Theor Appl Genet (2003) 107:1337–1349
DOI 10.1007/s00122-003-1386-x

ORIGINAL PAPER

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Isolation and characterization of simple sequence repeat markers in the hexaploid forage grass timothy (*Phleum pratense* L.)

Received: 27 April 2003 / Accepted: 27 June 2003 / Published online: 15 August 2003

Abstract To develop simple sequence repeat (SSR) markers for the hexaploid forage grass timothy (*Phleum pratense* L.), we used four SSR-enriched genomic libraries to isolate 1,331 SSR-containing clones. All four libraries contained a high percentage of perfect clones, ranging from 78.1% to 91.6%. From these clones, we developed 355 SSR markers when tested from 502 SSR primer pairs. Using all 355 SSR markers we tested one screening panel consisting of eight timothy clones to detect the level of polymorphism and identify a set of loci suitable for framework mapping. The SSR markers detected 90.4% polymorphism between the parents of a pseudo-testcross F_1 population. These SSR markers will provide an ideal marker system to assist with gene targeting, QTL (quantitative trait locus) mapping, and marker-assisted selection in timothy.

Keywords Simple sequence repeats (SSR) · SSR-enriched library · Molecular markers · Polymorphism · Genomic constitution · *Phleum pratense* L.

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00122-003-1386-x

Introduction

Timothy is an important forage grass that is grown widely in the cool, temperate regions of the world, including North America, Europe and Asia. Although the diploid species of timothy (*Phleum bertolonii* DC.) is cultivated in some countries, cultivated timothy is mostly *Phleum pratense* L., a hexaploid (2n=6*x*=42). It has a moderately large genome size (e.g., 1C=4,067 Mb; Bennett and Smith 1991), but the genomic constitution of this species (autopolyploid vs allopolyploid) is unclear (Muntzing and Prakken 1940; Leven 1941; Myers 1944; Nordenskiold 1945; Stebbins 1950; Wilton and Klebesadel 1973; Cai and Bullen 1991). There have been very few molecular studies of timothy (Cai and Bullen 1994; Ogawa et al. 2001), because this species is a hexaploid with a large genome size and, moreover, it is cross-pollinated.

The genomes of all eukaryotes contain a class of sequences termed microsatellites (Litt and Luty 1989) or simple sequence repeats (SSRs) (Tautz et al. 1986). Microsatellites with tandem repeats of a basic motif of <6 bp have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang et al. 1994). Unlike other markers, SSR markers have the advantages of being PCR-based and multiallelic, and possessing high polymorphism. SSR markers have been developed in many plant species, including most major crops such as rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Röder et al. 1998; Bhattramakki et al. 2000; Cordeiro et al. 2000; Ramsay et al. 2000; Temnykh et al. 2000; Jones et al. 2001; Tang et al. 2002). SSR markers have also been applied to studies of diversity and evolution.

We report the isolation, characterization, and development of 355 SSR markers from four SSR-enriched genomic libraries. One screening panel consisting of eight timothy clones was tested with all 355 SSR markers to detect polymorphism levels and identify a set of loci suitable for framework mapping. We also discuss the reason for the high level (40.7%) of redundancy and the genomic constitution of this species (autopolyploid versus allopolyploid).

Communicated by R. Hagemann

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Materials and methods

Plant materials

A timothy clone, SK, was used to construct the SSR-enriched genomic library. A panel of eight timothy clones native to Japan was used for screening SSR polymorphism. These clones were SK, NJ, 243, 341, 111, 117, 118 and 124. SK and NJ were from the collection of the Japan Grassland Farming and Forage Seed Association, Forage Crop Research Institute, and the other six clones were from the timothy collection at Hokkaido Kitami Agricultural Experiment Station.

A pseudo-testcross F_1 population consisting of 78 individuals derived from a single cross between clones 243 and 341 was used to confirm the segregation ratio of SSR markers.

Construction and sequence analysis of SSR-enriched genomic libraries

Four SSR-enriched genomic libraries (CA, GA, AAG and AAT) were constructed by Genetic Identification Services (GIS, Chatsworth, Calif., USA) from SK timothy. Briefly, 1 μg of genomic DNA was partly restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr*B1, *Pvu*II, *Stu*I, *Sca*I and *Eco*RV). Fragments ranging from 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG Inc., Lincoln Park, N.J., USA) using biotinylated capture molecules. Libraries were prepared in parallel using biotin-$(CA)_{15}$, biotin-$(GA)_{15}$, biotin-$(AAG)_{12}$ and biotin-$(TAGA)_8$ as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hin*dIII to remove the adapters. The resulting fragments were ligated into the *Hin*dIII site of pUC19. These were the original SSR-enriched libraries.

Recombinant molecules were electrophoresed into *Escherichia coli* DH10B. The recombinant clones were selected at random for sequencing, and the sequences were obtained on a MegaBACE1000 (Amersham Biosciences, Freiburg, Germany) with a DYEnamic ET dye terminator kit (Amersham Biosciences, Freiburg, Germany) at Dragon Genomics (Yokkaichi, Mie, Japan). After sequencing, the Phred values (Brent et al. 1998) were calculated, and clones with Phred values > 15 and longer than 300 bp were used for primer design.

Sequence checking and primer design

Sequences containing at least five di-, tri-, tetra-, penta- or hexanucleotide repeats were selected. The SSR structure was defined in terms of four categories: perfect, imperfect, interrupted or compound repeat, according to Jones et al. (2001).

All sequences containing SSR were checked to identify duplicates by using Sequencher 4.02 software (Gene Codes Corporation, Ann Arbor, Mich., USA). Only unique SSR clones with sufficiently long flanking sequences to generate both forward and reverse primers were used for primer design. Primer pairs flanking the microsatellite motif were designed with the program Primer 0.5 (Lander, Cambridge, Mass., USA). The parameter of the annealing temperature was set at 55°C.

Polymerase chain reaction and fragment analysis

An M13-tagged forward primer (Rampling et al. 2001) was used in the PCR reaction. The primers used were 5 pmol labelled M13 (-29) primers (IRD700- or IRD800-CACGACGTTGTAAAAC-GAC, Li-COR, Lincoln, Neb., USA), 1 pmol 5′-tagged forward primer, and 5 pmol reverse primer. The 5′-tagged forward primer for each particular microsatellite had the M13 sequence added to the 5′ end of the forward primer.

SSRs were amplified under the following PCR conditions: a "touchdown" PCR consisting of 94°C for 5 min; 2 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 1.5 min; 10 cycles of 94°C for 1 min, 65 to 55°C for 1 min decreasing by 1°C/cycle and 72°C for 1.5 min; and 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min, followed by 72°C for 7 min and 4°C as the holding step.

To detect SSR markers, PCR products were analyzed using 6% denatured acrylamide gel with a LI-COR sequencer (Li-COR, Lincoln, Neb., USA).

Primer evaluation

All primer pairs were screened on the panel of eight timothy clones for their ability to yield an amplification product of the expected size and to detect polymorphism. For primers that detected polymorphism, the number of alleles and the average number of alleles per strain were calculated.

Results

SSR isolation

A total of 4,000 clones (1,000 clones from each of four libraries) were sequenced. Of these, 2,492 clones (62.3%) contained SSR sequences, showing a high level of redundancy (1,016 clones, 40.8% of SSR-contained clones). In addition, we found that the sequences flanking the SSR motifs of 145 clones (5.8% of SSR-contained clones) were too short to design both forward and reverse primers. Finally, 1,331 unique (53.4% of SSR-contained clones) SSR clones were identified and used to design primers (Table 1). The percentage of unique SSR clones in library B (motif, GA/TC, 74.6%) was higher than in the others (44.4% to 49.9%).

Characterization of SSR loci

SSR loci were classified by repeat type and structure (Table 2). All four libraries contained perfect clones with very high frequencies, ranging from 78.1% to 91.6%.

Table 1 Efficacy of SSR isolation and working primers from four timothy SSR libraries

Library	Motif	Clones sequenced	SSR clones	Unique SSR clones	Primers tested	Strong amplification	Weak amplification
A	CA/TG	1,000	680	311 (45.7%)[a]	302	172 (57%)	45 (15%)
B	GA/TC	1,000	635	474 (74.6%)	100	50 (50%)	19 (19%)
C	AAG/TTC	1,000	431	215 (49.9%)	50	27 (54%)	8 (16%)
D	AAT/TTA	1,000	746	331 (44.4%)	50	27 (54%)	7 (14%)
Total		4,000	2,492(62.3%)	1,331 (53.4)	502	276 (55%)	79 (16%)

[a] % of SSR-containing clones

Table 2 Frequency of repeat types isolated from four timothy SSR-enriched libraries

Repeat type	Library A	Library B	Library C	Library D	Total
Perfect	243 (78.1%)	420 (88.6%)	197 (91.6%)	267 (80.7%)	1,127 (84.7%)
Imperfect	5 (1.6%)	7 (1.5%)	4 (1.9%)	23 (6.9%)	39 (2.9%)
Compound	51 (16.4%)	23 (4.9%)	9 (4.2%)	19 (5.7%)	102 (7.7%)
Interrupted	11 (3.5%)	14 (3.0%)	2 (0.9%)	16 (4.8%)	43 (3.2%)
Interrupted and compound	1 (0.3%)	10 (2.0%)	3 (1.4%)	6 (1.8%)	20 (1.5%)
Total	311	474	215	331	1,131 (100%)

Table 3 Frequencies of motif types in the perfect SSR isolated from four timothy SSR-enriched libraries

Motif	Library A	Library B	Library C	Library D	Total (%)	Average repeat number
CA/TG	214 (88.1%)	2 (0.5%)			216 (19.2%)	18.0
GA/TC	21 (8.6%)	413 (98.3%)	7 (3.6%)	177 (66.3%)	618 (54.8%)	19.2
AAG/TTC			180 (91.4%)		180 (16.0%)	11.5
AAT/TTA				40 (15.0%)	40 (3.5%)	11.7
TCA/TGA	4 (1.6%)	3 (0.7%)	1 (0.5%)	46 (17.2%)	54 (4.8%)	14.1
GCA		2 (0.5%)		4 (1.5%)	6 (0.5%)	18.3
CAA/TTG	4 (1.6%)		3 (1.5%)		7 (0.6%)	20.7
AAAG/TTTC			4 (2.0%)		4 (0.4%)	9.0
AT			1 (0.5%)		1 (0.1%)	9.0
TTCC			1 (0.5%)		1 (0.1%)	5.0
Total	243	420	197	267	1,127 (100%)	

