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Genetic analysis of quantitative trait loci for cocoon and silk production quantity in *Bombyx mori* (Lepidoptera: Bombycidae)

BING LI[1], XIUYE WANG[3], CHENGXIANG HOU[1, 2], ANYING XU[2] and MUWANG LI[1, 2]*

[1] Sericultural Research Institute, Jiangsu University of Science and Technology, Zhenjiang 212018, Jiangsu, China
[2] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, Jiangsu, China
[3] Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

Key words. Lepidoptera, Bombycidae, *Bombyx mori*, cocoon and silk production quantity, genetic linkage map, QTL mapping

Abstract. Silk production quantity is the most economically important characteristic of the domesticated silkworm moth, *Bombyx mori*. It is controlled by multiple loci. The cocoon and silk production quantity of silkworm strains Jingsong and Lan10 have significantly diverged. A backcross population (BC_1) was bred using Jingsong and Lan10 as parents to identify quantitative trait loci (QTLs) for silk quality. In this research, a genetic linkage map of the silkworm was constructed using the BC_1 mapping population. The map contained 85 sequence-tagged site markers, 80 simple sequence repeat markers, and 16 single nucleotide polymorphisms. A linkage map was constructed from the data, which consisted of 181 markers distributed over 28 expected linkage groups and spans 2147.1 cM in total length. Fourteen QTLs were detected for cocoon filament length, whole cocoon weight, pupae weight, filament weight, and cocoon shell weight. The 14 QTLs were distributed in 5 linkage groups (linkage groups 1, 14, 18, 23 and 25) based on the constructed linkage map. In addition, five QTLs, which had the highest logarithm (base 10) of odds (LOD) values, were located on the first chromosome, three of which located at the same region in linkage group 1. These results represent an important foundation for the map-based cloning of QTLs and marker-assisted selection for improving the silk quality of economically important silkworm strains.

INTRODUCTION

The silkworm moth, *Bombyx mori* L. has been domesticated over the past 5,000 years from the wild progenitor, *Bombyx mandarina* (Moore) (Xiang, 1991). The silkworm is important for genetic research as well as an economically important insect. More than 400 mutations have been identified in silkworms, and more than 1,000 silkworm strains are maintained as genetic resources (Ito et al., 2009; Meng et al., 2009). It is also a key model species of the order Lepidoptera, the second largest group of holometabolous insects, which includes many beneficial species and the most destructive agricultural pests.

Cocoon quality is very important because it influences sericulture yield and the selection of silkworm lines for silk production. Currently, modern techniques such as transgenesis and marker-assisted selection are the most effective ways of improving silk properties and they are applied widely in silk production (Lu et al., 2004). Molecular markers are powerful tools for genome analysis; they are applied comprehensively in the mapping functional genes and the molecular breeding of *B. mori* (Li et al., 2005b; Sreekumar et al., 2011). The construction of linkage maps is a fundamental aspect of gene analysis. It provides guidelines for marker-assisted selection and map-based cloning. An accurate linkage map is also necessary for further genetic analysis. In recent years, several linkage maps for *B. mori* have been constructed based on various molecular marker techniques, including restriction fragment length polymorphism (RFLP) linkage mapping (Shi et al., 1995), random amplification of polymorphic DNA (RAPD) linkage grouping (Promboon et al., 1995; Yasukochi, 1998), amplified fragment length polymorphism (AFLP) frame mapping (Tan et al., 2001; Lu et al., 2004), simple sequence repeat (SSR = microsatellite) linkage mapping (Miao et al., 2005), and single nucleotide polymorphism (SNP) (Yamamoto et al., 2006; Sreekumar et al., 2011). In many cases, maps from different parental populations and species have been integrated (Yasukochi et al., 2005; Zhan et al., 2009). However, some maps have relatively low density and could not be used effectively as guides for breeding *B. mori*.

In the present study, STS (sequence-tagged sites), SSRs, and SNPs were adopted to construct a linkage map of silkworm to map quantitative trait loci (QTLs) for silk quality because of their good transferability, high reproducibility, and co-dominant inheritance. These markers are particularly suitable for high-throughput genotyping, which allows the rapid analysis of hereditary monogenetic traits and QTLs (Maddox et al., 2001; Ihara et al., 2004; Lee et al., 2005; Huang et al., 2006). A linkage group was constructed and the QTLs were localized based on the cocoon filament length, cocoon shell weight, whole cocoon weight, pupae weight, cocoon filament weight, and cocoon shell ratio.

