAGCCAGAGAT |             |                |                          |                       |                           |        |                     |
| V100  | F: CTGTGACCACTCTTCATCTT | (CA)<sub>8</sub> | 56             | 220                      | 211–235               | α-3-phosphoglycerate dehydrogenase 2, chloroplastic-like [Sesamum indicum] | 1.20E-293 | MH825852 |
|       | R: TGGAAATCCTTCTACACGAC |             |                |                          |                       |                           |        |                     |

Note: T<sub>a</sub> = annealing temperature.

Table 2. Genetic variation in the 14 polymorphic EST-SSR markers in three Vitex negundo var. heterophylla populations.

| Locus | Population A (n = 5) | Population B (n = 5) | Population C (n = 5) |
|-------|----------------------|----------------------|----------------------|
|       | H<sub>o</sub> | H<sub>e</sub> | H<sub>o</sub> | H<sub>e</sub> | H<sub>o</sub> | H<sub>e</sub> |
| A     | 7 | 0.655 | 0.746 | 7 | 0.786 | 0.815 | 9 | 0.692 | 0.844 |
| V07   | 7 | 0.759 | 0.804 | 7 | 0.714 | 0.751 | 8 | 0.692 | 0.834 |
| V15   | 7 | 0.536 | 0.573 | 7 | 0.500 | 0.670 | 6 | 0.615 | 0.698 |
| V25   | 15 | 0.828 | 0.873 | 12 | 0.714 | 0.865 | 13 | 0.692 | 0.719 |
| V30   | 4 | 0.655 | 0.624 | 5 | 0.500 | 0.466 | 5 | 0.577 | 0.679 |
| V49   | 10 | 0.586 | 0.757 | 10 | 0.714 | 0.836 | 11 | 0.538 | 0.771 |
| V55   | 7 | 0.759 | 0.687 | 9 | 0.714 | 0.733 | 7 | 0.538 | 0.562 |
| V59   | 3 | 0.621 | 0.540 | 3 | 0.500 | 0.517 | 3 | 0.538 | 0.514 |
| V70   | 6 | 0.828 | 0.719 | 8 | 0.714 | 0.746 | 6 | 0.731 | 0.724 |
| V76   | 12 | 0.690 | 0.699 | 11 | 0.786 | 0.811 | 8 | 0.615 | 0.754 |
| V95   | 6 | 0.379 | 0.479 | 7 | 0.643 | 0.629 | 10 | 0.500 | 0.509 |
| V97   | 8 | 0.345 | 0.717* | 9 | 0.393 | 0.790* | 8 | 0.385 | 0.798 |
| V99   | 4 | 0.241 | 0.456 | 5 | 0.536 | 0.573 | 2 | 0.385 | 0.426 |
| V100  | 8 | 0.724 | 0.744* | 6 | 0.357 | 0.651* | 8 | 0.500 | 0.743 |

Note: A = number of alleles; H<sub>e</sub> = expected heterozygosity; H<sub>o</sub> = observed heterozygosity; n = sample size.

*Locality and voucher information are provided in Appendix 1.

**Significant deviation from Hardy–Weinberg equilibrium (P < 0.001).**
observed heterozygosity ranged from 0.241 to 0.828, and the levels of expected heterozygosity ranged from 0.426 to 0.873 (Table 2). Significant linkage disequilibrium was detected between loci V15 and V30 (P = 0.0109) and loci V25 and V70 (P = 0.0266). Loci V97 and V100 showed significant deviation from Hardy–Weinberg equilibrium in two populations (P < 0.001; Table 2).

To test the transferability of the 14 primers between taxa, they were used with DNA samples from *V. rotundifolia* and *Leonurus japonicus*. All primer pairs successfully amplified products from *V. rotundifolia*, but only four primer pairs amplified products from some *L. japonicus* individuals (Table 3).

**CONCLUSIONS**

We assembled 52,072 unigenes of *V. negundo var. heterophylla* following transcriptome sequencing and used this data set to develop 14 novel polymorphic EST-SSR primer pairs. All of these primers amplified products in the related species *V. rotundifolia*. These markers represent a useful resource for reproductive and genetic ecology studies of this species and may provide a valuable tool for revegetation and management in northern China.

**ACKNOWLEDGMENTS**

The authors thank Dr. Shuping Zhang for field assistance and Prof. Fengning Xiang for technical guidance. This work was supported by the National Natural Science Foundation of China (no. 31470402, 31770361), the Basic Work of the Ministry of Science and Technology of China (no. 2015FY1103003-02), and the Fundamental Research Funds of Shandong University (no. 2017GN0018). The authors also thank PlantScribe (www.plantscribe.com) for editing this manuscript.