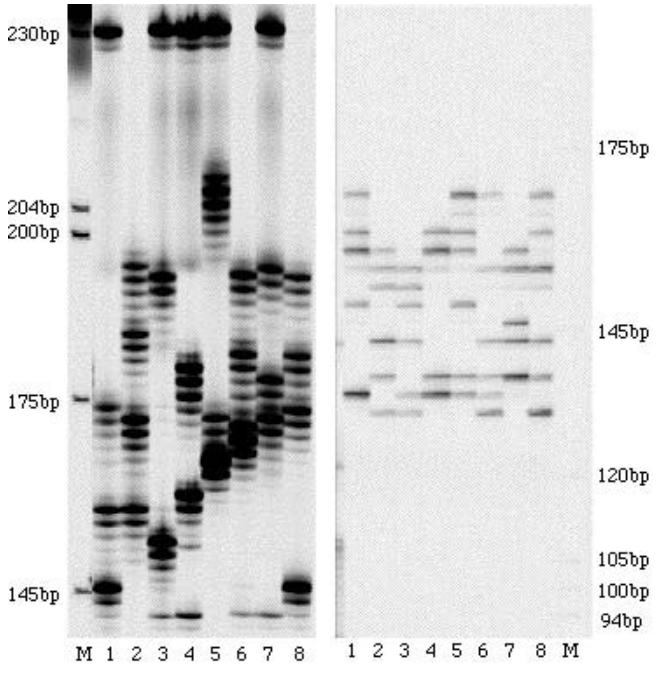

Fig. 1 PCR products amplified by the loci A10-A07 (*left*) and C01-E10 (*right*) for eight clones of timothy. *Lane 1*, clone 124; *lane 2*, clone 118; *lane 3*, clone 117; *lane 4*, clone 111; *lane 5*, clone 341; *lane 6*, clone 243; *lane 7*, clone SK; *lane 8*, clone NJ

Compound and imperfect clones were contained with higher frequencies in libraries A and D, respectively, than in other libraries.

The data in Table 3 show the proportions of different repeat motifs in each of the structural categories, by library. In libraries A, B and C the predominant motif was the expected type, e.g. CA/TG for A, GA/TC for B and AAG/TTC for C. Library D contained several types of motifs—GA/TC, AAT/TTA and TCA/TGA—probably because the selected oligonucleotide $(TAGA)_8$ was an imperfect compliment to the SSR sequences.

In the most-frequent dinucleotide- and trinucleotide-motif-containing clones, the average repeat numbers were 18.0 (CA/TG), 19.2 (GA/TC), 11.5 (AAG/TTC), 11.7 (AAT/TTA) and 14.1 (TCA/TGA) (Table 3).

Primer evaluation

Of the 502 primer pairs tested, 276 pairs (55%) could amplify very strong products and showed polymorphism in the eight timothy clones used. Seventy nine (16%) additional primers amplified weak products but also showed polymorphism.

Some primers produced a maximum of six alleles per locus from one timothy clone (Fig. 1 and Table 4).

Polymorphism of SSR loci

Many authors have used polymorphic information content (PIC) to evaluate the polymorphism of SSR markers (Ott 1991; Saal and Wricke 1999; Jones et al. 2001). In our study, PIC was not suitable because we could not distinguish homozygous and heterozygous states from the electrophoresis patterns owing to the hexaploid nature of timothy; therefore, the allele frequencies of SSRs could not be calculated. Most markers had a large number of alleles, and the average number of alleles per strain was higher than two (Table 4). Of the 355 working primer

Table 4 A selection of primer sequences designed for SSR loci that yielded amplification products of the expected size across eight timothy strains

SSR marker	Repeat motif/repeat class	Primer sequence (5′-3′)		Expected size	Total no. of alleles	Range of no. of alleles per clone	Average no. of alleles
A01-D03	(TG)12 Perfect	F R	*CTAGCTCGATCCTAGGCAG* *AGCTTCCATGGAGCTAGC*	150	7	1–3	1.9
A03-A01	(CA)13 Perfect	F R	*CCGAACGAGAAACAGTTG* *CAGACTCTATGGTCGAAAGTG*	392	12	3–5	3.6
A09-H08	(TG)16 Perfect	F R	*GCAAGGTTAATTGCCACTAC* *TAATGCTGCACCAAAGGT*	255	7	3–5	3.9
A10-A07	(TG)22(GA)10 Compound	F R	*TGGATGTTTGGATGTTGG* *GTGCGGGTGAGATACATAAT*	170	16	3–4	3.8
A10-A10	(CA)31 Perfect	F R	*AGTACTGCCCCAAGGTTC* *GTTGCCCCAGTTTATTCC*	232	11	2–5	3.0
B02-D11	(TG)19(GA)20 Compound	F R	*TTTCCTAAGTTTCTAGCCAATC* *ACCCATCCCTGACTGTTC*	144	11	2–4	3.0
B03-F07	(TC)14 Perfect	F R	*GGGAGAAATCATCTTGCTC* *GTCAATGCGGTAATTAAGCT*	130	8	1–3	2.0
C-T7	(TTC)15 Perfect	F R	*TGGGGAGTCTTCTCCTTC* *ATGTGGATGGACAGAGGTAC*	100	5	1–2	1.4
C01-E10	(TTC)13 Perfect	F R	*CGATGTGATGTTTGAGCTC* *ACGTTCCAAAGCGGAC*	127	11	4–6	5.5
C01-G04	(AAG)11 Perfect	F R	*TCAGACGTTGTTGGTCAGTA* *GACAATCCCTCCTGATATGA*	118	4	1–3	1.9
D01-E04	(CAA)8(TAA)10 Compound	F R	*TCTGTTGCCTATTCTGCTG* *GCATTTCACTAAGACTGTGACA*	158	13	4–6	4.2
D01-G10	(TGA)7(CGA)4 N(TGA)16 Compound and interrupted	F R	*ATAGATTGGGTGTTGGAGC* *ATTCCATGTTAGCATGTCATC*	231	16	2–5	3.9
D01-H08	(AAT)13 Perfect	F R	*GCTTTAAGGATTGGCTCAC* *CAACTTTCAGACTCAAACCTG*	147	9	1–3	2.0
D13-C02	(CGA)6(TGA)15 Compound	F R	*GATGACATGCTAACATGGC* *CCATTCAGTAGTCTCCCTCA*	146	9	1–5	3.1

pairs, 321 primers (90.4%) were found to detect polymorphism between the parents of our F_1 mapping population.

Discussion

Efficiency of SSR marker development

Our results showed that 62.3% of the clones were SSR-containing clones, and 53.4% of SSR-containing clones were unique. The percentage of SSR marker-isolation efficacy was similar to the results obtained in sunflower (*Helianthus annuus*, Tang et al. 2002) and Italian ryegrass (*Lolium multiflorum*, Hirata et al. 2000) using SSR-enriched libraries produced by GIS. The percentage of working primers in our study was 70.7% lower than for Italian ryegrass and sunflower but higher than for wheat and barley (Röder et al. 1998; Ramsay et al. 2000).

As pointed out by Cardle et al. (2000), the most common dinucleotide motif found in plants is AT/TA, followed by GA/CT and CA/GT, and the most common trinucleotide motifs are AAT/TAA and ATC/TAG, but AAG/TTC dominates in *Arabidopsis thaliana*. In our study, of 1,127 perfect SSRs, the motifs occuring at the highest rates were GA/TC (54.8%), CA/GT (19.2%), AAG/TTC (16.0%), TCA/TGA (4.8%) and AAT/TAA (3.5%). These results are close to those reported by Cardle et al. (2000). Although AT/TA is the most common dinucleotide motif in plants, this motif is not usually used in SSR-enrichment procedures owing to its self-complementary nature.

Redundancy in SSR enrichment library

Our level of redundancy was 40.8%, which was higher than that in other species (24% in tea tree, *Melaleuca alternifolia*: Rossetto et al. 1999; 16% in *Lolium perenne*: Jones et al. 2001; and less than 1% in sugarcane, *Saccharum* sp.: Cordeiro et al. 2000). Redundancy was found mostly within the same library, although duplicates across libraries were also observed, especially between libraries D and A. This redundancy may be caused by clone duplication, locus duplication or allelism, which is an inevitable problem for cross-pollinated and polyploid

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Clone name    sequence
              1                                                  50
>a01-h09      GCTATGCAAAATTGAGCCCATAAAGAATGCGTTTAGTTCTCCTGGTTTAT
<a14-c08      GCTATGCAAAATTGAGCCCGTAAAGAATGCGTTTAGTTCTCCTG:TTTAT
<a8-c09       GCTATGCAAAATTGAGCCCGTAAAGAATGTGTTTAGTTCTCCTG:TTTAT
>a3-e01       GCTATGCAAAATTGAGCCCGTAAAGAATGCGTTTAGTTCTCCTGGTTTAT
>a15-b07      GCTATGCAAAATTGAGCCCGTAAAGAATGTGTTTAGTTCTCCTGGTTTAT
<a12-g09      GCTATGCAAAATTGAGCCCGTAAAGAATGTGTTTAGTTCTCCTG:TTTAT
>a14-a02      GCTATGCAAAATTGAGCCCGTAAAGAATGCGTTTAGTTCTCCTGGTTTAT
>a12-f08      GCTATGCAAAATTGAGCCCGTAAAGAATGCGTTTAGTTCTCCTGGTTTAT
<a12-b10      GCTATGCAAAATTGAGCCCGTAAAGAATGCGTTTAGTTCTCCTG:TTTAT
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>a12-b11      GCTATGCAAAATTGAGCCCGTAAAGAATGTGTTTAGTTCTCCTGGTTTAT
<a7-e07       GCTATGCAAAATTGAGCCCGTAAAGAATGTGTTTAGTTCTCCTG:TTTAT
              51                                                 100
>a01-h09      GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
<a14-c08      GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
<a8-c09       GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
>a3-e01       GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
>a15-b07      GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
<a12-g09      GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
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<a7-e07       GGTTCTTCCATGTGAAGAAGAACAACCACCACAGTCGGAATGTGGCAGGT
              101                                                150
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<a14-c08      CCAAACTGATAAGATGCAGTGTTCTTTGGG::::TGTGTGTGTGTGTGTG
<a8-c09       CCAAACTGATAAGATGCAGTGTTCTTTGGG::::TGTGTGTGTGTGTGTG
>a3-e01       CCAAACTGATAAGATGCAGTGTTCTTTGGG::::TGTGTGTGTGTGTGTG

