



Short Communication

Genome-wide identification and characterization of simple sequence repeat loci in grape phylloxera, *Daktulosphaira vitifoliae*

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ABSTRACT. A genome-wide sequence search was conducted to identify simple sequence repeat (SSR) loci in phylloxera, *Daktulosphaira vitifoliae*, a major grape pest throughout the world. Collectively, 1524 SSR loci containing mono-, di-, tri-, tetra-, penta-, and hexanucleotide motifs were identified. Among them, trinucleotide repeats were the most abundant in the phylloxera genome (34.4%), followed by hexanucleotide (20.4%) and dinucleotide (19.6%) repeats. Mono-, tetra- and pentanucleotide repeats were found at a frequency of 1.3, 11.2 and 12.9%, respectively. The abundance and inherent variations in SSRs provide valuable information for developing molecular markers. The high levels of allelic variation and codominant features of SSRs make this marker system a useful tool for genotyping, diversity assessment and population genetic studies of reproductive characteristics of phylloxera in agricultural and natural populations.

Key words: Grape phylloxera; Simple sequence repeat marker; Genetic diversity; Population genetics

INTRODUCTION

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Homoptera: Phylloxeridae), is a viticultural pest specialized in feeding on grapevine (*Vitis* spp). Phylloxera has been destroying vineyards around the world for the past 140 years (Granett et al., 1996). It is found throughout the Americas where it appears to have coevolved with the endemic *Vitis* spp (Wapshere and Helm, 1987). This pest was accidentally introduced to European viticultural regions from North America in the mid 1800s. By 1900, two-thirds of all *Vitis vinifera* vineyards in Europe had been destroyed. Since then, phylloxera has spread to most grape-growing areas of the world, including New Zealand, Australia, South Africa, South America, and Canada (Skinkis et al., 1995). It is regarded as one of the most important viticultural pests in the world (Granett et al., 1996).

Although resistant rootstocks have effectively defended vineyards against this pest, the durability of resistant grape plants depends on the variability and adaptability of pest populations rather than the resistance gene itself. In California, for example, the emergence of more aggressive strains of phylloxera, called biotype B, overcame the limited resistance of the AXR#1 rootstock (Granett et al., 2001). Damage intensity caused by plant pests most likely correlates with diversity and population size. The insect varies genetically relative to its performance on hosts. The use of insecticides has limited effects on controlling the population, and other control methods are unproven. Therefore, information regarding the genetic diversity, population structure, and reproductive characteristics of grape phylloxera within and among various grape rootstocks is important for understanding host selection and adaptation and designing appropriate pest management strategies.

Simple sequence repeat (SSR markers), also known as microsatellite makers or variable number tandem repeats, are considered a powerful tool for distinguishing genetic diversity, studying populations, and determining reproductive characteristics in various organisms. The high level of polymorphism, easy adaption of high throughput capability, extensive genome coverage, and small amount of DNA required for analysis make this marker system favorable over other genetic markers. SSR makers have been isolated from grape phylloxera in previous studies. Corrie et al. (2002) initially isolated four SSR makers. Lin et al. (2006) also isolated 19 SSRs, but only seven were polymorphic across sample populations in California and Europe. In this study, we identified and developed a large set of new SSR markers for grape phylloxera.

MATERIAL AND METHODS

Phylloxera samples were collected from own-rooted Chardonnay (defined as biotype A) at the University of California (Davis, CA, USA) vineyard and AxR#1 rootstock (defined as biotype B) in Mendocino County, California. Genomic DNA was isolated following a procedure described elsewhere (Lin and Walker, 1996). DNA quality was evaluated using 1.2% agarose gel. DNA samples were then quantified using a fluorometer and the PicoGreen method. The phylloxera genome DNA samples were then used for 454 pyrosequencing with Titanium kit by Roche GS-FLX Sequencer (Roche, Branford, CT, USA) according to manufacturer instructions. Sequencing data were *de novo* assembled with Newbler version 2.0 (Roche).