* Corresponding author; e-mails: muwang_li@hotmail.com; muwangli@163.com

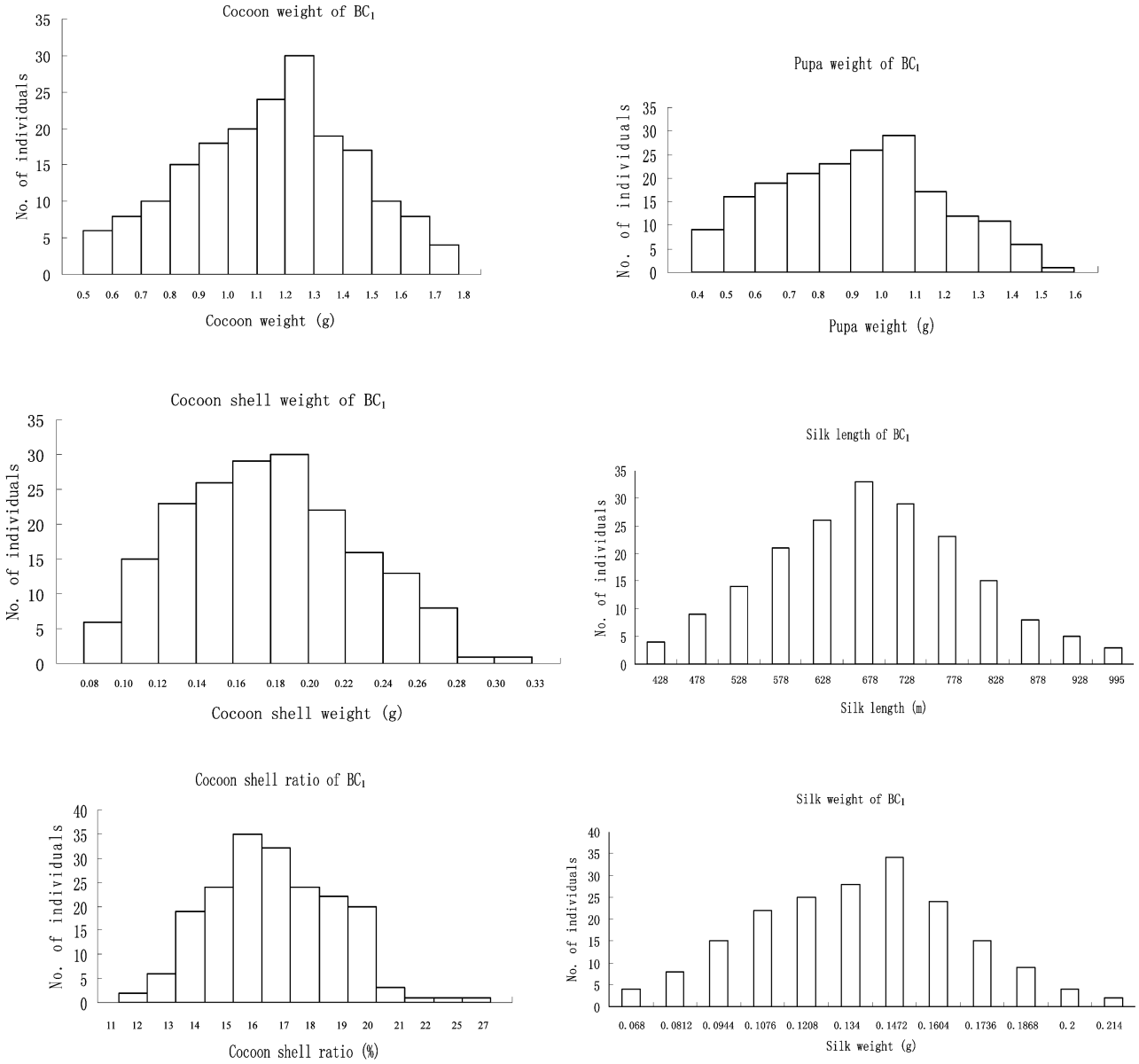

Fig. 1. The distribution of the cocoon and silk quality in male individuals in BC_1 population.

MATERIAL AND METHODS

Silkworm strains

Significant hereditary and ecological divergence occurred in the genes responsible for the silk quality of silkworm strains Jingsong (JS) and Lan10 (L10), which are preserved in the Sericultural Research Institute, Chinese Academy of Agricultural Sciences. The JS strain is used widely in various applications and its silk properties are advantageous for silk production. In contrast, L10 has high stress resistance, but produces smaller cocoons and has poor silk-producing properties. Additional crosses between strains from different origins may increase the mapping efficiency of markers because of the increased potential for genetic diversity (Zhan et al., 2009). A backcross population (BC_1) was bred from JS and L10 parents. The 93 male individuals were selected randomly from the resulting BC_1 population.

Silk quality collection

The fresh cocoons were weighed before dipping in 0.6% sodium silicate solution (Na_2SiO_3) for 60 min at 24°C. The cocoons were transferred in pure water and then the silk fibers were reeled. The length of the cocoon filament was measured during reeling. The silk fibers were washed and dried after measurement. The cocoon filament weight and pupal weight of each cocoon were also determined. The cocoon shell weight was calculated using the following formula:

Cocoon shell weight = whole cocoon weight – pupal weight – weight of castoff skin inside the cocoon.

DNA preparation and PCR amplification

DNA was extracted from the parent moths, and pupae of the F_1 progeny, and the 93 BC_1 progeny as described by Xia et al. (1998) The purity and concentration of the prepared DNA were determined with a BioPhotometer Plus (Eppendorf, Hamburg, Germany) and diluted with 1 × TE buffer (10 mM Tris-HCl, 1 mM EDTA), pH 8.0.

A total of 1120 pairs of STS/SNP primers were designed based on the silkworm genome database (The International Silkworm Genome Consortium, 2008), and 428 pairs of SSR primers were selected from the established SSR linkage groups

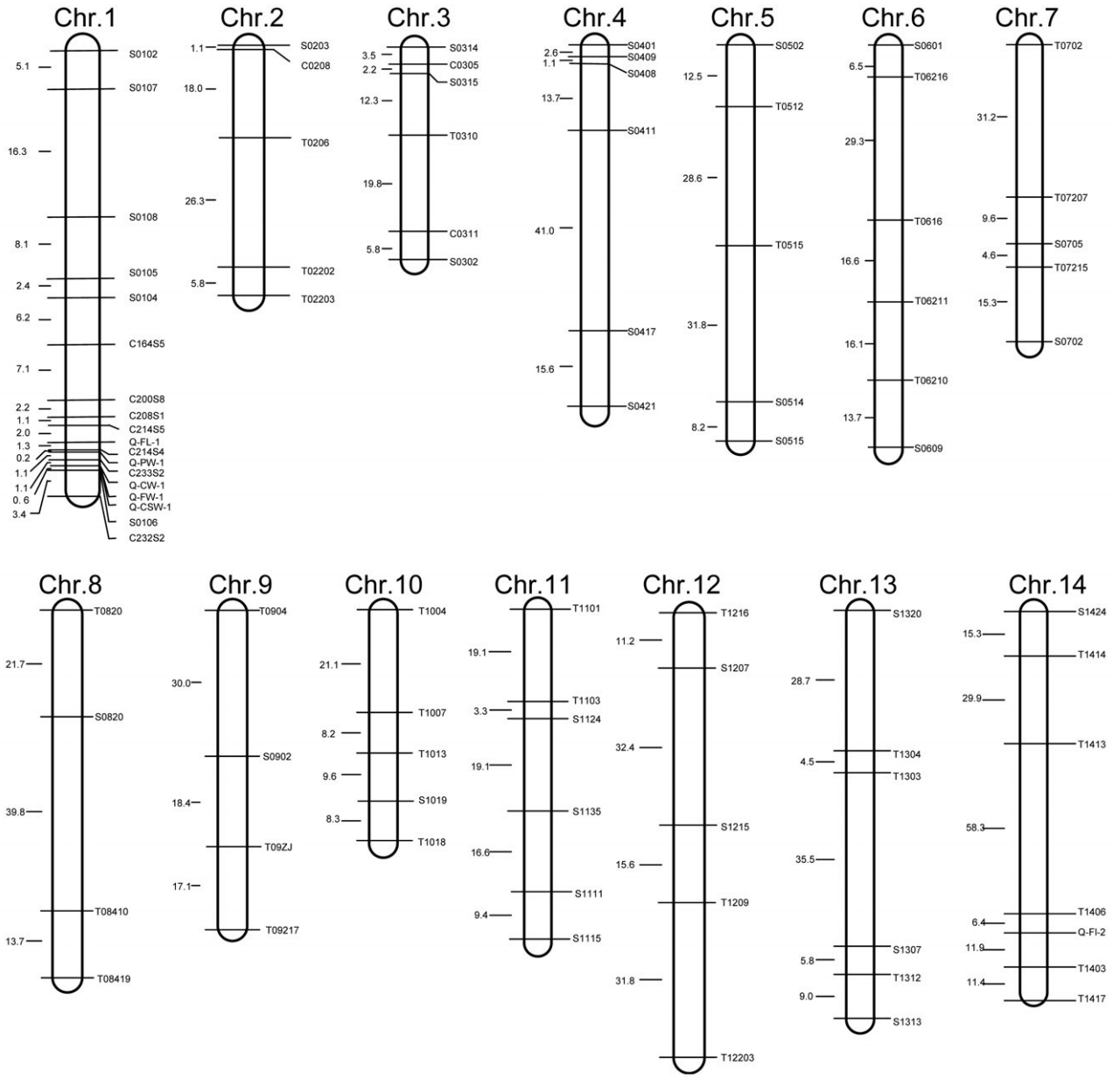

Fig. 2. Linkage map of *Bombyx mori.* Marker names were labeled at the right side of the groups, and the distance between markers are indicated at the left side.

