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Genetic diversity of sea-island cotton (*Gossypium barbadense*) revealed by mapped SSRs

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ABSTRACT. In order to evaluate the genetic diversity of sea-island cotton (*Gossypium barbadense*), 237 commonly mapped SSR markers covering the cotton genome were used to genotype 56 sea-island cotton accessions. A total of 218 polymorphic primer pairs (91.98%) amplified 361 loci, with a mean of 1.66 loci. Polymorphism information content values of the SSR primers ranged from 0.035 to 0.862, with a mean of 0.320. The highest mean polymorphism information content value for the SSR motifs was from a compound motif (0.402), and for the chromosomes it was Chr10 (0.589); the highest ratio of polymorphic primers in Xinjiang accessions was from Chr21 (83.33%). Genetic diversity was high in Xinjiang accessions. AMOVA showed that variation was 8 and 92% among populations and within populations, respectively. The 56 sea-island accessions were divided into three groups in the UPGMA dendrogram: Xinhai5 was in the first group; accessions from Xinjiang, except the five main ones, were in the second group, and the other 34 accessions

were in the third group. Accessions from the former Soviet Union and Xinjiang main accessions were closely related. Both PCA and UPGMA confirmed that Xinhai5 was distinct from the other accessions, and accessions from Xinjiang were in an independent group. Given the differences between principal components analysis and UPGMA results, it is necessary to combine molecular markers and pedigree information so that genetic diversity can be objectively analyzed.

Key words: Sea-island cotton; Genetic diversity; Mapped SSR

INTRODUCTION

Cotton (*Gossypium* spp), a unique cash crop that produces fiber from seed, provides the world's largest number of natural textile fibers. The cotton genus *Gossypium* L. consists of about 45 diploid and five tetraploid species (Fryxell, 1992). Among the five tetraploid species, two species have been domesticated. They are *G. hirsutum* L. (predominantly distributed in Meso-America and the Caribbean) and *G. barbadense* L. (mainly distributed in South America and the Caribbean) (Westengen et al., 2005). Upland cotton (*G. hirsutum*) and sea-island cotton (*G. barbadense*) contribute to 90 and 8% of the world's cotton production, respectively (Song et al., 2004).

Sea-island cotton is tall and robust, with large leaves and a long life period. Its prominent feature is its perfect fiber quality. Besides, this species has many other important traits, such as cold tolerance, drought tolerance, disease resistance, and insect resistance (Mehetre et al., 2003), which are absent in the high-yield upland cotton. Sea-island cotton includes annual and perennial types, and the annual ones are mainly cultivated. Cultivated annual sea-island cotton is classified into three ecotypes: Egyptian type, American type and Middle-Asia type (Mei et al., 2001). Annual sea-island cotton was introduced into China in 1954, and it has been experimentally planted in Yunnan, Guangxi, Guangdong, Fujian, Shanghai, Henan, Jiangsu, Xinjiang, etc. (Kong, 2002). Now, Xinjiang is the only growing region where sea-island cotton is planted. Besides the introduced cultivars, cotton breeders in China have developed several new sea-island cotton cultivars with high-yield production and good fiber quality, for example, Junhai 1 and Xinhai series cultivars.

Several molecular markers such as AFLP (Rana et al., 2005; Westengen et al., 2005), RAPD (Vafai-Tabar et al., 2004), SSR (Lacape et al., 2007; Kantartzi et al., 2009; Luo et al., 2010) and ISSR (Dongre et al., 2004) have been used in upland cotton germplasm analysis, which is helpful to reveal the genetic diversity of these varieties in future breeding. Molecular markers such as SSR (Diao et al., 2002; Chen et al., 2005; Li et al., 2009; Wu et al., 2010), ISSR (Jiang et al., 2008) and SRAP (Li et al., 2008) have also been applied in determining the genetic diversity of sea-island cotton.