To identify putative microsatellite regions in the phylloxera genome, we used the Tandem Repeats Finder software (Benson, 1999). After the identification of various motif

repeat regions, sequences that flanked the prospective repeat motifs of 200 bp upstream and downstream were extracted from the output file. Standalone BLASTn analyses were performed to compare sequences derived from biotype A and biotype B phylloxera. SSR-containing sequences were separated into three groups: 1) SSR sequences present in biotypes A, 2) SSR sequences present in biotypes B, and 3) SSR sequences present in both biotypes. After removing redundant sequences from each group, we identified more than a thousand SSR loci with various repeat motifs. Prospective SSR primers were designed from the identified loci using the Molecular Beacon Designer software (version 7.0) with the following criteria: 35-55% GC content, 50°-62°C melting temperature, and 132-290-bp amplification products containing repeat units ≥ 6 .

RESULTS AND DISCUSSION

In total 1524 SSR loci with various repeat motifs were identified. Among them, 21 (1.3%), 299 (19.6%), 524 (34.4%), 172 (11.2%), 197 (12.9%), and 311 (20.4%) were observed as mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats, respectively (Figure 1).

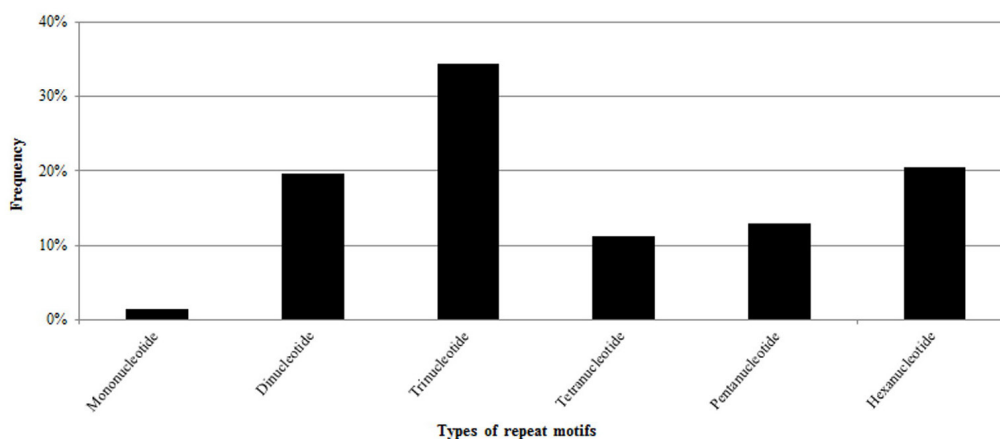


Figure 1. Types of simple sequence repeat frequencies throughout the genome of grape phylloxera (*Daktulosphaira vitifoliae*).

From these various SSR repeat regions, a total of 112 SSR primer pairs were tentatively designed (Table 1). Among them, 49 and 48 primer pairs were designed from the sequences obtained from biotype A and biotype B, respectively. In addition, 15 SSR primer pairs that detect sequence polymorphism between biotype A and biotype B were also designed (see Table 1).

Combining a next-generation deep-sequencing strategy with an *in silico* mining approach provided an effective way to identify SSR loci in the phylloxera genome. The new set of markers enhances the ability to characterize population structure, reproduction mode, and adaptation of grape phylloxera to various rootstocks in grape-growing regions around the world.

Table 1. Descriptions of 112 simple sequence repeat (SSR) makers developed from the genome-wide sequence search of grape phylloxera (*Daktulosphaira vitifoliae*).