(Miao et al., 2005; Zhan et al., 2009). The 93 DNA samples were diluted to a final volume of 10 ng/μl with sterilized double-distilled water and stored at 4°C prior to amplification using the polymerase chain reaction (PCR). The PCRs and the amplification using the SSR markers were performed according to Miao et al. (2005). The PCRs were performed via STS markers with an initial 3 min period at 94°C (denaturation), followed by 35 cycles of denaturation at 94°C in 30 s, 30 s of annealing at 56°C, 70 s of extension at 72°C; and a final extension time of 5 min at 72°C. The PCR products were electrophoresed using 2% agarose gel to determine their lengths. PCR products of the SNP markers were purified and then sequenced using an ABI 3130xl sequencer to detect SNPs.

Linkage map construction and data analysis

A genetic linkage map was constructed using MapMaker/Exp V3.0 (Lincoln et al., 1992). The QTLs for silk length, whole cocoon weight, pupal weight, silk weight, and cocoon shell weight were scanned using MapMaker/QTL v1.1b.Whole chromosomes were scanned every 2 cM for the presence of QTLs. Positions with LOD ≥ 2 were considered QTLs (Basten et al., 1994).

RESULTS

Phenotypic variations of five characteristics in the BC_1 population

Whole cocoon weight, cocoon filament length, cocoon shell weight, cocoon filament weight, and pupal weight showed patterns of continuous and normal distributions, which indicate the quantitative inheritance of these characteristics (Fig. 1).

Genetic relationship among different characters of cocoon and silk production quantity

The genetic relationships among cocoon and silk qualities were analyzed. All characteristics were positively

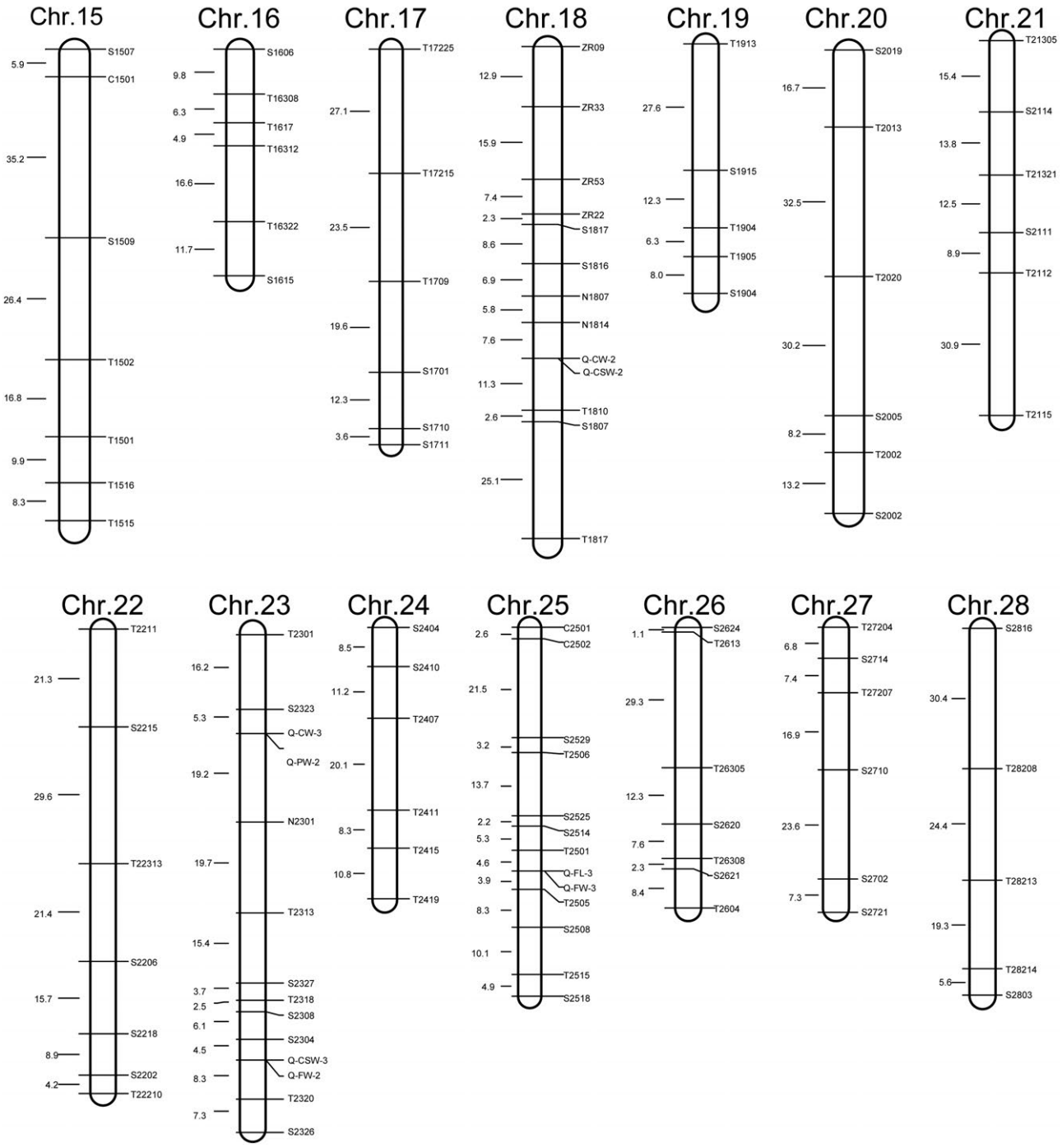

Fig. 2. continued.

correlated (Table 1). The genetic correlations between silk weight and cocoon shell weight, as well as between whole cocoon weight and pupal weight, were extremely high (0.999 and 0.996, respectively). The lowest correlation was between cocoon filament length and pupal weight (0.699). These results suggest that the QTLs for some characteristics are linked.