In our previous study, Li et al. (2008) used SRAP markers to analyze the genetic diversity of sea-island cotton accessions from China and other countries. Because the chromosome assignment of SRAP remains unknown, it is hard to understand the genomic diversity of these accessions. In recent years, more than 10,000 SSR markers have been released (CMD website, http://www.cottonmarker.org) and mapped in cotton (Nguyen et al.,

2004; Rong et al., 2004; Frelichowski Jr. et al., 2006; Guo et al., 2007; He et al., 2007; Yu et al., 2007; Zhang et al., 2008). In this study, to better understand the population structure of sea-island cotton in China, we used commonly mapped SSR markers to study the genetic diversity of sea-island cotton.

MATERIAL AND METHODS

Plant material

The 56 sea-island cotton accessions used in this study were those previously studied by Li et al. (2008), including the cultivars cultivated in different regions in China and some accessions introduced from foreign countries. DNAs used in this study were from Li et al. (2008).

SSR analysis

SSR markers were selected from the seven most informative and high-density interspecific linkage maps of cotton (Rong et al., 2004; Nguyen et al., 2004; Frelichowski Jr. et al., 2006; He et al., 2007; Guo et al., 2007; Yu et al., 2007; Zhang et al., 2008). The selection criteria were that 1) SSR markers were mapped in at least three maps and 2) a single locus was detected by each SSR. A total of 237 pairs of SSRs were selected from 25 chromosomes with 4 to 17 markers from each chromosome (Supplementary Table 1). No markers were selected from Chr22 because they amplified multiple loci on other chromosomes.

PCR mixtures (10 μL) consisted of 1X reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.3 μM primers, 25 ng template DNA, and 0.5 U Taq DNA polymerase (MBI). Amplification was carried out in a BIO-RAD thermal cycler, with an initial 5 min at 94°C, which was followed by 34 cycles of 1 min at 94°C, 1 min at X°C, and 1 min at 72°C, plus a 5-min final extension at 72°C. X°C refers to the annealing temperature specified for each primer. The amplified products were separated on 6% (w/v) denaturing polyacrylamide (1:19 bis:acrylamide) gels and visualized with silver staining.

Data analysis

SSR fragment was coded as "1" for presence, "0" for absence and "9" for missing data. An original data matrix was then generated for further study. PIC (polymorphism information content) was calculated according to Zhang et al. (2007).

The genetic diversity statistics of sea-island cotton was analyzed using the frequency procedure of the GenAlEx6.2 software (Cruz et al., 2007), including: sample size, number of different alleles, number of effective alleles, Shannon's information index, and expected heterozygosity. Analysis of molecular variance (AMOVA) was used to estimate molecular diversity at the hierarchical level between and within populations, and principal coordinates analysis (PCA) was based on Nei's genetic distance.

Genetic similarity coefficient matrices were obtained by SIMQUAL of the NTSYS-pc 2.1 statistics package (Rohlf, 2000) based on Jaccard's algorithm. The similarity coefficient was used to construct a genetic distance matrix using the SAHN procedure based on unweighted pair-group method analysis (UPGMA).

RESULTS

Amplification efficiency of SSRs

The 237 SSR primer pairs yielded 381 loci; among them, 218 primer pairs (91.98 %) generated 361 polymorphic loci with an average of 1.66 loci per primer pair (Supplementary Table 2).

PIC values of primers ranged from 0.035 to 0.862 with a mean of 0.320. The higher average PIC values of SSR repeat motifs were: compound (0.402), interrupted (0.373) and trinucleotide (0.369) (Table 1). When taking chromosomes into account, the higher average PIC values of the chromosomes were for Chr10 (0.589), followed by Chr21 (0.465) and Chr04 (0.438) (Supplementary Table 2).

Table 1. Amplification of different SSR repeat motifs.