>a15-b07      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
<a12-g09      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
>a14-a02      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
>a12-f08      CCAAACTGATAAGATGCAGTGTTCTTTGTG::TGTGTGTGTGTGTGTGTG
<a12-b10      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
>a12-h07      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
<a10-b02      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
>a14-b09      CCAAACTGATAAGATGCAGTGTTCTTTGGG::TGTGTGTGTGTGTGTGTG
>a12-b11      CCAAACTGATAAGATGCAGTGTTCTTTGGGTGTGTGTGTGTGTGTGTGTG
<a7-e07       CCAAACTGATAAGATGCAGTGTTCTTTGGGTGTGTGTGTGTGTGTGTGTG
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              151                                                200
>a01-h09      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
<a14-c08      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
<a8-c09       TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
>a3-e01       TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
>a15-b07      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
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<a12-b10      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
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<a10-b02      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
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>a12-b11      TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
<a7-e07       TGTGTGTGTGTGGCTTCATGAGACAAGTTATACTAGTAGCGTTTTTGTAG
              201                222
>a01-h09      TTGTGTCAGATTGCTTGTGGAT
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<a12-g09      TTGTGTCAGATTGCTTGTGGAT
>a14-a02      TTGTGTCAGATTGCTTGTGGAT
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<a12-b10      TTGTGTCAGATTGCTTGTGGAT
>a12-h07      TTGTGTCAGATTGCTTGTGGAT
<a10-b02      TTGTGTCAGATTGCTTGTGGAT
>a14-b09      TTGTGTCAGATTGCTTGTGGAT
>a12-b11      TTGTGTCAGATTGCTTGTGGAT
<a7-e07       TTGTGTCAGATTGCTTGTGGAT
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Fig. 2 A contig consisting of 14 clones with four alleles. The TG repeats are *underlined*. > and < show the directions of the original sequence for 5′ to 3′ and 3′ to 5′, respectively

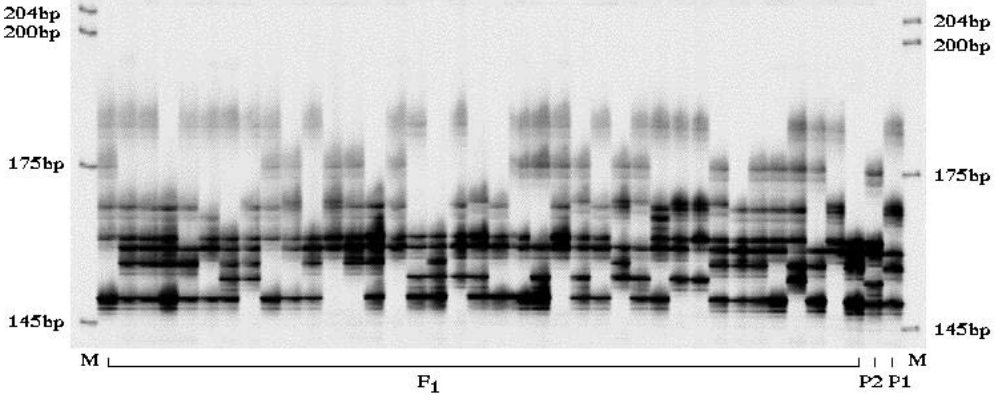

Fig. 3 Segregating pattern of SSR alleles detected by locus A11-G11 in two parents and 38 F_1 progeny of the 243/341 mapping population