**AUTHOR CONTRIBUTIONS**

W.G., L.L., and Y.C. conceived and designed the experiments. L.L., J.W., M.Y., X.G., X.Y., and N.D. contributed to sample collection. L.L. and J.W. performed the molecular laboratory work. L.L., J.W., M.Y., and Y.C. participated in data pre-processing. L.L., Y.C., N.D., X.Y., and W.G. analyzed the data. L.L. drafted the manuscript and all authors participated in manuscript modifications and gave final approval for publication.

**DATA ACCESSIBILITY**

All sequence information was uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (accession no. PRJNA491662); primer sequences were uploaded to GenBank (accession no. MH825839–MH825852 and MH892533–MH892570; Table 1 and Appendix 2).

**LITERATURE CITED**

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Du, N., W. Guo, X. Zhang, and R. Wang. 2010. Morphological and physiological responses of *Vitex negundo* L. var. *heterophylla* (Franch.) Rehd. to drought stress. *Acta Physiologiae Plantarum* 32: 839–848.

Du, N., R. Wang, J. Liu, X. Zhang, X. Tan, W. Wang, H. Chen, and W. Guo. 2012. Morphological response of *Vitex negundo* var. *heterophylla* and *Ziziphus jujuba* var. *spinosa* to the combined impact of drought and shade. *Agroforestry Systems* 87: 403–416.

Du, N., X. Tan, Q. Li, X. Liu, W. Zhang, R. Wang, J. Liu, and W. Guo. 2017. Dominance of an alien shrub *Rhus typhina* over a native shrub *Vitex negundo* var. *heterophylla* under variable water supply patterns. *PLoS ONE* 12: e0176491.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29: 644.

Liu, L., N. Du, C. Lei, X. Guo, and W. Guo. 2018. Genetic and epigenetic variations associated with adaptation to heterogeneous habitat conditions in a deciduous shrub. *Ecology and Evolution* 8: 2594–2606.

Ohtsuki, T., T. Shoda, Y. Kaneko, and H. Setoguchi. 2014. Development of microsatellite markers for *Vitex rotundifolia* (Verbenaceae), an endangered coastal plant in Lake Biwa, Japan. *Applications in Plant Sciences* 2: 1300100.

Peakall, R., and P. E. Smouse. 2012. *GenALEx* 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28: 2537–2539.

Rousset, F. 2008. GENEPOP’007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Su, H., L.-J. Qu, K. He, Z. Zhang, J. Wang, Z. Chen, and H. Gu. 2003. The Great Wall of China: A physical barrier to gene flow? *Heredity* 90: 212–219.

Thiel, T., W. Michalek, R. K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—New capabilities and interfaces. *Nucleic Acids Research* 40: e115.

Zhang, Z.-Y., X.-M. Zheng, and S. Ge. 2007. Population genetic structure of *Vitex negundo* (Verbenaceae) in Three-Gorge Area of the Yangtze River: The riverine barrier to seed dispersal in plants. *Biochemical Systematics and Ecology* 35: 506–516.
APPENDIX 1. Voucher information for Vitex negundo var. heterophylla, V. rotundifolia, and Leonurus japonicus individuals used in this study.

| Species                      | Population | N  | Collection localitya | Geographic coordinates | Voucher specimenb |
|------------------------------|------------|----|----------------------|------------------------|-------------------|
| Vitex negundo L. var. heterophylla (Franch.) Rehder | A          | 29 | Fanggan              | 36.4317°N, 117.4516°E | 01611001          |
| Vitex negundo var. heterophylla                        | B          | 28 | Mengshang            | 35.5376°N, 117.9895°E | 01611002          |
| Vitex negundo var. heterophylla                        | C          | 26 | Yaosiang             | 36.3213°N, 117.1200°E | 01611003          |
| Vitex negundo var. heterophylla                        | —          | 2  | Jinan                | 36.6317°N, 117.0347°E | 01709001c          |
| Vitex rotundifolia L. f.                               | —          | 13 | Muping               | 37.4574°N, 121.6826°E | 01801001          |
| Leonurus japonicus Houtt.                              | —          | 8  | Jinan                | 36.7239°N, 117.0207°E | 01801002          |

aCollection localities are in Shandong Province, China.
bAll voucher specimens were collected by Lelel Liu and are deposited in the Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University (JSPC), Qingdao, China. The sample with irregularly pinnatifid leaflets (c) and the sample with slightly incised leaflets (d) were used for transcriptome sequencing.