Locus	Primer sequences (5'-3')	Repeats	Fragment size	Ta (°C)	GenBank accession No.
49 SSR markers developed from biotype A					
DVSSR_A001-F	CAGTGATATTTGTCGGAGAGG	(CA) ₁₂ TA(CA) ₆	166	53	GF111391
DVSSR_A001-R	CCGGTCATTGGTTGTTATGG				
DVSSR_A002-F	CGGCGAACACGTATAATG	(GT) ₁₅	250	50	GF111392
DVSSR_A002-R	AACGGCATAAGCAATAAGC				
DVSSR_A003-F	GCGCTGGCCGAGAGCAAGAG	(AT) ₁₁ TTATGT(AT) ₄	221	63	GF111393
DVSSR_A003-R	CGGCGCGAACGCTCTATCTTG				
DVSSR_A004-F	CGGTGCCAGATTGCTTATGAAC	(TA) ₁₀ TGTAC(TA) ₃	234	57	GF111394
DVSSR_A004-R	AGTCAATCGAGATAAGCTGAAAGAG				
DVSSR_A005-F	CACTTAGCTTCCTTTCTATACTTGG	ATAC(AT) ₁₀ GT(AT) ₃	139	55	GF111395
DVSSR_A005-R	CAGCATGTCACCTAGGGATTGG				
DVSSR_A006-F	ATCCTCACTTCTCTTCTG	(AT) ₁₃ GT(AC) ₄ AT (AC) ₅ C(AT) ₁₃	236	55	GF111396
DVSSR_A006-R	CACGGCGTAGTGGATATGC				
DVSSR_A007-F	ATAGGGATAAGGAAACGATGGG	(AT) ₁₂ T(AT) ₅	214	55	GF111397
DVSSR_A007-R	GAGGCGATAGCAGAGTATGG				
DVSSR_A008-F	TGCGCTGGACTTAGTGTTAC	ATCT(AT) ₃ ACT(AT) ₁₂	132	54	GF111398
DVSSR_A008-R	TATCCACTGTTTACGGTTGAAC				
DVSSR_A009-F	CGGCAAGCAGCATCAAGC	(AC) ₁₀ A(AC) ₂ AT(AC) ₄	190	57	GF111399
DVSSR_A009-R	TTCCAGGTGTGTATGTGTTG				
DVSSR_A010-F	TACTCTAAAAGAAGCATAACATAGG	TAAA(TA) ₁₁ ...(TA) ₃	184	54	GF111400
DVSSR_A010-R	ATAATTTGCATGGTGGAAAGTTAG				
DVSSR_A011-F	TTATTGCCGTCGGAGGATCG	(TG) ₁₃ TAC(TG) ₂	206	57	GF111401
DVSSR_A011-R	TGGATTGTGGCGGTGATGG				
DVSSR_A012-F	AAGGCATTAACCTGTCGCATTC	(CA) ₃ ...(CA) ₁₅ TA(CA) ₂ TA(CA) ₇	223	56	GF111402
DVSSR_A012-R	GTAGCATGTGGACTTGACTGG				
DVSSR_A013-F	GCTTTCACCAACTACCGTACC	(AC) ₁₂	146	56	GF111403
DVSSR_A013-R	TCCCTCATACTACACTC				
DVSSR_A014-F	TGGTCCCTGGTGGCTTTGG	(AT) ₁₁ GT(AT) ₂	132	56	GF111404
DVSSR_A014-R	TCCACTGCCTCGATCTTGC				
DVSSR_A015-F	ACACGCTATAIATGATGGTTGG	(AT) ₃ ...(AT) ₁₁	221	54	GF111405
DVSSR_A015-R	CACGTTTAGTACAACAGACCTC				
DVSSR_A016-F	TTGTCAGTTAGGCTGAGATAC	(TA) ₅ ...(TA) ₉	183	52	GF111406
DVSSR_A016-R	CAACCATCTTAATCTTCTACC				
DVSSR_A017-F	ACAGTTAGCAGATGATTGGAAC	(TAT) ₆ AT(TAT) ₃	159	54	GF111407
DVSSR_A017-R	CACAAGCATCTTCAGATAGGC				
DVSSR_A018-F	TATGATCGTCACAGAGGAAACC	(ATT) ₁₁	230	55	GF111408
DVSSR_A018-R	ATCTTCGCCAATGTCAAGTG				
DVSSR_A019-F	GGCAGTGACCCATGACAG	(TAT) ₈	177	54	GF111409
DVSSR_A019-R	GGAIACGGTACACAGAAAACG				
DVSSR_A020-F	CTCTAGGACACTCATGATTGC	(ATA) ₂ ...(ATA) ₈	234	54	GF111410
DVSSR_A020-R	TTTCTACTGAGCTGTA AAAAGC				
DVSSR_A021-F	AAAGTGAGCCCAAAGTATAAGC	(TTA) ₂ TAA(TTA) ₆	170	54	GF111411
DVSSR_A021-R	TTTATTACTACGGTCCGCAAAC				
DVSSR_A022-F	TTTTAAAATAAAATCATCATCATCC	(ATC) ₃ CTC(ATC) ₂	150	53	GF111412
DVSSR_A022-R	TATTTGTTACTTACATACAGATATGATG				
DVSSR_A023-F	AGTCCACTTTCGCTGTTGTG	(TAT) ₉	141	56	GF111413
DVSSR_A023-R	CATCACGGTTCATAAATCAC				
DVSSR_A024-F	TTCGACTTGTCCGGCCTAATC	(AAC) ₉	200	53	GF111489
DVSSR_A024-R	TTTTACAGACAGTTTAGTGACG				
DVSSR_A025-F	GGTTCGCGTTCAGAATCG	(ATT) ₈	159	53	GF111414
DVSSR_A025-R	AACATTCGACTCTAGCAATACC				
DVSSR_A026-F	ACTGAATGTGTGCGTTTGTG	(TAA) ₇ TT(AT) ₈	243	54	GF111415
DVSSR_A026-R	AAGACCCTTGGCGAATACAG				
DVSSR_A027-F	TATCATAGCTTTCCACTTGAAC	(TTA) ₂ AA(TTA) ₈	164	52	GF111416
DVSSR_A027-R	TCCGAATTAACAGCGTAGG				
DVSSR_A028-F	TAATTTTGTAAAAGCCGTTTGG	(ATA) ₂ (TAA) ₇ (TA) ₂	201	52	GF111417
DVSSR_A028-R	ATTCCGAATAGGGAGTTTGG				