Table. 5 Supplementary material: List of SSR markers developed in this study

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
A01-A06	AB108066	*GTTGATGTCTGCAGATAAATTG*	*AAACGATAGATCAGGGCAC*	(TG)12	145	S
A01-A08	AB108067	*AACATTAGACCATAGAGATGGC*	*CCTCTAGACCCGGATAGTTT*	(CA)13	144	S
A01-A09	AB108068	*TAGTGAATACAGGGCAAATACA*	*GATAACCAAGTTGAGGCAAG*	(CA)35 (TA)8	128	S
A01-A11	AB108069	*AATCTGAGAGAGAAAGAGGGA*	*AAAACATCTTTGGGTCAGG*	(TG)15 (GA)12	120	S
A01-B02	AB108070	*TCTAACATTATGGCTAACGATG*	*AGTATGATTGGGTAACCACG*	(TG)12	124	S
A01-B03	AB108071	*ACGAGGATAATTTCATGGTG*	*CCATAACCAAAAGTTTTAGCA*	(TG)11	104	W
A01-B05	AB108072	*TGCAGTAGGCTGCCTAAC*	*CATCTACCAAAGACATGAAGC*	(TG)32	115	S
A01-B11	AB108073	*ATCCTGCATCTGGATCATAC*	*CCTTTGGGGGATAGCTTATA*	(CA)43	134	S
A01-B12	AB108074	*CTCCTTGTAGTTTGGGGAG*	*CAAGAGAAGAGAAATAACCAGG*	(TG)7	128	S
A01-C02	AB108075	*CAAAGTGGTCATACAAAGAGG*	*CCCGCAAGATTCATAAATAC*	(CA)18	185	S
A01-C03	AB108076	*CTGCAATTGCTACGACTACA*	*ATGTGAAACAAAGAGAGTGGA*	(TG)12	149	S
A01-C07	AB108077	*AACCTGCTCCAGGGTTAC*	*CTATTTTACCATTTTCATTCCC*	(CA)11	199	S
A01-C11	AB108078	*CACAAAGACAAGATGCCAC*	*TGCTTCAGTCCCTGTTAGTT*	(CA)10	107	S
A01-C12	AB108079	*AAAGACGAGCTCTAGCAGC*	*CCTGCAATTTTGGTCTTTAC*	(TC)7(CA)7 (CGCA)12 (CA)16	246	S
A01-D03	AB108080	*CTAGCTCGATCCTAGGCAG*	*AGCTTCCATGGAGCTAGC*	(TG)12	150	W
A01-D05	AB108081	*ATTATACCAATACACCCACCC*	*TGTTCTTTCCATTTCGAAGT*	(CA)11	142	S
A01-D07	AB108082	*CAACCTAATAGTGCCAAGATG*	*AACAACTTGTGTGGCATGTA*	(CA)15	308	S
A01-D09	AB108083	*TGGACAGTTTGGTAATTTCC*	*CTAATTGTTGGTCACACACG*	(TG)11	121	S
A01-D10	AB108084	*ATTTTTCATTACCAGTCAGCA*	*AACTTAGGTTGGAACCGG*	(CA)12	149	W
A01-D12	AB108085	*CAGAGATGGACCCTATCGTA*	*ACCTCGCCCCCTGGTGG*	(TG)20	133	S
A01-E01	AB108086	*TCGGAGCTAGATTCTTCTTG*	*TCCAGCTAGCTACATTTGTTC*	(TG)11	110	S
A01-E02	AB108087	*TGCTGTCTCCATCGATTC*	*AATCTCACCTAGCCCCAC*	(TG)8... (TG)8	145	S
A01-E04	AB108088	*AGCAAAGTCCTTGGTGAATA*	*TCTTAGATCATGTTGGACAAGA*	(TG)18	128	W
A01-E11	AB108089	*ATATGAATGATCTTCACTGCC*	*GGTGAATCTGTAACTGATGGA*	(CA)15	121	W
A01-E12	AB108090	*GTCTGTTGTTGTCGCACTTA*	*TCAATTAGCAAGAACACGC*	(TG)16	111	W
A01-F02	AB108091	*GGGGTTCTAGCAGCTCTC*	*TTTCATGGTCTATTCTAGCCA*	(TG)35	141	S
A01-F03	AB108092	*GCATCCGTGCATTAACTC*	*TACACCTTTCACAAGGAATACA*	(TG)21	139	S
A01-F04	AB108093	*TTGTTCCCATGACAGGAC*	*GTGTTAAGGTTGCATTAGGC*	(TG)13 (GA)13	131	S
A01-F05	AB108094	*AGAGGTTCGATATATTTTGGTG*	*GTTGGATGCTGAAATTCG*	(TG)5... (TG)6	131	W
A01-F06	AB108095	*AGAAGTTTCCTGAATCTCCC*	*CCGAGCTATCAGTAATTCATG*	(CA)10	69	W
A01-F07	AB108096	*TGATCCATCTTTATCTTTGTGA*	*CTTTCGCGTTGATAGATACC*	(TG)58	235	W
A01-F08	AB108097	*CGGCAGAATGTAACACAAC*	*GGAGTAATTATTCGGCGTC*	(TG)11	145	S
A01-G01	AB108098	*AATTTTCGCCGAGCTATC*	*ACATGATCTGAGTTGTGCAA*	(TGCG)17 (TG)32	236	W
A01-G03	AB108099	*AGTCGATCGAGTGAGGATC*	*TCCTCTGGAATGACCTATTG*	(TG)12 (GA)12	127	S
A01-G04	AB108100	*ATCTCCGAAACAACATCAAT*	*AACGCATAGGACTCCCAC*	(TG)25	146	S
A01-G07	AB108101	*CATATGAAAGTGGCGAGG*	*GATTCTCGACGATCTAGGTG*	(TG)7(GA)6	189	S
A01-G10	AB108102	*TTGCAAAGATTATCATGCTG*	*ATGTGATTTGGTTCTAGCTGA*	(CA)25	291	S
A01-H07*	AB108103	*GAAATATTCCCAGAATAGTGTTAAA*	*CACCGGTGCATAACTTTAATAAAGG*	(TG)46	144	S
A01-H08	AB108104	*TACCCAGCTAAAGGATTGAA*	*TGGATCTTCTAACCAATGCT*	(TG)31	150	S
A01-H12	AB108105	*GATAGAAGTGAAGCGGAATG*	*CTGACTACTGATTCTTGAAGCA*	(CA)17	139	S
A03-A01	AB108106	*CCGAACGAGAAACAGTTG*	*CAGACTCTATGGTCGAAAGTG*	(CA)13	392	S
A03-A04	AB108107	*TAGTAGCATTTAGCGATGCA*	*ACGCATGTCATCAGTAGTGA*	(TG)10	167	S
A03-A05	AB108108	*AGGAATTCATGGAAGAGTTATG*	*TTACATGGACATGTACAACACA*	(TG)12T24	204	W
A03-A07	AB108109	*GTTGTTATTCGTGCCGAC*	*GCAATGGAGTATTCAAAAGC*	(TG)33	140	S
A03-A08	AB108110	*AAACATCAACAAATTTGAATGA*	*CGTTTGAGATGACTACAGCA*	(TG)17	126	W
A03-A10	AB108111	*TCCAGAGTTCAACACAAACA*	*TTCATGCTTGAAGCTATGTG*	(GA)18	140	S
A03-A11	AB108112	*TAGAAGTTTTGCACCACCTT*	*CGAGTTGTCCTACATGATAGC*	(TG)12	381	S
A03-B04	AB108113	*TCGAGTCATTTGAAATGTGA*	*AAAGCATGTAAGATGGCTGT*	(TC)15 (CA)27	226	W
A03-B05	AB108114	*GCTCCTCATCAGGGACTT*	*AAGACTTTGCAGCTAAGATAGG*	(TG)27	200	S
A03-B11	AB108115	*CCAATTCGATCAACAAGAAG*	*TGGAGGGAGTAGTCGATAAA*	(TG)23	218	S
A03-C01	AB108116	*ATGTATGTTGTATTACTCGGCA*	*ACTTCACTGCTTGCTACTACG*	(TG)13 (GA)9	128	S
A03-C03	AB108117	*CACACGGTTCAGGTTAGC*	*CTTCCCATTCCCATAATACA*	(CA)11	328	S
A03-C08	AB108118	*ATCTCTGTGACCCATTGAAG*	*TTGTGATAATCCCATTTATGG*	(CA)15	314	S
A03-C10	AB108119	*GTACATTATGTGCTCCTTTGC*	*CAACAATATGCCCCATAATT*	(TG)9	98	S
A03-D01	AB108120	*ATTTATGAGTGACATTCCCG*	*GCAACTAATCGAATCAAACTG*	(CA)13	166	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
A03-D03	AB108121	*TATGTTGCTGAGCTGTACATG*	*ATGTTTGGTCAAAATTCAATG*	(TG)20 (GA)12	297	S
A03-D06	AB108122	*TCAACTCCTAATCAGGATTACC*	*TATATGCTTGAGCCTCGTG*	(TG)34	239	W
A03-D08	AB108123	*TCTGAGCATTTGAGCATATG*	*TCTGCCAAAGCTACTACATC*	(GA)21 (TG)12	251	S
A03-D11	AB108124	*GGTTCTCTCAAACATTAGCG*	*ACTAGATGGTCACTTGTAGGAA*	(TG)25	144	S
A03-E04	AB108125	*TCAACGTACACGTTTAGACG*	*TACGAATTTCAGAGGATCTGA*	(TC)27	296	S
A03-E06	AB108126	*GTGCGGATCCAAGTAAGTAG*	*ATCTACCAACGAATAGGAAATG*	(TTG)28	238	S
A03-F02	AB108127	*ATGTATGCAGCTAGCTAGTGG*	*ATGCAGTGGACACACCTC*	(TG)8	195	S
A03-F03	AB108128	*GATTCTCCAAAGATGCATGT*	*GACCGAGTGAAGCACTTG*	(GA)30	239	W
A03-F04	AB108129	*ATGAATGATCTTCACAGCTACA*	*GTCATTTGCATCAGACAGTG*	(CA)14	247	S
A03-F11	AB108130	*TGGATCCGTAATAAAGTTTGA*	*CCTTGTGCTAGTATATAACGCA*	(TG)12	241	S
A03-G01	AB108131	*CTCATGCTATCCAATCCG*	*ACCAAACTCATGCCACAC*	(TG)11	125	S
A03-G03	AB108132	*CCAGGTATAGCAACTTCCAG*	*CTATTTAAACCCCAAGCATG*	(TG)10	321	S
A03-G04	AB108133	*CCTCACATCAATTTAGACAGC*	*AACGTGATAACCTCCACAAC*	(TG)10T12	221	S
A03-G05	AB108134	*TTGTTACATCTTATTTCCTCCC*	*CAACCACTGCAATTTAGATTC*	(TG)18 (GA)22	345	W
A03-G09	AB108135	*GTCGTATGAGATATGGTCATCA*	*ACTCGGAATTCCTGTGATC*	(TG)15	107	S
A03-G10	AB108136	*ACATCCTTGGTGCTTATCC*	*GTCTGTGCTTCCAATATTGC*	(CA)11	101	S
A03-H03	AB108137	*TGCATGGTAGGGTCAGAC*	*TTGTGCCATCACAAATTTT*	(TG)32	288	S
A03-H12	AB108138	*TGCCAACCATGATATGGT*	*TTTTGAAGATTGTGTTGTCG*	A8(CA)9	100	S
A07-A04	AB108139	*CTAGGAAGAGGACGTAGGGT*	*GGACTACTTGTGCTCTGTCC*	(CA)10	149	W
A07-A05	AB108140	*ACTAACTCATCGTTTGAACGA*	*TTGAGATTCCAGCCAATG*	(GA)22	179	S
A07-B05	AB108141	*CGATAAGACCAGTCTTTTGG*	*TATGGTGCTTGCTAGTGGAG*	(CA)6	114	S
A07-C01	AB108142	*ATCCACTCATTTTAATCTGCA*	*TTGCGTGCATGATCTTATAG*	(TG)11	222	S
A07-C03	AB108143	*AATATCTCCTTTCCACAATCTG*	*TGTCGGAAGTAGCTAAGTTTG*	(CA)14	193	S
A07-C09	AB108144	*ACAATGCTGATATTTGGACC*	*ACCACCAATATAGAGGCTAGG*	(TG)17	185	W
A07-C12	AB108145	*TCCGTCTTCAATATCCATTC*	*ATGGCACCAAATATATGAGG*	(TG)12	120	W
A07-D04	AB108146	*TCCTACTCACTGAGGTGAAGA*	*TACACAACTGATCCTCATTCC*	(TG)20	218	S
A07-D10	AB108147	*TGAATTAACGAAGCCAGC*	*TTGGTGTTGCCTTCTGTT*	(CA)17	128	S
A07-D12	AB108148	*GGACAAAGAAATAGGCTGC*	*CAAGAGAACCCACTGCAC*	(TTTTG)6... (TG)12	244	W
A07-E01	AB108149	*CGAACAATTGAAATTTTGGT*	*AAGACGCATGTACAAGCC*	(TG)14 (GA)11	143	W
A07-E06	AB108150	*GCTCAAGGCTTACCATGTAC*	*ACACCTGGAGAATGAGAGG*	(CA)19	150	S
A07-E08	AB108151	*GTGCCAGAGTTATCGACG*	*CGCTAGCTAGATTCCATATCA*	(TG)24	129	W
A07-F04	AB108152	*AGGAGGTTGTATTTCATGACTC*	*TCATTGAAACGCAATAATATTC*	(TG)27	143	S
A07-F06	AB108153	*CATTAACTCTCCGATTCAGTG*	*TATCGCTAGCGTCTACCTTC*	(TG)60	387	W
A07-F10	AB108154	*AACGAAATAACTTCCTGCAA*	*AGTCCCACAACATACAAATTG*	(CA)13	260	S
A07-G01	AB108155	*ATTACATGCACTTTTCCTTCA*	*TTCGGCAAAGTCTCTGAG*	(CA)18 imperfect	193	W
A07-G04	AB108156	*ATCCAAATTTACATCACATGC*	*GTCAGACAAATAATTAGCTGCA*	(CA)11	139	S
A07-G06	AB108157	*GTATGACAGGCTTGTTGATTT*	*CGTACCTTCGACGTCATAGT*	(TG)45	149	W
A07-G09	AB108158	*CGATGTAGGATGAAGAGACTG*	*GGACTCGTGAGTACCAACC*	(TG)12 (GA)18	154	S
A07-H01	AB108159	*ACATCTAGGCACTCACGC*	*GTTGAGTCCCCTCGTCTC*	(TGA)20	129	W
A07-H06	AB108160	*ATAGAATAAATGAGGTGTGCG*	*TTCAACACAGAAAATGCAAG*	(TG)10 (GA)24	140	S
A07-H10	AB108161	*AATGGCTACCAAATATATGAGG*	*GAACACGTTCTATGCATGC*	(CA)12	100	W
A08-A03	AB108162	*TCGTCGAGCTATCAGTAATTC*	*GGAGCAGTGGTACGTGAG*	(TG)12	270	S
A08-A07	AB108163	*ACTATTCCACTGGACAGCC*	*TACACTGGAAAGAACCTCTCA*	(CA)40	174	W
A08-A11	AB108164	*CGTCTATCAGCAGACAATCTC*	*AGATGATTTACAGAATGCTCAA*	(TG)13	142	S
A08-B01	AB108165	*AATGATCACACGGAAATAGG*	*GCTCGACGACATGAGAAC*	(TG)10	239	W
A08-B05	AB108166	*TTCTCAACCAGTTACCTGCT*	*GTTACAACCATGGCTGATG*	(TC)28	147	S
A08-B12	AB108167	*ATACGATTCACAAGTTACCACA*	*ATGTAGGGTTTCCGGATC*	(GA)26	129	S
A08-C02	AB108168	*TGAAAAGAGTTCCTTTCATGA*	*GTCAGACACCCTGACTGTTT*	(CA)10	226	S
A08-C05	AB108169	*ATCCTTATCGGGACGATC*	*GTAATATCACTCCCGATCCA*	(TG)7... (TG)13	240	S
A08-C07	AB108170	*GATATTCCTTCCATGAGAACC*	*GTTCTCATGTCGTCGAGC*	(CA)8... (CA)11	107	S
A08-C11	AB108171	*TATCTGTTGGGACCATGC*	*CATGGGTATGCGTTACTTG*	(CA)15	216	S
A08-D06	AB108172	*CGTACGTCCGAAACAGAC*	*AGATGTAAGTTGCAGGCACT*	(GA)15	252	S
A08-D07	AB108173	*TATGGAATCACTTCGCTTCT*	*GGGAAAGTAGGCAACCAT*	(TG)45	250	S
A08-D10	AB108174	*ATACACATCCATTGAAACCTG*	*ACCTTTCGCTGTTCAGTTT*	(CA)15	134	W
A08-D11	AB108175	*TTGGGTTGTTATTCGTGC*	*ATACGTGAATCCATTTCAATG*	(TG)13	132	S