APPENDIX 2. Characteristics of the 38 monomorphic EST-SSR markers developed for Vitex negundo var. heterophylla.

| Locus | Primer sequences (5′–3′) | Repeat motif | T_a (°C) | Allele size (bp) | Putative function [Organism] | E-value | GenBank accession no. |
|-------|--------------------------|--------------|----------|-----------------|-------------------------------|---------|----------------------|
| V3    | FGATGCTGCCCTCCACACTGTC   | (TC)_15      | 56       | 150             | Unnamed protein product [Coffea canephora] | 2.80E-17 | MH892533             |
| V4    | TCTCTCTCTCTCTCTCTGCGCC   | (CAA)_6      | 56       | 207             | Uncharacterized protein LOC105175754 [Sesamum indicum] | 1.50E-60 | MH892534             |
| V8    | ACCGGCACTCTGGAAGATGTTG   | (CT)_15      | 56       | 171             | Hypothetical protein M569_03371, partial [Genlisea aurea] | 2.50E-56 | MH892535             |
| V56   | ACAGTCACAGTGTGGCACAT     | (AAG)_4      | 56       | 248             | G-type lectin S-receptor-like serine/threonine-protein kinase At4g27290 [Sesamum indicum] | 1.20E-15 | MH892536             |
| V13   | TAAGACTCCCACTGCAAGGCA    | (CCA)_6      | 56       | 212             | Uncharacterized protein LOC105974765 [Erythranthe guttatus] | 3.20E-50 | MH892537             |
| V18   | GGAACACGTAATGCGGGTTCC   | (TC)_15      | 56       | 193             | No hit                         | —       | MH892538             |
| V21   | CCGGAAAGCAAGCTAAGCCCA   | (CT)_15      | 56       | 209             | Unnamed protein product [Vitis vinifera] | 5.10E-06 | MH892539             |
| V26   | CAGCGACCCCCAAAAATTTCGAA | (GGC)_6      | 56       | 241             | 26S proteasome subunit RPT2B [Arabidopsis thaliana] | 5.10E-06 | MH892540             |
| V36   | TACGCTATGGTTGGCCCA      | (AG)_10      | 56       | 182             | Pentatricopeptide repeat-containing protein At5g67570, chloroplastic [Sesamum indicum] | 7.00E-118 | MH892544            |
| V39   | CCGGGGACCGAACAAAGATGAT  | (GA)_10      | 56       | 245             | Myosin-14 [Sesamum indicum] | 2.90E-127 | MH892544             |
| V43   | AGCAGGGGGAGGTTAATGGCA   | (AAC)_6      | 56       | 221             | Transcription factor GTE7-like [Sesamum indicum] | 1.50E-111 | MH892545            |
| V47   | TGGAAAGCCTGTGGTGTTGGA   | (GA)_17      | 56       | 152             | Haloacid dehalogenase-like hydrolase domain-containing protein SGPP [Erythranthe guttatus] | 9.90E-75 | MH892546             |
| V48   | CCAACAAAGCGGATTGACTCA   | (CAG)_6      | 56       | 103             | Unnamed protein product [Coffea canephora] | 9.90E-198 | MH892547             |
| V50   | CCAATTACACGCAACAGGAC    | (TC)_15      | 56       | 258             | Phospholipase SGR2-like isofrom X1 [Sesamum indicum] | 3.60E-187 | MH892548             |
| V51   | CCGGGGAGGTTAATGGCA      | (GGC)_6      | 56       | 165             | Uncharacterized protein LOC105172005 isofrom X1 [Sesamum indicum] | 2.10E-83 | MH892549             |
| V52   | CCAAGGGGAGGTTAATGGCA    | (AG)_18      | 56       | 260             | Uncharacterized protein LOC105171026 [Sesamum indicum] | 1.30E-36 | MH892550             |
| V53   | AACACCGGGAGGTTAATGGAG   | (AG)_14      | 56       | 115             | Hypothetical protein POPTR_0007s12520g [Populus trichocarpa] | 7.10E-33 | MH892551             |
| V54   | CGCTCTCCACTGCAACTGCC    | (TG)_15      | 56       | 149             | No hit                         | —       | MH892552             |
| V56   | ACCATTGCTCCCTGCAAGC    | (GGA)_6      | 56       | 135             | No hit                         | —       | MH892553             |
| V58   | AACGCTCTCACCACGGTGA    | (GTG)_15     | 56       | 269             | Protein E6-like [Sesamum indicum] | 2.00E-24 | MH892554             |
| V61   | GCCCTCAGAAGCCCAAGCAG    | (GA)_11      | 56       | 159             | Galacturonokinase [Sesamum indicum] | 5.10E-165 | MH892555            |