Construction of molecular linkage maps

A total of 182 markers were polymorphic between the two parents JS and L10. The DNA polymorphisms from the 93 individuals of the BC_1 population were amplified. The genotyping results of the 181 polymorphic primers (Supplement 1), including 85 STS markers, 80 SSR markers, and 16 SNPs accorded with the 1 : 1 ratio of the population. These markers were analyzed using Mapmaker/Exp (Version 3.0) and assigned into 28 linkage groups (Fig. 2). The total genetic distance was 2147.1 cM. The mean distance between adjacent markers was 8.5 cM.

QTL analyses for cocoon and silk production quantity

A total of 14 QTLs were detected for cocoon filament length, whole cocoon weight, pupal weight, cocoon fila-

TABLE 1. Comparative genetic correlations of cocoon quality in *Bombyx mori*.

	Whole cocoon weight	Cocoon filament length	Pupal weight	Cocoon filament weight	Cocoon shell weight
Whole cocoon weight	1				
Cocoon filament length	0.749	1			
Pupal weight	0.996	0.699	1		
Cocoon filament weight	0.896	0.902	0.852	1	
Cocoon shell weight	0.898	0.901	0.854	0.999	1

ment weight, and cocoon shell weight using Mapmaker/QTL (Version 1.1b) (Table 2). The QTLs were distributed in 5 linkage groups (linkage groups 1, 14, 18, 23 and 25). Interestingly, the 5 QTLs located on the first chromosome had the greatest logarithm (base 10) of odds (LOD) values, which meant they were highly reliable, 3 of which located at the same region in linkage group 1 (232S1 and S0106). These findings indicate that some silk traits are significantly correlated with each other.

The clustered distribution of the QTLs is in accordance with the genetic relationship among these traits, which in turn is consistent with the relationships among whole cocoon weight, cocoon shell weight, and pupal weight. These findings highlight the significant relationship between the traits for cocoon production quantity, and explain why the highest QTLs were mainly detected in the same linkage group.

DISCUSSION

The two parent silkworm strains used to construct the linkage map have numerous genetic differences, which facilitated the expression of numerous extensive polymorphism markers leading to the construction of a molecular linkage map. The silkworm is one of the lepidopteran species in which no genetic exchange occurs in females (the heterogametic sex) during the formation of female gametes. Using the BC1 population as the mapping population circumvented this problem and allowed analysis using mapping software. In our research, the silk fibers were reeled from the cocoons while the pupae were still alive. Data, including the cocoon filment length data and cocoon filament weight, were recorded to utilize live pupae to extract DNA for linkage and QTL analysis. In addition, JS is one of most economically important strains and it has been used in silk production for nearly 30 years. Therefore, the study has a more direct applied importance to sericulture and the production of silk-related products.

The silkworm has attracted increasing attention as an important genetic and biomaterial resource. China has a long history of sericultural research and an accumulation of genetic data on silkworms. Xia et al. (2004) reported whole-genome shotgun sequencing and provided public access to the assembled silkworm genome data. In 2008, The International Silkworm Genome Consortium published the fine scale genome sequence of the silkworm, which is necessary for molecular genetics research in this insect. Cocoon quality is an important characteristic of silkworms; however, the genes of this insect are more difficult to map than single Mendelian factors (Zhan et al., 2009).

A total of 14 QTLs for the silk quality characteristics were identified. Cocoon filament length, cocoon shell weight, whole cocoon weight, pupal weight, and cocoon filament weight, which are controlled by multiple genes, are the economically significant characteristics of importance to the silk industry. The present investigation pro-

TABLE 2. Information of QTLs detected from BC_1 in *Bombyx mori*.

Trait	Locus	Linkage group	Mark interval	LOD value
Whole cocoon weight	Q-CW-1	1	C33S2-S0106	11.0
	Q-CW-2	18	N1814-T1810	2.3
	Q-CW-3	23	S2323-N2301	2.1
Cocoon shell weight	Q-CSW-1	1	C33S2-S0106	6.2
	Q-CSW-2	18	N1814-T1810	2.4
	Q-CSW-3	23	S2304-T2320	2.3
Cocoon filament length	Q-FL-1	1	C214S5-C214S4	8.8
	Q-FL-2	14	T1406-T1403	3.1
	Q-FL-3	25	T2501-T2505	2.0
Cocoon filament weight	Q-FW-1	1	C33S2-S0106	11.2
	Q-FW-2	23	S2304-T2320	2.6
	Q-FW-3	25	T2501-T2505	2.2
Pupae weight	Q-PW-1	1	C214S4-C233S2	5.2
	Q-PW-2	23	S2323-N2301	2.2

vides an excellent foundation for the map-based cloning of major genes that control silk production. Compared with previous research on QTLs for cocoon quality (Lu et al., 2004; Li et al., 2005a; Sima et al., 2005), the locations of the QTLs in the chromosomes were not identified because of the difficulties involved in duplicating both the AFLP or RAPD results. In research, the exact location of the STS, SSR, and SNP markers were determined from the silkworm fine scale genome sequence, and these markers can be duplicated in other silkworm strains. Our results are thus consistent with those by Zhan et al. (2009). Firstly, the detected QTLs were distributed mainly in groups 1 and 23. Secondly, the LODs of the QTLs in group 1 are extremely high. These results indicate that the data are accurate and repeatable. Both results show that one or more QTLs on chromosome 1 affect silk and cocoon quality. Our results also show that all of the QTLs on chromosome 1 are located at a region of 290 kb, and there were 12 predicted genes in that region. We are now trying to clone all the genes and compare them between Jingsong and Lan10. The findings by Zhan et al. (2009) indicate two QTLs for cocoon shell ratio in groups 18 and 19; however, we only detected two QTLs at the same locus and which controlled whole cocoon weight and cocoon shell weight on chromosome 18, but did not contribute to cocoon shell ratio, and no other QTLs were detected for cocoon shell ratio either. This result could be attributed to the fewer markers available in those groups, which were insufficient for detecting QTLs. Although we constructed a linkage map that covers all 28 chromosomes of the silkworm, the marker density was still low, and many QTLs that control cocoon and silk production quantity characters were not detected. More SSR and STS/SNP markers will be designed in future studies to finely map the QTLs and allow map-based cloning thereby leading to a better understanding of the mechanism of silk production in silkworm).

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SUPPLEMENT 1. The Primer Sequences in the Linkage Groups.