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Table 1. Continued.

Locus	Primer sequences (5'-3')	Repeats	Fragment size	Ta (°C)	GenBank accession No.
DVSSR_A029-F	GACAGGTAATGAGGTGTGAGG	(AAT) ₂ ...(ATA) ₉	144	55	GF111418
DVSSR_A029-R	TTATGCTATGCGACGACGAC				
DVSSR_A030-F	TAGTTGTTCGGCGCAAGC	(ACT) ₂ ...(ATT) ₁₀	159	54	GF111419
DVSSR_A030-R	CAGCATAACCATGTAATTTGTGG				
DVSSR_A031-F	TGTTGTGTTGTTGTTGTTGTTAG	(GTT) ₃ ...(GTT) ₈	151	54	GF111420
DVSSR_A031-R	CCGTTACCTATGTGCTATTGC				
DVSSR_A032-F	GGACAGGAGAGGAATACTTCG	(GTT) ₂ GTG(GTT) ₃ ...(GTT) ₂	227	54	GF111421
DVSSR_A032-R	GAGCAGCGGTACAAGGAG				
DVSSR_A033-F	TGGAGTCTTGAACAACGTATGG	(GTT) ₄ GCT(GTT) ₃	209	56	GF111422
DVSSR_A033-R	ACAGCAACCATACGCAAGC				
DVSSR_A034-F	CCCTGTATTGTGCCCTCTG	(GTT) ₈	158	55	GF111423
DVSSR_A034-R	TACCGTATGCGAGAGTAATGG				
DVSSR_A035-F	AAAAGGGCACAAATGGTTCC	(AGC) ₅ (AGG) ₂ (AGC) ₂	200	55	GF111424
DVSSR_A035-R	TGATATGCAACATTTCTCAGCTTG				
DVSSR_A037-F	TTTACGAGAAGAGTCTGTACCC	(GTA) ₃ (CAG) ₂ (TAG) ₅ .. (TAG) ₁₅	237	54	GF111425
DVSSR_A037-R	ACGACCACATCTACATTA AAC				
DVSSR_A038-F	CTAAAGGTACACACAGATTCC	(AAT) ₁₁	219	54	GF111426
DVSSR_A038-R	GGCGGAATAAATGAGAAAAGTG				
DVSSR_A039-F	ACTGTTGACTCCGACAGAGC	(ATA) ₈	216	56	GF111427
DVSSR_A039-R	CCACACGTATAGGTACACAAGC				
DVSSR_A040-F	ACTGCGATAATGCCACTGC	(AAT) ₉ ...(GT) ₂ (AAT) ₃	236	55	GF111428
DVSSR_A040-R	CGAGATAGCCTAGCGTATGTG				
DVSSR_A041-F	TTTTGGTCTCAGCATCTTTTCC	(GGT) ₅ (GGC) ₃ GGA(GGT) ₄	153	54	GF111429
DVSSR_A041-R	TTGTTACAGGCCATATTTACCC				
DVSSR_A042-F	CAGATGGCTGGAGGAATGG	(ATT) ₇ ...(ATT) ₂	180	54	GF111430
DVSSR_A042-R	TTCTATGGTGTAGGATGACGAG				
DVSSR_A043-F	ATTCAATGTAATTTATTTCTTGGTTC	(ATT) ₈ ...(CT) ₂ .. (CA) ₂ ...(ATT) ₂	224	54	GF111431