A08-E11	AB108176	*GCAACCCTCTGTATAACGAG*	*AATCAGCAATTGGGGATAG*	(TC)7(TG)49	196	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
A08-F02	AB108177	*TTCTACTCTGCCAGTGCAC*	*AGAGTATAGCGGAGCCTCTT*	(TG)12	101	S
A08-F08	AB108178	*ACCTCGAAACAGGGTTTC*	*GTTGTATTGGGCTTACTTGTG*	(CA)10	140	S
A08-F09	AB108179	*GCTCTTGGAGATGCTCTTAA*	*TTTCACTAGGTTCATCATTTCA*	(CA)12 (CACG)9	277	S
A08-G01	AB108180	*GTAACTGTTGCCAGAATTCC*	*TGGTACTCCATATAACTGAGCA*	(CA)10	343	S
A08-G05	AB108181	*ACTCCAATCGAACAAAACAT*	*ATGGCTACTCTGGTTCTGAA*	(TC)17	107	W
A08-G08	AB108182	*TCCGGGGAGTTAGATTTC*	*TAACGGACTACTCAGATGATCA*	(TG)16	239	S
A08-G10	AB108183	*GTTCTCATGCACAGCTCC*	*TTGCAATATTTGCTCCTTG*	(CA)13	388	W
A08-H05	AB108184	*GGTTGGATATGTTGGGAAC*	*CTAGTTCAGGGCGTCATG*	(CA)8	140	S
A08-H11	AB108185	*AACAGAAAGAGAGGGAAAAGA*	*TGTAGTCACATTCCGTCAGA*	(GA)40	174	S
A09-A05	AB108186	*GGATGATGGTGGAGTTTAAA*	*CATTGCATCAAGCCAATATA*	(CA)34	139	S
A09-A06	AB108187	*TATCCATTTGTGAAGGAAGG*	*AAATGATCACTTCTCCCATG*	(TG)25 (GA)14	175	W
A09-A12	AB108188	*CTTGGTGCTAGTATATAACGCA*	*ATAAGTCTATGACGGTGAGCA*	(CA)12	397	S
A09-B01	AB108189	*GTAAGCATGTGATTCCGC*	*ACAGGCAGGAAAATGGATAG*	(TC)26	128	S
A09-B09	AB108190	*GGGAGATCTCTCACTTATAGGA*	*AGTGGAATCAAATCACGAAC*	(TG)22	106	S
A09-C07	AB108191	*ACCGTATGACAGGCTTGTT*	*TCTTCGTACCTTCGACTCAT*	(TG)41	147	S
A09-C08	AB108192	*ACTATGGCTATGGGTGTGAC*	*TGCAACTAGCTTTCTCTCATC*	(TG)15	231	S
A09-D02	AB108193	*AAGTTGGCTATGATGCACTT*	*GCTTTCGATAGTTGCATAAGA*	(TG)16	264	S
A09-D03	AB108194	*TTTGCAAGCAAGCAAGTATA*	*TGGAGATGGTATGGAGTATTG*	(CA)9	378	S
A09-D10	AB108195	*GTAACCGTGGCATTCATACT*	*AGAGATGGCGGCTGATAT*	(TG)37	148	S
A09-E07	AB108196	*ATCCGTACTACATTGTCCTAGC*	*GAACATAAAACGGCATGC*	(CA)18	139	S
A09-E09	AB108197	*CACTGCTTGGATCGATTC*	*TCCATCAACTCCTATATGTGG*	(CA)12	129	W
A09-E12	AB108198	*GCTTAAATTTTCGCGGAG*	*CTAATATGACAATCCGTGCTC*	(TGCG)15 (TG)30	180	W
A09-F03	AB108199	*GAAAACCAAGTAGCAAGTGC*	*ATGCTGAAGAAATGGAGCT*	(CA)12 (GA)15	324	S
A09-F05	AB108200	*ACCCATGGTTTTGTAAGTAGTC*	*GTTTTCTCCCTCGACTTTG*	(CA)66	308	S
A09-G01	AB108201	*TACAGCCAGGTTCGAATC*	*GCAACAAAATTATGAAAGAAAA*	(CA)5	114	S
A09-H02	AB108202	*TGTTAGGATACCACTGTGCA*	*GTCGCTTCCTCTATTGAGG*	(CA)9	248	S
A09-H05	AB108203	*GCAACGAGGTTACAATGC*	*AAGCCAATTACAGTGAGAACA*	(TC)16 (CA)17 (CG)5... (CA)7	244	S
A09-H08	AB108204	*GCAAGGTTAATTGCCACTAC*	*TAATGCTGCACCAAAGGT*	(TG)16	255	S
A10-A03	AB108205	*AGGAGACATGCATCCATG*	*CCTAGTGTAGCTTCGCAGAT*	(CA)21 (CGCA)9 (CA)17	264	S
A10-A07	AB108206	*TGGATGTTTGGATGTTGG*	*GTGCGGGTGAGATACATAAT*	(TG)22 (GA)10	170	S
A10-A08	AB108207	*CTACAAGAGCCATCATTCG*	*TTGGAGTTGCAGAACCTATT*	(TG)31	146	S
A10-A10	AB108208	*AGTACTGCCCCAAGGTTC*	*GTTGCCCCAGTTTATTCC*	(CA)31	232	S
A10-B01	AB108209	*CGGCTACACTGCATATGAC*	*GCCAGAAGGTACATCACCT*	(CA)12	216	S
A10-B02	AB108210	*CACAAGCAATCTGACACAAC*	*CAGGTCCAAACTGATAAGATG*	(CA)15	123	S
A10-C01	AB108211	*TTCTGAGATGGCTGTGGT*	*AGAGACTCCGGCAGAACT*	(TTG)7 (TGC)7 (TTG)22	249	S
A10-C02	AB108212	*AATAGATCTTGACTTTTGGGG*	*AATCGTGAGAATCGAGACC*	(CA)13	361	S
A10-C07	AB108213	*AGCTCCATCACTAGACAGTGA*	*ATCATCCCCTCACCACTT*	(TG)13 (GGA)5	172	S
A10-D01	AB108214	*GTTCTTGCTTGGGAATACAG*	*GCATATCACAGACGAACTAGG*	(TG)12	146	S
A10-D04	AB108215	*TGGCTAGCTAGGTGTTGTG*	*GCGGTATCATGGAGTATCAT*	(TG)9	129	S
A10-E07	AB108216	*TTAAGCAGAGTGCCGAAC*	*TCGTTCGCCACTACTCAT*	(TG)56	392	W
A10-E08	AB108217	*AGAATCGAGCGTAGTGTCAT*	*TGTTGTATTCCTTTCTCACTGA*	(CA)12	158	S
A10-E10	AB108218	*GCAGCTTGTGAGCTGATT*	*GTAAATTTGCTCACCATCTTG*	(CA)10	114	W
A10-F06	AB108219	*AAGGGATGAAAGATGCATC*	*GTGGAGATGTAAGCGAAGAC*	(TG)25 (GA)14	294	S
A10-F08	AB108220	*GCAATACATTCAGTTGAGAGG*	*AATTTTCGCCGAGCTATC*	(CA)12	117	S
A10-G08	AB108221	*TAGTCAGATCAACAAATTTGGA*	*ACTATGCCATCTCCTGTCC*	(TG)12	168	S
A10-G11	AB108222	*ATAAGCCACAGTAAACCGAA*	*TTTGGTGGTAGATCTATATGCA*	A10(CA)10	236	S
A10-H01	AB108223	*ACTCCATGGCTGGTAGATC*	*AACTCCGATATTCCCTGC*	(GA)17	260	S
A10-H03	AB108224	*TGCGTGTTTATGTTCCCT*	*GTCATTCATCAATCCAAGAGA*	(TG)9	101	S
A10-H08	AB108225	*CAGAATGTATTGAGTCTGAACG*	*TGTTACATTCCCTGTGTGG*	(CA)11	110	S
A11-B12	AB108226	*GTGTGGGACGTACGATTAGT*	*TATGACCCCATATATAAGAGCC*	(CG)7 (TG)14	114	S
A11-C07	AB108227	*CCTCTGTGAGCTCCCTCT*	*AGAAATTCCTTCCATCGC*	(CA)27	133	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
A11-E01	AB108228	*TTATTGCCTTTGCTAATCGT*	*TTGGTTCCTTGTCCATATTC*	(TC)26	145	S
A11-E06	AB108229	*CGCATTTCGTTCTGATTC*	*GGTCATCATCAAACACATGA*	(TG)14	249	S
A11-F06	AB108230	*TAAGGAGTTTCCAACTTCCA*	*GACGAGCTAGCTGAGTGTCT*	(TG)16	197	S
A11-F07	AB108231	*GTGTATTCAAGCATGTGACG*	*CGATGACCTAGTGTAGCCTC*	(TG)9	252	S
A11-G09	AB108232	*GGTATTCATCAATCCCACC*	*TAGCTTAAGCCTGTTCTTAACC*	(TG)12	258	S
A11-G11	AB108233	*CGAGATGGTTAGTGTGTAAGG*	*ATTTAGATTCTCGTTGGAACC*	(TG)15 (GA)11	216	S
A11-H05	AB108234	*GGTGGTTGATAAGAATGCAT*	*CTCCAGATGGTTATATTGCTG*	(TG)15	245	S
A11-H06	AB108235	*TCATGTTGGACAAGAATTCA*	*GCAAAGTCCCTTGTGAATAG*	(CA)18	120	W
A11-H10	AB108236	*AATCCGTATGAATATGTTAGGG*	*GCAATCTTGTAATTCAGTTTGA*	(CA)13	126	S
A12-A04	AB108237	*ACTTGGAATCCAAGGTGAG*	*AACTGGAGTCAGGTTGGAG*	(CA)17	227	S
A12-A07	AB108238	*GGATCAAGCTATCTAGTTTTGG*	*GCTGGACACACCAGCTAC*	(TG)8	111	S
A12-A11	AB108239	*CTCTGTAGCTACAATTGCTACG*	*CGCAGTCAATAATGCCTC*	(TG)10	124	S
A12-B01	AB108240	*TACTAGTACGTGACCAGCCC*	*TGCAGCCTTACTTGTTGTC*	(TG)8	117	W
A12-C04	AB108241	*CCACCTCCTAGACCTGCT*	*AGAGGCAGGAGAGTGAGG*	(CA)9	111	W
A12-C08	AB108242	*TCACAAGGCACAGATTGAT*	*CCATCAAGTTGGTTTTGATT*	(TG)11	123	S
A12-D01	AB108243	*CTGTGAGGTGGGTGTGTT*	*GAATTAACGAAGCCATCAAC*	(TG)7	128	W
A12-D04	AB108244	*GGACCAATAGCAGGTGAGT*	*CTCCATCATCTCCTCCCT*	(GA)30	150	S
A12-E10	AB108245	*GCTCTGATACCATGTTGGTT*	*AGAGGAGAAGAGAAATCGAGA*	(CA)10	128	S
A12-F04	AB108246	*AGAAGCTGGTCAAGCACTAG*	*AATAAAGTGGAACAGAGACCC*	(TG)41	142	S
A12-G04	AB108247	*CGACAAATCATAACAAAGAGG*	*GTCGTCTCGATCCTAAACAG*	(CA)12	150	S
A12-H09	AB108248	*TCTATCCAGCGTGCTACTG*	*GCCACTTCCCTACTTGAATT*	(TG)31 imperfect	143	S
A14-C02	AB108249	*TTTGCGATGATTATTTCCTT*	*TTCGTAAGCAAGCCAATC*	(TG)16	155	S
A14-C07	AB108250	*ATGTGCATCACGAAAATACA*	*GCTGTTCCATTATTCTGACTG*	(CA)19	132	S
A14-D12	AB108251	*GTACATTATGTGCTCCTTTGC*	*AATATGCCCCATAATTAGAACA*	(TG)14	102	S
A14-E09	AB108252	*TCCACACGAATCAAATGTAA*	*GCCTATAAAAGTCCTTCATTTG*	(CA)14	184	W
A14-F05	AB108253	*ACAGCTAGCTAGCTTGGTCA*	*TTGCTGCTGTTAGGAAGC*	(CA)16 (CGCA)11 (CA)44	242	S
A14-F10	AB108254	*CTTTTGATTTCAGTTGCACA*	*TGCATGTAGTCCACATTGTT*	(CA)12	125	S
A14-G05	AB108255	*CTCAGCATGCATACTCTCAA*	*CCACATTAGGCTGACAATG*	(CA)17	100	S
A14-G09	AB108256	*ATCCTAAGTGTCCAGAAAACC*	*GGGAACCACTATCTGTCAGA*	(CA)13 (GA)16	173	S
A14-H06	AB108257	*AGAGAGAGCAAACAACACTTG*	*GTACTCTGTGGTGAAACGGT*	(CA)12	113	W
A15-A06	AB108258	*CGGTGTAGACGACGACTC*	*GTACGAGCTAGGACCCATG*	(GA)17	277	W
A15-A11	AB108259	*TCTCTGCTCCTGGTTCTTT*	*TTCACTGGCGATAGAGATTT*	(TG)22	214	S
A15-C09	AB108260	*CTTGTCCTCCTTCGCTCA*	*CTAGGTCAGGTCTTGCTCC*	(TC)7 (CA)15	182	S
A15-D09	AB108261	*GCCTGCATGATTAGAGTAGG*	*GGAGTAGGACAAGTATGGACC*	(TG)9	116	S
A15-D10	AB108262	*CATGGTAAGCGCTCTGTC*	*CATCCTGCTACAGATGGC*	(TG)27 (TGCG)9 (TG)6	366	S
A15-D11	AB108263	*CTTACGAGTAATAAAACGCACA*	*TCCACCATACTACAAGAAATGA*	(TG)18 imperfect	251	S
A15-E10	AB108264	*GAGCTTCATATGTTCTTGGTG*	*CTCCTTCCAATAAGGTTGTG*	(TG)44	146	S
A15-F01	AB108265	*CGTCTATCAGCAGACAATCTC*	*TTACCCACAGATGATTTACAGA*	(TG)10	146	S
A15-H07	AB108266	*CCAAGAAGGTGAAACTTGAG*	*CTCTGGTGAGCATGAAATG*	(CA)8	261	S
A16-A08	AB108267	*CTACAAAGGCAGTAGGATGG*	*TGACAGAATGACCAACTTGA*	(TG)13	202	W
A16-B07	AB108268	*TATTCCCCTGGAAGAACAC*	*GTGCCTTAGAGCTGGAATC*	(TG)11	180	S
A16-C03	AB108269	*CTCTCTCCTCCAACTCAGC*	*TATGGTGCTTGCTAGTGGAG*	(CA)17	162	S
A16-C07	AB108270	*GTGGACTCATGAGGTTTATCA*	*TAAACCTCGCTTGTCAGC*	(CA)17	245	S
A16-D04	AB108271	*TTATTTCCCTTCCAATAGCA*	*GGAAGTTAGCTATCCACGTG*	(CAA)45	266	S
A16-D05	AB108272	*GTTCAGACTCGTCTCCTTTG*	*TCAACCAACGTAGGTAGCTT*	(TG)9	141	S
A16-F01	AB108273	*TTCTAGGCTTTTCCCTCTCT*	*GAGTGCATACGCTCATCC*	(TC)10 (TG)12	150	S
A16-F08	AB108274	*GTATCGGGAAGTAAGTCGTG*	*GCGTGTGTACATTATGCAAC*	(TG)8	148	S
A16-G07	AB108275	*CTGCACCCTTATACACCG*	*AATTATGATTGAGTGGGTGTG*	(TG)15	110	S
A16-G10	AB108276	*CTTTATTATGTGATAGCAGGCA*	*GACTTGTAATTTGAGACCCG*	(CA)35	250	S
A16-H06	AB108277	*GTGAATCGGCATGACATG*	*TCCAAGTCACACAGACAAGA*	(TG)5	173	S
A16-H11	AB108278	*ATCTGGATTCTGGGGTGT*	*TTTCTCTCTTGTAAGCTGGC*	(GA)10... (GA)10... (GA)19	185	S
A17-G01	AB108279	*GTATTTGCCAACGAGGAAT*	*TCGGTATAAGGATGTTGAGG*	(TG)16	132	S
A-T04	AB108280	*ATATACTCTCGTCGAAAACCC*	*TCATTCGACCACCTACCTAC*	(CA)25	133	S
A-T22	AB108281	*GCAATTAGCTACTACCATGGCA*	*CAATCGGATTTATGGCTTTAGA*	(TC)19	147	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
A-T26	AB108282	*CTTCCTAAGCCACAGTGAAC*	*GCATTTTCTTCTTTTCTCTCC*	(CA)12	262	S
B01-A03	AB108283	*CATGGGATCTCGACGAT*	*GAGGTCGAGCTCCTCG*	(TC)23	130	W
B01-A05	AB108284	*AGAAGATGTGTTCCTGTGC*	*TGGCTCTTTCAATCATCTCT*	(GA)36	148	W
B01-A07	AB108285	*CGAGTCTCATTTTCATGAATC*	*GTACATGTGCCCTTCCTTT*	(TC)13	136	S