Loci name	F primer (5'—3')	R primer (5'—3')	Scaffold	Locus	Marker type*
S0102	TTGAAACCAGCTCCACATAATAGG	AATAGGAAGTTTTGAAGTATCTCGACC	nscaf2210	4153kb	SSR
S0107	CCCTATCCCTAATGATTCTTGTCCA	CGATGCTCGATCTGATTTACCA	nscaf1690	243kb	SSR
S0108	TTTATTTGGAATTAGTGGGGTTA	CATTATCGCTCACATCGCTCCT	nscaf1690	4529kb	SSR
S0105	CAGATTTCGCCAGGACTACACTTT	GAGAAGTGCAGAGTGCCCATATT	nscaf3040	1385kb	SSR
S0104	ATAACAGTGACGCAAAGAACTAAACA	TTAGGAGCGTCTATTTTAATAATCTGTTC	nscaf3040	1535kb	SSR
C164S5	GGAACCAAATGAAATGCTCTTT	GGGGTTGGTTTTAGCTGTCA	nscaf3040	2862kb	SNP
C200S8	GAGATGCCGAATGGATCTGT	GGAATCGGTGTTTTTCATGC	nscaf3040	3272kb	SNP
C208S1	AGGTCATTTTCACAGTCATGTTC	CGAAACAGCAAAAGAAGAAACA	nscaf3040	3362kb	SNP
C214S5	CCAATTTTGCTTCGCTTCTC	TAAAAGAGCGGACCGTTAGC	nscaf3040	3472kb	SNP
C214S4	TTTCGTGCGTCTTTCATTTTT	TATTCCATGGGCGCTTTTTA	nscaf3040	3468kb	SNP
C233S2	CATTTTGCAGCGGATAAGGT	AATTACCGCATCCACGTGTT	nscaf3040	3755kb	SNP
S0106	GCATTCAAACCTAAGTCAAATCAAA	TGGCACAGTCTTTATGTTTGGCT	nscaf3040	3758kb	SSR
C232S2	CATTTTGCAGCGGATAAGGT	AATTACCGCATCCACGTGTT			SNP
S0203	TGTTCAGAAATGAGGTTGCCCA	GAGTTGTTGACCCTTGCGATTAT	nscaf2964	4944kb	SSR
C0208	TTCAGTATGTCCGATGGGTT	GCTTATAGTGATTATGGAGTGCTT	nscaf2964	4896kb	SNP
T0206	GTATCATGTGAGCCAACGACC	GACCAAGAAACTGCTATGTAAGAAC	nscaf2964	1596kb	STS
T02202	AGCCGAGGTGAGTTGGAATC	GATGAATGTGAGGACAGAGATGAC	nscaf2623	1386kb	STS
T02203	ATCTCTTCTGGTGTTCTTAGGTCT	CGATAGATGTCACTACTGCGATTC	nscaf2623	1104kb	STS
S0314	CTTTAACCCTTTGACTGCCATGTA	CGCCTACTGTCACAAGTGTATCTGC	nscaf2883	2671kb	SSR
C0305	AGTTTGGCCTACAAATACAGC	TCTAATGAAAGCATTCCATCTT	nscaf2886	1114kb	SNP
S0315	CGACGCACTCCACCAAGC	GCTACGGTATTCGCACTGGAG	nscaf2886	713kb	SSR
T0310	GTCTGCATTCCTGAACTCGT	CTGGCTGTCGTGCTTACTTT	nscaf2930	3634kb	STS
C0311	CGACTGAGTTGAGGCTGGAG	CAAATTCATGCCAAACGAGA	nscaf2930	4894kb	SNP
S0302	CGGCGTGACCGATTTCCA	GTGGTATGGACATAATATGATGCGTA	nscaf2925	654kb	SSR
S0401	AGCAAACAAACCGCCTGATG	GATTCCCACATCGGGCAAA	nscaf2847	4243kb	SSR
S0409	CGAAGGCAGAGCAAAGTGGAC	TCGTAGTTATCACTTGCGAATGC	nscaf2847	4546kb	SSR
S0408	GCGAGAAATTCGTTTCCAAATG	AACCCCGGTTCTATGCAATCT	nscaf2847	4625kb	SSR
S0411	TCGTACTAGGGTGACAGTCCAAAG	CGGTTGTCTGGAAGAGGTCGC	nscaf2847	4851kb	SSR
S0417	TTAGCGATAAGACCGCCTATTGTA	CAATCATCAATTAGGTCGTCTATCCA	nscaf2589	1639kb	SSR
S0421	TGGGTCATTTTGATTTGGGG	TCATTAGAGTGGGATAGCGTGTTT	nscaf2681	463kb	SSR
S0502	CCAAGGGCATCGGTGACA	ACTTAGTTCGCCCACCTGACC	nscaf2838	1066kb	SSR
T0512	ACGAGTTCATCTGCCGATCC	CACGCACTTTAATGTCTTCCTG	nscaf2674	14kb	STS
T0515	CAAACAGCGGAGGTCGTATA	TTCGCACCATTATCGTCAAA	nscaf2674	3460kb	STS
S0514	CAGGCTTTGGATGATGATAAATACA	GAGGATGTTGTGCGGACGC	nscaf2529	1867kb	SSR
S0515	CCAAATGTCGCGTATTAAGCAAA	TGTCGTGCGTTTGAAAAGGTT	nscaf2529	3007kb	SSR
S0601	CCTAAGCTAAAGGAGTCCTAATCACA	GATGGTGAGTGGTTAGGGACGT	nscaf2556	452kb	SSR
T06216	CTACGCCTTGACCTGTGACT	CAGACAATGCCGAGAATATAATGC	nscaf2556	472kb	STS
T0616	ATTGGATATTCTCGGCAGTTT	CGTTGACATTCCTCGTTGG	nscaf2852	2152kb	STS
T06211	GCAACTGTCCATTCCTCCTAAT	CCTACCGTAATCTACCGTATCTTC	nscaf2852	1969kb	STS
T06210	CTCCAGGCAGTCCTCTCAAG	TTAGGCACACTACACATCACAAG	nscaf2852	1621kb	STS
S0609	TGCCAGCCCTCGCCTAA	GCACGGAAGCTCCAACGAC	nscaf2853	1862kb	SSR
T0702	GGGCGTTACGAAACCAAA	CTTCAAAGGAGGGTAAGGGAC	nscaf2983	286kb	STS
T07207	GAAGCGTCGGCTCTAAGGTA	TTGAACATATCGGTCGTATAGTCC	nscaf2986	2089kb	STS