DVSSR_A043-R	TCAACAAAACAATTATCTATCAAAGTTC				
DVSSR_A044-F	CGGCTCGCTAACATATTGC	(TATT) ₆	144	54	GF111490
DVSSR_A044-R	AAACTTACCTTGTGCAGCAC				
DVSSR_A045-F	CGTGGCGTTTTGAGAGTTAC	(TACA) ₁₁	239	55	GF111432
DVSSR_A045-R	ACGATAGTTACCATTGACAAGC				
DVSSR_A046-F	CACGACCGACCCGAGACG	(CGTA) ₈	213	58	GF111433
DVSSR_A046-R	TCGGAAAACGGCAGAGTCC				
DVSSR_A047-F	CCGCCC GCCTATAAATGTC	(TATC) ₁₂ (TA) ₈	235	55	GF111434
DVSSR_A047-R	GCGTTGCCAGTAGAAGG				
DVSSR_A048-F	TGACGGCTGCTAACTTACC	(CTGC) ₆ (CAGC) ₃	158	57	GF111435
DVSSR_A048-R	CCACGGTTGTGAGGAGTCC				
DVSSR_A049-F	TAGTGTGCTGTCTTGTGTTG	(TACA) ₄ TGCA(TACA) ₄	217	54	GF111436
DVSSR_A049-R	CGCAAATGGCTACCGTATC				
DVSSR_A050-F	CCATTGAATGCGGTACTTCC	(GTAT) ₆	161	55	GF111437
DVSSR_A050-R	TGCGTTATGACAGTCTAGTCTC				
48 SSR markers developed from biotype B					
DVSSR_B001-F	GAGCTACAAAGATCTAGACAGG	(TA) ₃ T(TA) ₁₀ T(TA) ₁	162	53	GF111438
DVSSR_B001-R	CCGTGGAACTGTCAAACC				
DVSSR_B002-F	GCGGACAAACCAATAATAACC	GTAT(GT) ₃ T(GT) ₈	191	54	GF111439
DVSSR_B002-R	CGTCGTCTCGGATGAATCG				
DVSSR_B003-F	CCGCTGCTGGCAATACAC	(TA) ₁₂ (AT) ₂ TT(TA) ₄	191	55	GF111440
DVSSR_B003-R	CATGCGTTGAGGAGGTAAGG				
DVSSR_B004-F	CACTATAATATGACAAAACCTGGTAATC	(TA) ₁₀ TT(TA) ₂ (AT) ₂	176	53	GF111441
DVSSR_B004-R	GACCGACTTATGACAATGAACTG				
DVSSR_B005-F	GACAATGCACAAGAAGTAAACG	(TA) ₁₁ T(TA) ₂ AG(AT) ₂	145	54	GF111442
DVSSR_B005-R	ATTACCACCAGAAGCCAGTC				
DVSSR_B006-F	GCTATGCGTATTCCGTAAGTCG	(GT) ₁₀ GC(GT) ₃	141	57	GF111443
DVSSR_B006-R	GCTACCACCACAGACCTGAG				
DVSSR_B007-F	ATAACGCCACTGAAACATTGATG	(TA) ₂ TT(TA) ₁₀ TTATT(TA) ₃	214	56	GF111444

Continued on next page

Table 1. Continued.