(Continues)
### APPENDIX 2. (Continued)

| Locus | Primer sequences (5′–3′) | Repeat motif | $T_a$ (°C) | Allele size (bp) | Putative function [Organism] | $E$-value | GenBank accession no. |
|-------|--------------------------|--------------|----------|-----------------|----------------------------|-----------|----------------------|
| V63   | F: CCATGACGTCGGAGGAGATG   | (AG)$_n$     | 56       | 275             | Uncharacterized protein LOC105970868 [Erythranthe guttatus] | 8.20E-51 | MH892556             |
|       | R: TCTGTCACAAACACCGCCATT |             |          |                 |                            |           |                      |
| V64   | F: ACCGACCTGGATTTCGACAC   | (AG)$_n$     | 56       | 168             | No hit —                    | —         | MH892557             |
|       | R: GCAGCAACAACAAGACCAT    |             |          |                 |                            |           |                      |
| V66   | F: TCTTGATCAGCTGCCCACCAT | (TCA)$_n$    | 56       | 234             | Uncharacterized protein LOC105157368 [Sesamum indicum] | 1.10E-31 | MH892558             |
|       | R: GASCTGTGTTGATGTGGCGAGA |             |          |                 |                            |           |                      |
| V71   | F: CACTCGCAACACTTGAAGCT   | (TCT)$_n$    | 56       | 162             | Protein IQ-DOMAIN 32-like [Sesamum indicum] | 5.90E-60 | MH892559             |
|       | R: GTGAAGCGGAGAGACCAACA   |             |          |                 |                            |           |                      |
| V72   | F: TCAAGGGCGTCCTGTAGAGTC  | (TCT)$_n$    | 56       | 123             | Uncharacterized protein LOC105170218 [Sesamum indicum] | 1.30E-74 | MH892560             |
|       | R: CATCAGGCGGGAGAAACAGTG  |             |          |                 |                            |           |                      |
| V82   | F: GCAAGAGCTCTGAGCTTTC    | (ATG)$_n$    | 56       | 248             | RNA exonuclease 3 [Gossypium arboreum] | 1.90E-22 | MH892561             |
|       | R: AGCTCATGAGCTTCCGACAAAT |             |          |                 |                            |           |                      |
| V83   | F: TCCACCAACACTCAAAGAGC   | (CT)$_n$     | 56       | 139             | Protein GAR2 isoform X1 [Sesamum indicum] | 7.90E-98 | MH892562             |
|       | R: CCTGCAAACCTCTCATTCCGT  |             |          |                 |                            |           |                      |
| V84   | F: CAGTGAAGAGCGAGAGAGAGA  | (GGC)$_n$    | 56       | 183             | Uncharacterized protein LOC105176253 isoform X1 [Sesamum indicum] | 2.00E-253| MH892563            |
|       | R: CCTCCTCTCGCTTCCATCAC   |             |          |                 |                            |           |                      |
| V88   | F: TTGTTCTCTGCAAGCATAGCA  | (AG)$_n$     | 56       | 189             | No hit —                    | —         | MH892564             |
|       | R: TGCCAACGCGGTCTTAAATCA  |             |          |                 |                            |           |                      |
| V89   | F: TCGCGTGTGCCAGCCTTCTCTC| (AG)$_n$     | 56       | 149             | Reactive oxygen species modulator 1-like [Sesamum indicum] | 1.90E-20 | MH892565             |
|       | R: ATAAACAGCACAACACCGC    |             |          |                 |                            |           |                      |
| V90   | F: ACCGATGCCTGTGAGGATT    | (GT)$_n$     | 56       | 151             | Probable leucine-rich repeat receptor-like protein kinase At1g35710 [Sesamum indicum] | 1.60E-185| MH892566            |
|       | R: CGTCTCCACACTCGATGCTTT  |             |          |                 |                            |           |                      |
| V92   | F: GGAAATGCTGTTCCCTGCCG  | (AGC)$_n$    | 56       | 186             | E3 ubiquitin-protein ligase RGLG2-like, partial [Pyrus x bretscheni] | 1.60E-87 | MH892567             |
|       | R: GCAAATCTGACGCCTTCAGGC  |             |          |                 |                            |           |                      |
| V93   | F: CAGATATCGCGGTGGAACCG   | (CGC)$_n$    | 56       | 251             | Uncharacterized protein LOC105173283 [Sesamum indicum] | 6.30E-51 | MH892568             |
|       | R: ACTTCTACTCTGCCGATCTC   |             |          |                 |                            |           |                      |
| V94   | F: CGGAGAAAGCCATCATCATG   | (GAA)$_n$    | 56       | 226             | Hypothetical protein MIMGU1 [Erythranthe guttata] | 1.90E-18 | MH892569             |
|       | R: TGGATTCAAGGAGACAGCCA   |             |          |                 |                            |           |                      |
| V96   | F: AAGGCAGAAGCAAGAGATG    | (GGC)$_n$    | 56       | 177             | Uncharacterized protein LOC105968457 [Erythranthe guttata] | 3.80E-40 | MH892570             |
|       | R: GAGTGGCCTGCTTCCAAATCG  |             |          |                 |                            |           |                      |

Note: $T_a$ = annealing temperature.