S0705	TCCAATTTGTGTGTTTGTATGTGTG	GGTTGAGAAGCAAGTCTGCGATA	nscaf2986	3772kb	SSR
T07215	CGCCACGACAACGAATAGAC	GAAGAACGATGAAGCCTTATACCA	nscaf2986	4526kb	STS
S0702	AGTTCCTTTCACAATGTCTTATGCTA	CACCATACATGGGCTCCGAT	nscaf2912	1428kb	SSR
T0820	AATGTATCTTCGACAACCTCCG	CCTACTCACGCTCCCTCATCT	nscaf98	14kb	STS
S0820	GAGGTACCAGTGATTGCAGACGT	CCCAGGTGTACTCGGAGTCATTTA	nscaf463	642kb	SSR
T08410	ACGGTGATGATGTGAATAGTGATG	TCCATCGTAAGAATCCATCCAGAA	nscaf2828	3893kb	STS
T08419	CCGTAACTGAAGGTGATTGAACTG	AGCCTATTAACGCCACTACTGAAT	nscaf2970	960kb	STS
T0904	TTCCTAATCCGAGTGCTGTCTA	TGCTGGTCGCTTGCTTGA	nscaf2889	3609kb	STS
S0902	CCTAAGACTACATAACTTGTCAATCTACGA	AAAAGTTTGATACTAAATGAAGAGCACA	nscaf3045	1405kb	SSR
T09ZJ	CCATGACTTTTGTAGGGGTAC	CATCGTTTTCTTGGTCGTTA	nscaf2511	590kb	STS
T09217	CCGCAAGGAGCAGTACAATT	GGAAGAGCCACCGCATAGAT	nscaf2511	5049kb	STS
T1004	CGTTCAAGTCCGAAGTCTGTC	CGAGGAAGGCTCACTCCATAA	nscaf2855	3444kb	STS
T1007	ACAGACTATGGCACTGAGATACC	GCATTACAACATTGGAAGACCTTC	nscaf2855	6414kb	STS
T1013	TTCTGAATCGGCAAGTCCAATC	CCATAGCAGCACGCAATATAGC	nscaf2860	409kb	STS
S1019	TCAGACTGTTGAGCCAAAGCG	GGCGTTTTGTAGTTGTTTTCAGTC	nscaf2575	137kb	SSR
T1018	ATTGAGAAGCCGACAAAGTAGGA	CGAATAAGTGAAGCGAAGCCATT	nscaf2575	2729kb	STS
T1101	ACGAAGTGGAGGTTCAGATACA	GGATGTCTATGTTCTCTGGAGTC	nscaf2176	237kb	STS
T1103	ACCTACCAGTTGATGATGTCACTT	ACAGTTCCTCGTGCCAATCC	nscaf2176	2360kb	STS
S1124	CGGACAGCTATAAATTGTACCATCG	CGATAAGACCGCCTATTGCAC	nscaf2176	2818kb	SSR
S1135	CGGCTTTGGCTATTGTTTACTTAC	CCAGTTTACATGACCCAAATTTAGAT	nscaf3032	1344kb	SSR
S1111	AACCTGTGAGCTGACCTAACCG	AACAAGATAGTCCGCAACCCAG	nscaf3031	1088kb	SSR
S1115	CCCTCAACCAGATGCAGCGA	ACCGTTATAGATGCTAAAGATATGATTGT	nscaf3031	4832kb	SSR
T1216	CTGGCGTTACAAGAATAGTAGGAC	GCAGAACAGTGAGGTATTGACAG	nscaf2842	1266kb	STS
S1207	CTCAGTTGGTCGCATTTCCG	TCCGTGGAATTTTATTTCAGTTGTA	nscaf2825	585kb	SSR
S1215	GCACTTTATCTCCTCAGCTACCG	TTGGAATAGAGTGGCACGGTTT	nscaf2993	4498kb	SSR
T1209	CTTATGCGGACTACATTGTGTCAA	AAGGACCAACGACGATTCTGAA	nscaf2993	7562kb	STS
T12203	GCTCCAATCTCGTTAGGTGTTC	TGGTTCAGCGGAAGGACTTG	nscaf2993	106kb	STS
S1320	GAGACTTTGTGTCGTGGGTCCTAC	CCTTCAAACCGAAACGCATTACT	nscaf3093	1130kb	SSR
T1304	GGCTATCCGAACGCTAATCAGA	GAGATTCCACAGGAGTGTCTTAGT	nscaf1898	14462kb	STS
T1303	ATACCGACCACACCATACAATACT	CATTCTGCTTCTGCCGTTGATAA	nscaf1898	15213kb	STS
S1307	TCCCTGGCATTGCTGACG	TTATCTAAAATGGCATCGCAAATC	nscaf1898	7459kb	SSR
T1312	CTAGTGTGATTGGATCGGTGAGTA	GGTGAAGGTGAACGGAGGAC	nscaf1898	6336kb	STS
S1313	TTGCTTCGTGACCTTGAGATAAC	TACACAGAGACCTGAACCTAGAAC	nscaf1898	5514kb	SSR
S1424	ACCTCGGGACAGATTCGTTTT	AACTAATAACCGTCTCCGACAAATAA	nscaf2948	2568kb	SSR