Locus	Primer sequences (5'-3')	Repeats	Fragment size	Ta (°C)	GenBank accession No.
DVSSR_B007-R	GCAACAGATATGAATACAGAGTAGC	ATA(AT) ₉ ...(AT) ₃ ...(AT) ₃	239	55	GF111445
DVSSR_B008-F	GCGTTACGAAGATGTGTGTC				
DVSSR_B008-R	GTTCTCCGGCCTTCCAC	TGTA(TG) ₂ TA(TG) ₁₁	235	56	GF111446
DVSSR_B009-F	CGTGTGCCGTTCAAGGTC				
DVSSR_B009-R	CCCCGCGTTCATCAGAC	(TA) ₁₄	223	56	GF111447
DVSSR_B010-F	AGACTGTCGTAACGCATTAC				
DVSSR_B010-R	GGCTGATAAAGGTGGCACTAG	(AC) ₁₄	166	54	GF111448
DVSSR_B011-F	TACAGGATACAATATTCACACTCAG				
DVSSR_B011-R	GTACAAACATATGATCTCGATTTCG	(GT) ₆ ..TT(GT) ₂ TT (GT) ₃ TT(GT) ₆	228	56	GF111449
DVSSR_B012-F	TCAGCACGAGTCTATTGAAACG				
DVSSR_B012-R	AGCGACGGTGATAATAAAGTGG	(AT) ₁₄ ...(AT) ₂	232	54	GF111450
DVSSR_B013-F	ATATTAAGTTCCTATGTTTCCTTACC				
DVSSR_B013-R	ACATCTACAATTATAGAACACACAAC	(AT) ₉	183	54	GF111451
DVSSR_B014-F	CACCTGTGTCTGGAATATACC				
DVSSR_B014-R	CCACATCATAGGTCAGTATTGC	(AT) ₁₀ AATT(AT) ₃	187	55	GF111452
DVSSR_B015-F	TCTAAACAGCCCTGAAATTAAC				
DVSSR_B015-R	AGCTCACACTTGTATTTATTCATTG	(TA) ₂ ...(TA) ₂ ...(TA) ₁₂	200	55	GF111453
DVSSR_B016-F	ATGGTCCAACAGGCTTAGTG				
DVSSR_B016-R	AATCGATGTGCTACTATGAACG	(TA) ₄ A(TA) ₂ A(TA) ₅ T(TA) ₃	177	53	GF111454
DVSSR_B017-F	AATACCACCCGCATGTAATG				
DVSSR_B017-R	ATAGTAAGGGACATAAGTACG	(CT) ₄ (AT) ₄ ...(CT) ₄ (AT) ₃ AA(AT) ₇	238	53	GF111455
DVSSR_B018-F	ATGGACGTACTTCAAGAATAGC				
DVSSR_B018-R	ACATTGTTTTATAGGACCAACG	(AT) ₅ A(AT) ₁₀ ...(AT) ₇	180	56	GF111456
DVSSR_B019-F	AAGATAATAAATGGCGGAGTAACAC				
DVSSR_B019-R	ATACGCATTCCGGCTCAACAC	(AT) ₃ ...(AT) ₁₂	238	53	GF111457
DVSSR_B020-F	CACATATCGGAATGTAATTTTAGTAC				
DVSSR_B020-R	GACTACCTTACAGAGAATAGACC	(AT) ₁₅	168	55	GF111458
DVSSR_B021-F	AGGTTATTGGTCAGTGGTGTG				
DVSSR_B021-R	TGAAGTGTCTCCGCATCG	(AT) ₁₄	137	56	GF111459
DVSSR_B022-F	GTTTTGTGTTGTATGTTTATATTCAGG				
DVSSR_B022-R	GCCTTAGACAATAAATACTAAAGAAGC	(AT) ₁₂	204	55	GF111460
DVSSR_B023-F	GCTTGAACGACGAACCTCATC				
DVSSR_B023-R	AAAACAAAACCTCCCTCTGC	(AT) ₂ AA(AT) ₄ ...(AT) ₁₃	250	53	GF111461
DVSSR_B024-F	CAACTACCAGTTTGTACTCAAG				
DVSSR_B024-R	ACACATGTCCAAAATGTCAATC	(TG) ₁₅	154	57	GF111462
DVSSR_B025-F	CGTTCGCCACTACAGGTAC				
DVSSR_B025-R	TTCGTCGCCAACCCAACC	(AC) ₉ AT(AC) ₂	163	56	GF111463
DVSSR_B026-F	AGGGCACACCAACAGTCC				
DVSSR_B026-R	GTCCAGTGCAACGCTAAGG	(ACAT) ₂ (AC) ₁₂	193	54	GF111464
DVSSR_B027-F	ACAGAGCCTTTACTTACAACC				
DVSSR_B027-R	TCAGCCGTGTAATACAATTAGG	(TG) ₁₂	234	55	GF111465
DVSSR_B028-F	GAACGACCGATGTGATTGC				
DVSSR_B028-R	TGTGTTGCGACCAAGTGTAC	(TAT) ₉	224	56	GF111466
DVSSR_B029-F	CTACACGCCATAAGAACCATAGG				
DVSSR_B029-R	ATGAACGCCTAGTTAACAGTGG	(TTA) ₂ (TAA) ₂ (ATT) ₈	240	54	GF111467
DVSSR_B030-F	AATTCAGCCTATCTTATGTGTCG				
DVSSR_B030-R	TAATTTTCAAGTAAAGATGGACTAGAG	(TA) ₃ (ATA) ₂ ...(ATT) ₈	183	54	GF111468
DVSSR_B031-F	TGCTTATTAGACATACATATTATCGC				
DVSSR_B031-R	AACACAATAGCTCAGAGATTACC	(ATT) ₉ ...(ATC) ₂ AGT (ATT) ₃	196	54	GF111469
DVSSR_B032-F	TTTATTTTCGACCGATCTCACC				
DVSSR_B032-R	TTGGACTATCTACCCTACATGC	(ACT) ₈	148	55	GF111470
DVSSR_B033-F	AGCCATACCATGAAAGTGTACC				
DVSSR_B033-R	GAACGGAGTCGAGGAAGAATC	(TAT) ₉	143	55	GF111491
DVSSR_B034-F	AGTTGTATTAGTTTGTAAAGTGTACG				
DVSSR_B034-R	TTTTGCCACGACGACCTC	(TTA) ₈	185	57	GF111471
DVSSR_B035-F	CACTTCAACCTACAGAATTGTTTCG				
DVSSR_B035-R	GCGTGGTGGACATTGATATTGG				