B01-A09	AB108286	*CGAACGTCAAACCAAGTC*	*GACAAACTTTTGACTGGGAA*	(TC)15	211	S
B01-A11	AB108287	*CATCGGTGAGAGACTATGGT*	*GCTTCTTGATGGCATATTTC*	(GA)16	235	W
B01-A12	AB108288	*AAATCACATGGGAGTGGTT*	*CTGGAGAGTGACTCTTGTAAAA*	(TC)22	129	S
B01-B01	AB108289	*TCATCCAGGACGCTAATTAC*	*CTCATATGTTTACCAACAATCG*	(TC)23	141	W
B01-B02	AB108290	*GGTCCCTAATTAAGCATGC*	*AGACGACAGCATTTCAACTT*	(TC)33	149	S
B01-B04	AB108291	*GTTTGCTTGGGTGAATATGT*	*AGCACAGCTTCTTCTAGGTG*	(GA)21	122	S
B01-C06	AB108292	*GTTGCAGTTGCTGCACTG*	*GTAGCCGCCGTATACTGTAC*	(TG)6 (GA)15	109	S
B01-C10	AB108293	*ACGCAAGGTGTTGCTTATAT*	*CAAACTTGTGCTTGTTCAAA*	(GA)20	129	W
B01-D03	AB108294	*TCACATGGGACAAAATCAC*	*TAGCGTGCAAATTAGAGAATC*	(GA)16	286	W
B01-D09	AB108295	*GCGATATTGCACCAACTC*	*ATGAACTGAACTCGTTTGCT*	(GA)15	299	S
B01-E09	AB108296	*ATATGAAGTTGGGTGGCAT*	*ACGACACCCAGATTAAACAC*	(TC)28	128	S
B01-F05	AB108297	*ACAGATGAGTTTCGGTATGG*	*ATTTGTTCTTCTCAGAACTCCT*	(TC)30	175	W
B01-F07	AB108298	*ATAAAACCGGCTGTAGTTGA*	*ACTCTAGAGCACCACCGTC*	(GA)15	241	S
B01-F10	AB108299	*TTTCATTTCCGTGCGTAT*	*GGAAGCAGTGTCGAGATG*	(GA)16	139	S
B01-G02	AB108300	*CTGCACAGTTACAAACACTCA*	*AGACATGACAATGTCCCTTC*	(GA)24	148	S
B01-G10	AB108301	*CCAGTACCAAATATGGAGGA*	*CAGAGACAGTGGCTTACCTC*	(TC)20	200	S
B01-G12	AB108302	*TTTGGACCTCTCTCTCCC*	*CTCATGCAAGATATGGCAC*	(TC)18	117	S
B01-H04	AB108303	*CCAGTCCTGTCCTTGAGAT*	*AAGGTAGTGTGCTGTATGATGA*	(TTC)6 (TC)17	249	S
B02-A05	AB108304	*TCCTCCTATATAAGGAGGTGC*	*GAAGCACCGGTAACACAG*	(TC)24	191	S
B02-A07	AB108305	*AATGTAAGCTGACTCTCTCCC*	*TATTCACCTTCCCACTGTTC*	(TC)17	230	S
B02-A08	AB108306	*TAGGATTATATATGCGGACCA*	*TTTCCCTTGCGAGTTACC*	(TC)11	114	S
B02-A09	AB108307	*ACCAAATAGGATTTCTCAAGG*	*TCATACCGAGGAATTTACTCA*	(TC)14	226	S
B02-A11	AB108308	*GTCAATCCAGGATGCAGAG*	*AAGTTAGAGTGCAAGTGGGA*	(GA)15	165	W
B02-B01	AB108309	*CACTATAGCTTCCTCACGCT*	*GAACTCTAGCCGCCATAAC*	(GA)24	210	S
B02-B04	AB108310	*TTTGACTGTAACTACGGCG*	*TTTCTCCCGTGTCTCTCC*	(GA)12	144	S
B02-B12	AB108311	*GTCCGACAGGCAGCTC*	*TTGAGCCTAACGATCTTGAT*	(GA)15	198	W
B02-C02	AB108312	*TCCCTATTGCGTGTTGTC*	*CGATGCATACGAAGAAGG*	(GA)15	150	W
B02-C07	AB108313	*TCCATCCCACAAAGTGATT*	*TATGTGACGTTGGTGAGAGA*	(TC)16	218	S
B02-C12	AB108314	*CGCCAGTACCAAATATGG*	*GTGCCACCATATCTCTTGAT*	(TC)22	163	S
B02-D11	AB108315	*TTTCCTAAGTTTCTAGCCAATC*	*ACCCATCCCTGACTGTTC*	(TG)19 (GA)20	144	S
B02-E04	AB108316	*CTCTCTATTCCTCTGTGTTGTG*	*CTGCGCACCAATTACATC*	(GA)11	101	W
B02-E10	AB108317	*GCGATGGTAGGATATGGATA*	*CACAGTAAAGGTGGTTCCAT*	(GA)15	259	S
B02-E11	AB108318	*AGAGAAGTACAGGCAAGCAG*	*ATTACCCAGCTTCCTTGG*	(GA)25	101	S
B02-E12	AB108319	*CGGACTGGTATCCTACCTC*	*GGCAATATCACCATTGAAGT*	(TC)16	146	S
B02-F05	AB108320	*CTCAGACGTACTACAGAAAGCA*	*TCCTATAAAATCCATTTGTTCC*	(GA)12	232	S
B02-F09	AB108321	*TCGAGCTGTCCGCCAT*	*GCATGCCGGAGAGCAC*	(GA)22	133	W
B02-F11	AB108322	*GCCCTGTGTATTTTGTGC*	*CAAATGTCCATACAGCTAGATG*	(TC)23	145	S
B02-G06	AB108323	*CACCAGATCAGGTGTGTGT*	*AGCAGCAAGACCATAGGATA*	(GA)20	236	W
B02-G11	AB108324	*CGATGCATAGGTAGTACTCTCA*	*CGAAGTGACCTTTAGAATCG*	(TC)25	137	S
B02-H04	AB108325	*GAACCTATTGATCACACCTCA*	*ATGCACACGGTAGGACAC*	(GA)19	109	S
B02-H06	AB108326	*AGGGAGCAGAGAGAGTGC*	*AAGGAAACCTGTTCGACAG*	(GA)11	142	S
B03-A01	AB108327	*GCTCATGCCAAAACAAAC*	*GTGGTCGCACAGAGATTC*	(TC)19	234	S
B03-A06	AB108328	*CTGGTGGAGCTACAGTGG*	*ATTATCTCCTTCCAAGCTCC*	(GA)24	140	W
B03-A09	AB108329	*AACTAGGTGAACCGTTTGG*	*CCCGTTATGTCCTTGTATGT*	(GA)19	226	S
B03-B04	AB108330	*TGGCAACTTGGCATAAAT*	*CTGCCCAGAGTCAGTCAG*	(GA)14	283	S
B03-B10	AB108331	*AGGTTTCATTCATGATTGATG*	*CAGACACCCAATAGATCGAT*	(GA)15	310	S
B03-C05	AB108332	*ACGATCTGACCATATGATCTG*	*CAAAGGTTATGTACTCGATGC*	(TC)15	186	S
B03-D03	AB108333	*GTCTACAGATCTTTTGCATGG*	*TTTTCAGGAACTCGTCAAGT*	(TC)17	174	S
B03-D04	AB108334	*CACCACAACTAGCCACATC*	*AATAGAGCAGTGGTTGTCAGA*	(TC)19	113	S
B03-D06	AB108335	*GTAGCACACAACGGCTTC*	*ATCACCAGTAGAGCACATCC*	(TC)26	142	W
B03-D08	AB108336	*CTGAACAATTGGTCTGGAAT*	*AGGATTTGCTCAAGGACC*	(GA)27	332	S
B03-D09	AB108337	*AGAGATGTGCATAGTGTGAAGA*	*CGTGATCATCCCCACTG*	(GA)27	106	W
B03-D11	AB108338	*GGGGATAACCAACCAAAC*	*AAGAGGCAGTAGTACCCTTTG*	(TC)10	116	W
B03-E01	AB108339	*GTGTCGTCTCCAACAGATCT*	*TATCCACGCGTACATCCT*	(TC)14	143	S
B03-E03	AB108340	*TATATATAGATGGGTTCCCTCC*	*TTATGGTGGAGACAACCTTC*	(TC)22	149	W
B03-E06	AB108341	*ATCCCATGCGCTATCC*	*GGAGTCGAGCTCCTCG*	(TC)13	223	S
B03-E08	AB108342	*CATGCATCGGTAGTCAGTC*	*CTTCAAACCACGTAACGG*	(TC)22	204	S
B03-E10	AB108343	*TAAGGGTTGAATTGACTTTGA*	*TTGGGGTAGCCATCTCTAC*	(GA)19	139	S
B03-E12	AB108344	*CGAGGTGGCTTAGTTGTG*	*TCTCCAGCTCTCATGCTC*	(GA)14	137	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
B03-F02	AB108345	*AACAAAGCAGAGGCTGAAG*	*GTAGCATGAGGATGAAGAGC*	(TC)19	312	S
B03-F07	AB108346	*GGGAGAAATCATCTTGCTC*	*GTCAATGCGGTAATTAAGCT*	(TC)14	130	S
B-T09	AB108347	*CGCAAACTTATCTAGGTCTGA*	*AACAAGGATGTCACTTCAGG*	(TC)24	135	W
B-T14	AB108348	*AACTTGTTAAGACTCTAAGCGC*	*CATTTTGCTTGACAACCAC*	(TC)7	210	S
B-T21	AB108349	*AACAATTACCTAGCGCAATC*	*TCACATGCTCCTTTATTTTTT*	(TC)11 (TACA)11	247	S
B-T23	AB108350	*CTCTAGCTAGCATCTAGCACG*	*TCAATTTAAACGTACTACACGC*	(GA)23	318	S
B-T25	AB108351	*AATAGGGAACAGGTTGCC*	*CTTTTGGATCTGGCAATG*	(GA)11	318	S
C01-B08	AB108352	*TTTGAGAGCGATCATGGT*	*GCTACGTCCGTGTTAATTTC*	(TTC)13	280	W
C01-B09	AB108353	*AATACAGTTCGATCGGACC*	*CTATCTGGAGTCTGGACTCG*	(AAG)16	221	S
C01-B11	AB108354	*GCTTCCCCATCTCTCACT*	*TCAGCTAATACCCGCAAC*	(TTC)16	194	S
C01-D01	AB108355	*GTTGGTTGCATTGTTTCAG*	*GGAGAGAAGTGCATGTGC*	(TTC)17	220	S
C01-E10	AB108356	*CGATGTGATGTTTGAGCTC*	*ACGTTCCAAAGCGGAC*	(TTC)13	127	S
C01-E11	AB108357	*TCGTCGTGGTGCTTCTAT*	*AGAGCAGAACCCAGTAGACA*	(TTC)11	123	S
C01-F04	AB108358	*GCCTCCAATGATACTGCTAC*	*ACATTCGTCTCTGCTGAAGT*	(AAG)12	157	S
C01-F09	AB108359	*CATGTTTTGTGCTCTCTTGAAA*	*TTCACTAACACATGGATCCTC*	(TTC)8	106	S
C01-G04	AB108360	*TCAGACGTTGTTGGTCAGTA*	*GACAATCCCTCCTGATATGA*	(AAG)11	118	S
C01-G06	AB108361	*TGTCATGTGATCCAGCTTAA*	*AGTTTAATTGAAGGAAATGCC*	(TC)14 (TTC)9	147	W
C01-G09	AB108362	*GTCAAAATCTTGAAACCGA*	*CCGCGATATTCAACTGTC*	(AAG)13	223	S
C01-G10	AB108363	*TCAACTCACCAACTTTCACA*	*GTCGGTCATAGCCTCAATAG*	(AAG)11	103	S
C01-H04	AB108364	*GAGAGGGCACTATTAAGCAA*	*CGTTGCAGTATTATCGACAA*	(AAG)22	136	S
C01-H07	AB108365	*GAATAGATGTTTAGTTCGCCA*	*GTGAAGGATCTCGAGGATAA*	(TTC)17	143	S
C01-H08	AB108366	*AAGTAGGGGATGGCTTAT*	*TAGATGTACCTCCGTCCAAC*	(TTC)15	190	S
C01-H09	AB108367	*ACCCCAGACCTAAACAGC*	*AAAGTTCCTCTCCATGGTG*	(TTC)14	318	S
C02-B06	AB108368	*TGAATGCTGTTGAAACTACG*	*GTACCTGACATTCCGGCA*	(TTC)12	142	S
C02-C02	AB108369	*GGTCAGAAGAGCTCATAAACTC*	*CACAAGGATGAGCTGAGG*	(TTC)9	306	W
C02-C03	AB108370	*GGATGGCTTATGTGATGG*	*CTCCTGGATGGTCTGCTC*	(TTC)16	150	S
C02-C06	AB108371	*AGGGTGGAGGAAGTCAAG*	*CACATGATTGCGCAGTAG*	(GAA/GGA)36 imperfect	252	S
C02-C08	AB108372	*TACAAACTCTGCCCACGT*	*ACTCAGAACAGGTATGGGC*	(AAG)13	241	S
C02-D09	AB108373	*GCGTGCTAGCTCTGTAAATT*	*AATACCAGTCCTGCATTAACA*	(AAG)22	249	W
C02-E06	AB108374	*AGCACCTCCAGTTTCACTTA*	*AAACTAACCTTCCTGGGAAG*	(TTC)10	297	W
C02-E08	AB108375	*ATCTATAGTAGGGCTGGATGG*	*CGTAAGTTGCTCAGTAGGCT*	(TTC)13	400	W
C02-F10	AB108376	*TTGGTTACAGTACCAGACTTGA*	*AGCTAGAGAGAGGAACTGAGC*	(AAG)11	222	W
C02-G06	AB108377	*TCGAGAGAATCTTGGGTTTA*	*CTTCCTCTCCTCCTCGTC*	(TGA)10... (GGA)9... (AAG)19	399	W
C02-H01	AB108378	*CACTGGTGATCTTACACGC*	*AGATCATCCACGAATTGATC*	(TTC)17	146	S
C02-H05	AB108379	*GGCAAAGCCTAACTCTCC*	*GAGAGCCATGCAGTAAAATC*	(TTC)13	278	S
C02-H09	AB108380	*TCTGTCTCCAATCTTGCTCT*	*AAAGGTCACTCGCAGAATAA*	(TTC)24	301	S
C03-A05	AB108381	*GGACATCTGAGGAGTCTGAA*	*CTTTTCTTCGTGTTTGCTTT*	(AAG)13	146	S
C03-A07	AB108382	*CGGTCACTATCATACACGTG*	*AAATACCCTACATCCCCG*	(AAG)17	203	S
C03-A09	AB108383	*ATTAGGGTTGCACACAACTC*	*GGTTTGCAGTTTCATAATTTG*	(AAG)14	134	S
C03-B10	AB108384	*CTCCGTGCATGTATGATATG*	*TCAGCATCATTGTCGTCTT*	(AAG)22	121	S
C03-C10	AB108385	*AGAAAACTAACCTTCCTGGG*	*TTTGCTTCTTGTACCTATTGC*	(AAG)11	128	S
C-T07	AB108386	*ACGTCCCTACCTAATACGATC*	*ATGTGGATGGACAGAGGTAC*	(TTC)15	180	S
D01-A04	AB108387	*CTCTACCATCATCGATCGTT*	*CACAATCTATATTTTCTCGAAATG*	(TTA)8	138	S
D01-A11	AB108388	*CAACAGCATGAAGCGATATA*	*TTTCGAGTCACCAGAAAGTT*	(TGA)13	105	S
D01-B09	AB108389	*CTAGTGCCGTTGTAGTCACA*	*GCGACAACAGAAAGAGAGAG*	(TC)17 (TTC)7	149	S
D01-D02	AB108390	*TGATACAAAATGCCAGATGA*	*ACGGAAACTAGGAGAGGATC*	(TTA)19	211	W
D01-D07	AB108391	*GCTCACTTGAATCAAAGAGG*	*ATAGCAACATATCCACCACC*	(TGC)4 (TC)17	137	W
D01-E02	AB108392	*AACCGCAGTTCAGAAAGTT*	*ACTAAACGCAAAATGGTGAT*	(TA)10... (TTA)15	147	S
D01-E04	AB108393	*TCTGTTGCCTATTCTGCTG*	*GCATTTCACTAAGACTGTGACA*	(CAA)8 (TAA)10	158	S
D01-F06	AB108394	*GTTCCTCTTGTACCTTGCTG*	*ATACACTTTATGTTGGCACTCA*	(TCA)16	177	S
D01-G10	AB108395	*ATAGATTGGGTGTTGGAGC*	*ATTCCATGTTAGCATGTCATC*	(TGA)7 (CGA)4... (TGA)16	231	S
D01-H01	AB108396	*CTCTTGCAAGATACCACACTT*	*ATCCACTGTGAACATGGTG*	(TGA)22	134	W
D01-H08	AB108397	*GCTTTAAGGATTGGCTCAC*	*CAACTTTCAGACTCAAACCTG*	(AAT)13	147	S
D01-H09	AB108398	*CCAACGTTTCAAGAAGGTT*	*CAACCTCAAATACTTGCATTC*	(TAA)10	164	S