T1414	GGTCACAGACGAGGTCATCAC	ACGGTGGTCTTACGAATGTCAT	nscaf2948	1245kb	STS
T1413	CTGGAGCCTGTAGTGGACTG	ATTGCTACTGGTTCGCTTGGA	nscaf2948	268kb	STS
T1406	ACAGGTAGGTCGGTGTAGCA	CTGGCGTGTCACTTGTCTCT	nscaf2943	335kb	STS
T1403	GGTTACCAGTGTTCATAGCAGAG	GTAGGCATACTCATAGGCATAAGC	nscaf2953	1534kb	STS
T1417	AACCATAGTCGGGGTCATTC	GTAGATAAATCCCACAAGCAAGA	nscaf2953	25kb	STS
S1507	TGCCCCACCCTTCAAACC	GCGTAGCCACTGAAGGTTTACTT	nscaf2888	473kb	SSR
C1501	TGGACAGTGGCACCTTTCT	GGAGCAGCCTCATCTTAACAT	nscaf2888	811kb	SNP
S1509	GTGGTTATTGGGAACTTTTATGATGT	CGTATTAGGGAAGAAAAGATGGCT	nscaf2888	5086kb	SSR
T1502	GGTGTTATGGAATATGCGGTAGG	TACTTGTTATTGGACCTCGTGGAT	nscaf2888	8349kb	STS
T1501	CACAGACAATTCCTCCACGAGTA	CGCTCCGCTATCTCATTACCTTAG	nscaf2888	9504kb	STS
T1516	CACGAGTCCATCAGAAACACAAT	CCGCAAGGTAGCATAACACAG	nscaf2655	3336kb	STS
T1515	TCACAGTCAGCACAGATGGTT	TAACAACTCACAGTCATCGCTAAG	nscaf2655	2642kb	STS
S1606	AAATAGAAACTGACGATGAGGTAATAGC	ACAGATTGATTCCAGAAACGAGC	nscaf3058	1638kb	SSR
T16308	GCGAGAGGCTATTATTCTGACTTC	CGAATGTGCGTGATGTAACCAA	nscaf3058	629kb	STS
T1617	ATCAGCGAACGAAAATAGACG	GGCGATAACTTTGGGATACAC	nscaf3058	67kb	STS
T16312	TCGCACACTCGCTCTCATTG	TGACTTCCAGTTACACGCATCTA	nscaf3062	246kb	STS
T16322	AAGCATCAGGCAGTAATGTGGTA	CCGAGGAAGAGATAGTTGGACTC	nscaf3063	1417kb	STS
S1615	CACCTAGTTTTACTTCACGGACCAT	CCCTTGGGCTTAAGTCGGTTT	nscaf3063	331kb	SSR
T17225	ACTGTTATTCGGGGCTTGTG	TACTTACCCTCCGCTGTTCG	nscaf2766	900kb	STS
T17215	AAACTCTGTAGAAGTAGGGGAACC	AACAATAAGCCACTGGACGC	nscaf2865	4938kb	STS
T1709	TCAGGTGGCATACAGAGAAGAC	GAAGAGGCGGTGTTGGCTAA	nscaf2873	45kb	STS
S1701	GTCATTGGGAGTTTGAAGTTTCG	ACGGGCTTTCTTTGCTAGATGT	nscaf2829	3178kb	SSR
S1710	GGGATAAGTGGGTCGTTTTGATT	TGAGACCCAATAATGTCCCGAG	nscaf2829	3202kb	SSR
S1711	CGGCACTTAAAAGTTTTCATATCAATC	CTGACAGTGGTGAGTTAATAAAACAAAA	nscaf2829	3278kb	SSR
ZR09	CCACCAACCTTGCGACAC	GCCGAGCCTTCTCAAACG	nscaf2901	633kb	SSR
ZR33	AAGTCTAGTCGTGAAACTAGGGAG	TTCTTATCATTTATAGGGTGAGGC	nscaf2901	460kb	SSR
ZR53	AGGGAGGTAGTCAGGACAAGG	TCCGACGGCTATCAAACAC	nscaf2901	964kb	SSR
ZR22	GCGACCCTCACAACCAGA	AAGATGACAGCCAAGTTCCAC	nscaf2901	1582kb	SSR
S1817	GGCTACGTAAAATACATGGCAGTT	AGCGATAAGACCGCCTTTTGT	nscaf2901	947kb	SSR
S1816	TTCCCCTTTTGAATTTTAACGAG	TGGCTTGGCTCTGCTCCTG	nscaf2902	252kb	SSR
N1807	CTCAGATGAATCGCAAGA	ATGTGAACAACCCAAACT	nscaf2902	3456kb	SNP
N1814	AGTATCTATCGTGCCCTGAA	TGAAGCGTGTCGTATCGT	nscaf2902	4517kb	SNP
T1810	AACTCGGACATACTTGACTGGTT	TAACGGACGCTGTTGGCATT	nscaf2902	8692kb	STS
S1807	TTTATTTGTTAGGCTCGTTACTGTCA	CAAAAGCACAGCTACTTCCGC	nscaf2902	8871kb	SSR
T1817	AATAGAAGGGAGAAGGAAGGTCT	GTAAAGTTGCATACTCAGGTGTCA	nscaf2903	1582kb	STS
T1913	AGACTCCGAGCGGCTGTTAT	ACGGTCGCATAGCAGGATAGA	nscaf2770	526kb	STS