Continued on next page

Table 1. Continued.

Locus	Primer sequences (5'-3')	Repeats	Fragment size	Ta (°C)	GenBank accession No.
DVSSR_B036-F	AAATTAAGTCTGAACAGGTAAATCC	(GCA) ₂ (ACA) ₈	200	54	GF111472
DVSSR_B036-R	TTCTTTGCGTCTTTGATCTGG				
DVSSR_B037-F	CATTCGCCACAGCAACAAC	(CAA) ₈	215	55	GF111473
DVSSR_B037-R	ATGGTATCGTCGTCGTAATCG				
DVSSR_B038-F	AATACCATCGTCCATAAGAGC	(ATT) ₉	184	56	GF111474
DVSSR_B038-R	CGTGATCCGACTACTGTGTAAC				
DVSSR_B039-F	CGGCGTGAACCTTTGATTGG	(TAC) ₉	167	54	GF111475
DVSSR_B039-R	GCGGTTCCATTTTCATTATTC				
DVSSR_B040-F	AACTTGTGGTGGTTGATTGC	(CTG) ₂ CCT(CTT) ₈ .. (CTT) ₂ ..(CTT) ₂	162	55	GF111476
DVSSR_B040-R	GAATCTGATACTGCTGCTGAAG				
DVSSR_B041-F	GGAGAATAACTACAAGCAGAGC	(ACG) ₈	208	55	GF111477
DVSSR_B041-R	ACGAAGGGCGACAACAAC				
DVSSR_B042-F	GCTGAGAGATTAACGGAAAC	(GAG) ₇	160	54	GF111478
DVSSR_B042-R	ACCACCAATCGCAGTTACC				
DVSSR_B043-F	GGGATGGCATAATGGATTGG	(ATA) ₉	181	54	GF111479
DVSSR_B043-R	TTCTGCTGGTTGGTGAAGG				
DVSSR_B044-F	GTAACGACGACAACACAGC	(AAT) ₂ GAT(AAT) ₇	189	55	GF111480
DVSSR_B044-R	CAGGATAACAGCAGAATACACG				
DVSSR_B045-F	TTCTCGATCTGCTCCTTGG	(CTC) ₃ TT(CTT) ₁ (CCT) ₃ CTT(CCT) ₂	164	57	GF111481
DVSSR_B045-R	GCGATTGAAGTTGATACGAATTGG				
DVSSR_B046-F	ATGACAAGAAAGACAAACAATG	(ATTTA) ₆ GTTT(ATTT) ₂	232	52	GF111482
DVSSR_B046-R	GGCTTGTGTTAAAATAATCACC				
DVSSR_B047-F	GGCTCCGATTGGTTGTTC	(ACAT) ₅ TT(AT) ₇	200	56	GF111483
DVSSR_B047-R	GCGGTGTAGTAATGACGAAGG				
DVSSR_B048-F	CATCGAGATTAATAAGTAGTTAGGG	(GTTGG) ₃	229	53	GF111484
DVSSR_B048-R	ATTTAATAGTCATATACCAACAACCC				
15 SSR markers developed from the shared loci of biotype A and biotype B					
DVSSR_AB001-F	GTAATGTTTTGTCTGGATCTAATA	(AT) ₈ T(AT) ₂ C(AT) ₃	166	53	GF111485