SSR repeat motif	Markers	Polymorphic rate (%)	Average amplified loci	Range of PIC	Mean PIC
Dinucleotide	179	92.18	1.66	0.035-0.855	0.342
Trinucleotide	9	77.78	1.71	0.035-0.762	0.369
Pentanucleotide	1	100.00	1.00	0.035-0.035	0.035
Hexanucleotide	1	100.00	1.00	0.035-0.035	0.035
Compound	10	90.00	1.67	0.035-0.777	0.402
Interrupted	37	94.59	1.63	0.035-0.862	0.373

Assessment of genetic diversity statistics

In order to easily analyze the genetic diversity of 56 sea-island cotton accessions, we classified them based on their origins. Numbers 1-14 and 24-29 are foreign accessions, as Pop1; numbers 15-23, as Pop2, are domestic cultivars, which are mainly from the Yangtzi River region, Yellow River region and Southern cotton region, and numbers 30-56 are the cultivars developed in Xinjiang, as Pop3.

Genetic diversity statistics showed that the order of all statistic values was Pop3 > Pop1 > Pop2; moreover, Pop1 and Pop2 had similar statistic values (Table 2).

Table 2. Comparison of genetic diversity statistics of three pops.

Pop type	Polymorphic (%)	N_A	N_E	I	H_E
Pop1	42.78	0.86	1.16	0.16	0.10
Pop2	37.27	0.76	1.15	0.15	0.09
Pop3	86.61	1.73	1.71	0.21	0.12

N_A = number of different alleles; N_E = number of effective alleles; I = Shannon's information index; H_E = expected heterozygosity.

AMOVA and PCA results

AMOVA showed that 8% of the total molecular variance contributed to population divergence and 92% to individual differences. In the PCA graph (Figure 1), principal coordinates 1, 2 and 3 explained 26.72, 22.59 and 16.01% of variation, respectively. The most direct view of Figure 1 is that 37 (Xinhai5), which belonged to Pop3, was separated from Pop3, and far away from other accessions. Moreover, genetic distance of Pop3 was much larger than that of Pop1 and Pop2. To some extent, Pop3 was an independent group; however, gene flow phenomenon was evident between Pop1 and Pop2.

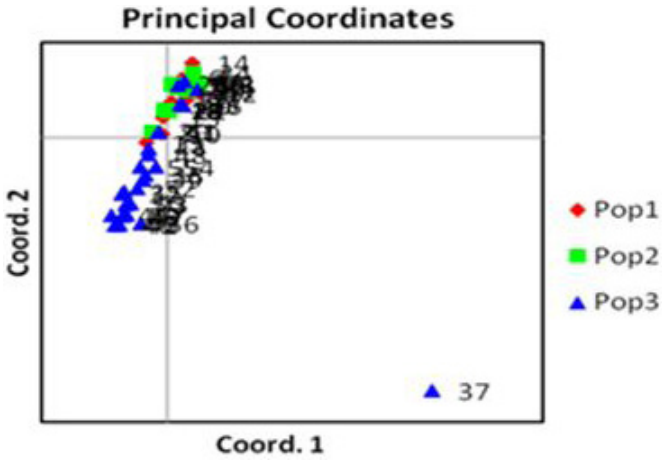

Figure 1. PCA graph of 56 sea-island cotton accessions based on SSRs. Red diamonds represented Pop1, green squares Pop2, and blue triangles Pop3.

UPGMA analysis by SSRs

Taking 0.22 as a threshold value, the 56 sea-island cotton accessions were divided into 3 groups (Figure 2). The first group was located at the bottom of the figure, only including 37 (Xinhai5). The second group was in the middle oval box including most of the latest cultivars from Xinjiang. The third group was in the upper oval box; this group included foreign accessions and other domestic cultivars except those followed by a dot, namely 30 (Shengli1), 31 (Tuhai2), 33 (Xinhaimian), 34 (Xinhai2), and 36 (Xinhai4), which are the basic cultivars in Xinjiang.