S1915	GCCGCTGCTAACCGAAAGA	TATGATCTTATCTTTTCAGAATTTGGG	nscaf2204	762kb	STS
T1904	ACTGTATGGTACTGTACGGTGTT	TTGACATTGATGGAGCCAAGTTC	nscaf2204	3312kb	STS
T1905	ACAAGCGATACTGCGACACA	ACCTAACAAGATGGCTGAGATACA	nscaf2204	1865kb	STS
S1904	AACTTAACATTTAAGCAAACCCA	CCGCACAGCCAACTGACAA	nscaf2204	4211kb	SSR
S2019	AAATTGCAGACATTGGCATCATC	AATCTTATCAGTTTTAGAAGGTACCGG	nscaf2938	350kb	SSR
T2013	CGACAGAGATACAAGAGAACACAA	CCAAGAGACCTAGAAGCCTGAA	nscaf2937	467kb	STS
T2020	GAAGATTGAAGGCGACGCATAA	GGATCAGAAACCAGGGAGTT	nscaf2789	826kb	STS
S2005	GCCGCAAAACAATCAAGTGG	CGTATTTTAGTGTATGACTCGGATGA	nscaf2795	246kb	SSR
T2002	GTAATACTTATCGGAGGACCACAA	GCACATTGGAAGCAGAGGTT	nscaf2795	2625kb	STS
S2002	ACTCACCAACAAACCGCAAGA	CACCGCAACATGCCTGCTATA	nscaf3089	90kb	SSR
T21305	GTGTGGCGTAAGAGCAAGCA	GGAAGGTTATGGCGACAAGTGA	nscaf3041	1569kb	STS
S2114	TCAAGGAAGAGTAGACGGTCAAAAC	CGTCTTATTGTTTGATTGCGGCT	nscaf3044	657kb	SSR
T21321	CCACGACGACTAAGGTATAGAGAA	AACAGGACGGCGATGAGATG	nscaf2868	676kb	STS
S2111	AGTCACAGATTTCGCCAATTAAGAT	ATTGTGCGGACGGAGGAGTA	nscaf2136	1035kb	SSR
T2112	TTGTGCGTTGTGCCTTAACTATC	ACTACTAGGACAGATGGAGGAGAC	nscaf2136	5255kb	STS
T2115	AATCCAGGCAAGCAGCACAT	GCAAGTGAAGTGTACCGATGG	nscaf2136	8279kb	STS
T2211	GACTCATCTCCATTCACCTCCAT	ATCAGCAAGTTGACGGTTCATAC	nscaf1681	572kb	STS
S2215	TGTCAGCCAAAGAATGTGTAAATGT	ATACAAATGTAAAACTTTGCCGTTG	nscaf1681	4208kb	STS
T22313	ACTCGCATAACCATAGTAAGAACC	CATACACATCGTCGCCAACAA	nscaf2980	49kb	STS
S2206	GGGTTAGAGGTCCCAAACGATG	CAGGTCACTTAGTCTTAACATTGCG	nscaf3008	1493kb	SSR
S2218	TTACTGGCGTAGATAGTTGAATGC	GGATACCTTCTCCGTGTTGCT	nscaf3005	1703kb	SSR
S2202	TTTAGTCATTCACAAAACAAACAAGG	TGAGATGACCTAAGGTAAGGGGAA	nscaf3005	819kb	SSR
T22210	CATCGTAGCACTTGTCGTTGTC	ACTTGGTAGTATGTGAAGCCTCTG	nscaf3055	15kb	STS
T2301	CACGGTCACGGCATTCTCTT	ACCTACCAGGACGGACTTACC	nscaf1962	294kb	STS
S2323	GCCTCTGTTTTATTCAACTCACGC	GGTTTTCTGATTTATTGTTCTATGGTATTA	nscaf3027	209kb	SSR
N2301	TTCGGATTACTTCTGTATTTGGTC	CCTTTCAGATGACCTGCCGTTGG	nscaf3027	2334kb	SNP
T2313	TGAAGGTCTTGCCGAAGTTATGA	TAGAAGGTGATGACAGGCTCAATT	nscaf3026	305kb	STS
S2327	GGAACGCTGAATCCACGGA	TTTAATGTATCGCACTGTTTATTTGTTT	nscaf3026	4843kb	SSR
T2318	GTAGCCACTCACAGCCAGAC	CTTGTCGCATAAGAACCACATCTA	nscaf3026	5133kb	STS
S2308	GTGCTCACGAACGACTTGTACG	TGGAAATTGTAGTGCAAGGTAGAGG	nscaf3026	5155kb	SSR
S2304	ATGACAATACGCTTTATCACCAGG	ATGGGTTAGTTCGCTCGTCG	nscaf3026	5670kb	SSR
T2320	CAGGACATACGCACGGAGTC	TGAGAGCCAACGGATACTAGGT	nscaf3022	143kb	STS
S2326	CGGGGACGGTGCTCCTTAC	TGCCCACGGTCTCCTCG	nscaf3022	1420kb	SSR
S2404	ATTAATATTTAGTATACAAAGCGTCCCAT	GAATGTCGGTAGTTTGTACTTTGTTTG	nscaf2891	436kb	SSR
S2410	TTTCTTTGTCCCCGCCTTCT	GGTAGAGGGGCAAGAGGTCG	nscaf3066	1295kb	SSR
T2407	TGTTAGGCTGTTGCGGAGTC	CAGGAATCGGTGAGTTCTTCTTAA	nscaf1108	1200kb	STS
T2411	CGTAGTCCTCGGTGTGATTGA	TTGATCGTGCGAATTGTTGCTA	nscaf2962	213kb	STS
T2415	TCTTCAGCACTCGTCATCTATACT	AGGACTAACTCACACTGTCATCAT	scaffold606	31kb	STS
T2419	TCCTTCAGACCACAGCATTCATT	GTTACAGGCTTCCAGAGTGAGAG	nscaf3035	199kb	STS
C2501	GTTCAAGCAAGGTCTTCAGTGT	ATCCGAGAACGCTAATGTATCAGA	nscaf1705	461kb	SNP
C2502	TGATGACACTGCTGCTTGGAA	GCTGACGACTATGCCTCAACA	nscaf1705	1172kb	SNP
S2529	GGGTGAAAAGTGATGACCAAAAC	CCAAGGAAAATCCCTGGAATCT	nscaf2822	963kb	SSR
T2506	TCTCTGGTATTCACTCCGACAAC	CGTCAGGTGCGTCACATAGA	nscaf2822	1666kb	STS
S2525	AATGAGCTGTCTTGCTTAGAAAACTTAT	ACCCTTAGCAGTCGAGTACAATTTT	nscaf2823	930kb	SSR
S2514	AAACATCAAACAGTTAGGTGGGC	ACTGGTTTCGCATTGCTTGTG	nscaf2823	1152kb	SSR
T2501	CATTGCTAGGGCTAGTGTTGG	TTGGAATTGATGACCCGTTT	nscaf2823	1176kb	STS
T2505	GGTTTCGGTGATTCTGATGT	TATTTGCTTCGGAGTGTCGT	nscaf2823	1711kb	STS
S2508	CTTCGCATATCATAATAGGCTTTTG	GGTTTTACGGGATTCCCTCAGT	nscaf2823	4234kb	SSR
T2515	TTCTCTGGTGAAGGCAAGTCTA	TGCTTCCAACCGATTAGTAGTTAC	nscaf2815	1155kb	STS
S2518	CCAGCGAACGTAGGGGACA	TAGCGATAAGACCGCCTATTGTAC	nscaf2815	1829kb	SSR
S2624	AATAGCGGAGGAACTGGGGA	AAACTTATCTCAAGGTAGGTGGCG	nscaf1071	1056kb	SSR
T2613	CACAGCAGATTATACACGCATTAC	TGTCTCGCTCATCCTCATACG	nscaf1071	935kb	STS
T26305	CTCGCCAGTGTTATGAAGAGAAC	TCACCTTGGAGCAGCACCTA	nscaf2330	3868kb	STS
S2620	TGAATACTGAAATAGCGGTCGTTG	GGCGCAATTTTGTCTGCACTTA	nscaf2330	2521kb	SSR
T26308	ATGCTCTGCTGAAGAAGGTGAT	CGATGTTCCTCTCCGAAGACTT	nscaf2330	2324kb	STS
S2621	GCCAAATGATTATTGTCAGCCTG	GGATGGACGGAAAATCGCAG	nscaf2330	1776kb	SSR
T2604	TGCCTCTGTAGTTGTTACTTGTGA	GCATTGGACGCAGTACCTCATA	nscaf3003	3204kb	STS
T27204	GGAACCTCTACCGCATCATCA	AAGACCTATCACCACCGACTG	nscaf3099	1867kb	STS
S2714	ACAGACTTAACTTAAAACGGATTGAAA	CGTTGTAGATGTCTATGGGCTCC	nscaf3099	2927kb	SSR
T27207	GTCGTAGCCAGGAACACTATTAG	GCATCTATTCCGTTCGCTTATATG	nscaf3099	4032kb	STS
S2710	GGCACCCGACCATTTGTTGT	CCAGGCAGTTGAGGTCGTGA	nscaf3097	568kb	SSR
S2702	TGAAGTGTCCTTAGGAGTCGTCG	CCAGAAATAGCGGGTGAATCC	nscaf3098	442kb	SSR
S2721	GGCAAAGACTGGATGTGAGATGTAA	ATATTCAGCACAGCGTTTCCTTAA	nscaf3098	1862kb	SSR
S2816	TGTTTGCTGTGGCGGTAGTG	CTGTAAATTAACACGGTTGTCTGGA	nscaf3071	86kb	SSR
T28208	GGTAAGGTGAGGTGGCGTAG	GCAGTGAGGTGGCAATGATG	nscaf2836	575kb	STS
T28213	TACCGATACACGACTCGCATA	AGGTGAGAATCTACTCCAGCAT	nscaf2801	386kb	STS
T28214	GGAACTGGCAATAGCGAACAA	CCGAATCTTCACAATTCTCCACAA	nscaf2797	582kb	STS
S2803	CGGAATTAGATGTCTCACTGCCA	GAAGCAGCAGCCCTCCGA	nscaf2797	1101kb	SSR

* SSR – Simple Sequence Repeat, PCR products size polymorphism; STS – Sequence taq site, PCR products size polymorphism; SNP – Single nucleosis polymorphism.