DVSSR_AB001-R	GGGCTCTAGGTTGTCCGATT				
DVSSR_AB002-F	TTTTGTGCGGCACGGTACTC	(AT) ₁₂	153	56	GF111492
DVSSR_AB002-R	GGTAATGATGAACACCACACA				
DVSSR_AB005-F	AGTTTATTGTGTCTGAAAACGCA	T ₆ A ₄ TA..T ₄ ..(GT) ₃	172	57	GF111486
DVSSR_AB005-R	AACCAACACAAGGGGGTCG				
DVSSR_AB008-F	GAGTATCACCGTAAAGTGAC	(TTAAAA) ₆	185	50	GF111502
DVSSR_AB008-R	CTGTCTTATTTTATTGACAATC				
DVSSR_AB009-F	GTTACCAACCTTTATTATCATTG	(AT) ₈ ..(CA) ₃	180	52	GF111493
DVSSR_AB009-R	TGCTCACACACACACCTTACT				
DVSSR_AB010-F	GAGGTGTTTCACCTACACAGT	(AC) ₁₁ GT(AC) ₃ GC(AC) ₃	189	52	GF111487
DVSSR_AB010-R	GAGTATGTGTTCAATAACTCG				
DVSSR_AB014-F	CTTTTGCTATCGGACGGCCC	(GGT) ₃ ..(GGC) ₄ ..(GGT) ₆	180	59	GF111488
DVSSR_AB014-R	TGCGCTAGTTCATCGACGTA				
DVSSR_AB018F	CTGTGCTTTGCCACAGTAATA	(ATTACT) ₂ (ATT) ₄	211	53	GF111494
DVSSR_AB018R	CCAACGCGTATAATACAGGTA				
DVSSR_AB019F	TCCAACATCGCACTCCTTGC	(TA) ₁ (TTAA) ₃ GT (ATT) ₃ ..(AT) ₃	289	54	GF111495
DVSSR_AB019R	TCTGAAAATCGATCGCGACCC				
DVSSR_AB020F	GCATTACTTGTAACCCGAGCC	(TAG) ₁₂	215	54	GF111496
DVSSR_AB020R	CAAAAGTCATAAGCGTTGTGC				
DVSSR_AB021F	ACTGTGTGCATGGAGAACCC	(TAAA) ₂ ..(TATAAA) ₂ ..(TA) ₂	207	57	GF111497
DVSSR_AB021R	TTGATACTTCGGGACGGGTG				
DVSSR_AB022F	ACGCCAATTAGGGCAAACAG	(AT) ₃ ..(AT) ₆	293	57	GF111498
DVSSR_AB022R	CTCTCCTGTAAATCGCATGCT				
DVSSR_AB023F	GCGCAGCATATTCGCAAATGT	(AAT) ₉	213	58	GF111499
DVSSR_AB023R	TCATCTCGGAGACCACCGAAA				
DVSSR_AB024F	CAACCGAACTCTTCAATCACC	(TAATA) ₂ ..(TAATA) ₄	286	52	GF111500
DVSSR_AB024R	AATGTGATACTCGCAACAC				
DVSSR_AB025F	GCTAACCAATACATCTTGTTTC	(TA) ₃ ..(TAA) ₄	237	52	GF111501
DVSSR_AB025R	CGTAGAGATCGTTCATTGCCA				

Ta = annealing temperature of the primer pairs.

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