Table. 5 (continued)

SSR Marker	DDBJ Accession No.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Repeat motif[a]	Expected size[a]	Amplification[b]
D02-A08	AB108399	*ATTTCGACACTTTGTGATCC*	*CTGATTGGTTTGAGTAAGTGTG*	(AAT)10	238	W
D02-A11	AB108400	*CCAGTTGTCAGTGGGTTATT*	*TTAAAGATGGGGAAACGAC*	(TTA)11	103	S
D02-B04	AB108401	*TGGTGTCTCTATTTTAAATCCC*	*TTTTAGGACATTGGTAGCGT*	(TAA)13	172	S
D02-C06	AB108402	*TACTTTGGTGGAACACACAA*	*GATCCCTGCGAATCTATCTA*	(TTA)19	145	S
D10-F04	AB108403	*TCAAATCCATTGGGAATG*	*TGGCATGATTTTCATCAATA*	(TGA)12	220	S
D10-H06	AB108404	*CCTATTTTCAACCCCAATG*	*ACATGGTGAATCATTTGACTC*	(TGA)21 imperfect	141	S
D11-C05	AB108405	*TTGATCGCAGTCGGTATT*	*AGCACGTCTCTAATGAGAATG*	(TGA)21	324	S
D11-E03	AB108406	*CGAGTCATGGGTGTCTTG*	*TCCATAAAATGTAGAGTATGCC*	(TAA)10... (TAA) 11 imperfect	273	S
D11-E07	AB108407	*AACAACGGTAGGCATGC*	*TGGTCGTGGTGGACAC*	(TCA)19	224	S
D11-F03	AB108408	*ACTCAGGTAACAACATGGAGA*	*TCCTGCCACACTGTTTTT*	(GCA)27	114	W
D11-G02	AB108409	*TATGTTATGCAGCAAACAGC*	*AGGCAATTAGACAGTTTCACA*	(TAA)17	177	S
D11-H12	AB108410	*AATGCTGGATAATCGTGTTC*	*CTAGCCCATGATGACCTTT*	(TAA)9	150	W
D13-C01	AB108411	*CAACTCGGTGAAGCATTC*	*CACGTATAAGGCATTGCC*	(TGA)11	137	S
D13-C02	AB108412	*GATGACATGCTAACATGGC*	*CCATTCAGTAGTCTCCCTCA*	(CGA)6 (TGA)15	146	S
D13-C07	AB108413	*GTGGCTTTGGGGTCTAGT*	*CTGTGATGGAGGAGGTACAT*	(AAT)13	210	S
D13-E10	AB108414	*CCCAATGGTAGAAGAACAAG*	*TCATCTCTGGTCTCCTCATC*	(TGA)8... (TGA)4	136	S
D13-F11	AB108415	*CAGGAACCATCACTATATCCA*	*AGCAGTGGAAGACTAGTCCA*	(TCC)6... (TCA/GCA)27 imperfect	241	S
D14-B11	AB108416	*TAACTTTAACACGGTAGCAATG*	*AACCATCCGCCAACTAGT*	(TGA)20	114	S
D14-C04	AB108417	*TATTCCTATTGTATCTGGCACA*	*CTTCATGGTGTTGCCTATG*	(AAT)9	395	S
D14-C10	AB108418	*GAGAAGGATGACGATGAAGA*	*CTAATTCTACTGACTCTTCGCC*	(TGA/TCA)16 imperfect	130	W
D-T04	AB108419	*AGGGATGCACTCCTTCTC*	*GTCGTTTGCACACTGAATC*	(TC)18	210	S
D-T21	AB108420	*TGCAGATAACCAAAGANC*	*TGTCGATCCAAACATGAAC*	(TCA)16	135	S

[a] The given repeat motif and expected size are based on the reference clone, SK
[b] S, Strong amplification, W, weak amplification
* This annealing temperature was set to 60°C

species. For example, a contig consisting of 14 clones included four alleles that each had 1, 3, 8 or 2 duplicate clones (Fig. 2). Timothy is reported to be an outbreeding hexaploid species. Our source of SSR-enriched library construction was the clone SK, and its heterogeneous state was confirmed by SSR analysis (Fig. 1).

Genomic constitution of timothy: autohexaploidy verus allohexaploidy

There are two hypothesises regarding the genomic constitution of hexaploid timothy. One assumes that the cultivated *P. pratense* is an allohexaploid containing two distinct genomes derived from *Phleum alpinum* (2n=4*x*=28) and *Phleum nodosum* (= *P. bertolonii*, 2n=2*x*=14) (Muntzing and Prakken 1940; Leven 1941; Stebbins 1950; Cai and Bullen 1991). The other assumes that timothy is an autopolyploid of *P. nodosum* (Myers 1944; Nordenskiold 1945, 1949, 1953, 1957; Wilton and Klebesadel 1973). The latter view seems to be generally accepted. On the other hand, Cai and Bullen (1994) pointed out that the degree of differentiation between *P. alpinum* and *P. bertolonii* is relatively low, judging from genome-specific sequences. This view seems to agree with the results of earlier studies by Nordenskiold (1945), who concluded on the basis of interspecific hybridization and cytological observation of hybrids that *P. bertolonii* might be partly homologous to *P. alpinum.*

In our study, timothy showed some autohexaploid properties. First, there were up to six multiple alleles in hexaploid timothy clones (Fig. 1 and Table 4). In the diploid species *P. bertolonii* and the tetraploid species *P. alpinum*, the maximum number of multiple alleles did not exceed 2 and 4, respectively (our unpublished data). Second, the segregation types of single-dose restriction fragments (SDRF, segregating at 1:1), double-dose restriction fragments (DDRF, segregating at 4:1 in auto-hexaploid species) and triple-dose restriction fragments (TDRF, segregating at 19:1 in auto-hexaploid species) (Liu et al. 1998) were detected in an F_1 pseudo-testcross population using SSR markers (Fig. 3; Cai et al., in preparation).

We have thus reported the isolation and characterization of a large number of SSR sequences and the development of 355 SSR markers for hexaploid timothy (Table 5). The detection of SSR polymorphisms in an F_1 pseudo-testcross population will enable us to construct a genetic linkage map and to detect homologous linkage groups in hexaploid timothy. These SSR markers will provide an ideal marker system to assist with gene targeting, QTL mapping, and marker-assisted selection in timothy.

Acknowledgements We thank Dr. H. Morishima (Tokyo University of Agriculture, Japan) for her critical reading of and valuable comments on this manuscript. This study was financially supported by a grant from the Japan Racing Association.

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