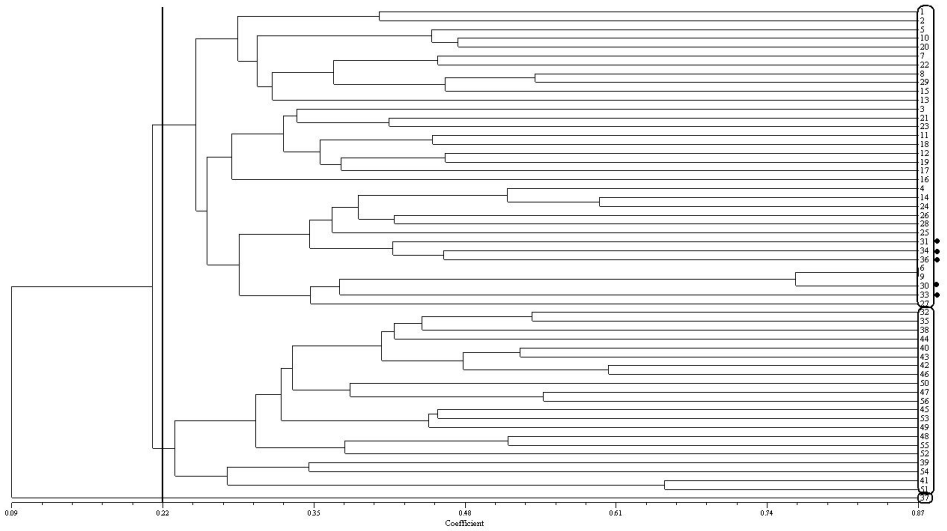

Figure 2. UPGMA dendrogram based on SSRs. Bold vertical line in the figure represents threshold lines. Oval boxes on the right represent different groups. The accessions are followed by a dot representing special individuals.

In order to characterize the diversity of cultivars from Xinjiang (excluding 37 and 56), the polymorphic primers among accessions from Xinjiang were picked out to calculate the polymorphic ratio of the 25 chromosomes (Figure 3). The variations of accessions from Xinjiang were more on Chr06, Chr08, Chr10, and Chr21 with up to 70% of polymorphism on these chromosomes, and less than 30% on Chr07, Chr09, Chr11, and Chr17.

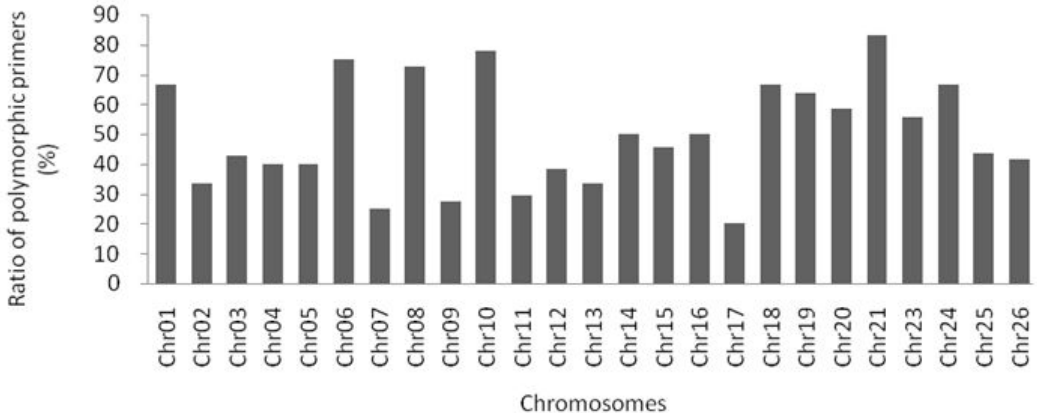

Figure 3. Ratio of polymorphic primers in Xinjiang cultivars except Xinhai5 and Xinhai25 on 25 chromosomes.

Comparing UPGMA trees and PCA results, we found some consistencies and differences. The consistencies included: 1) the latest cultivars from Xinjiang were clustered together, which was the same with the Pop3 results of PCA. 2) There were little differences between foreign and other domestic accessions, i.e., gene flow should exist, which was mainly induced by the introduction of foreign accessions. Differences included: 1) 37 (Xinhai5), which belonged to Pop3, was separated from the other 55 accessions in UPGMA results. 2) Basic cultivars, which belonged to Pop3, are clustered with the foreign ones in UPGMA results.

DISCUSSION

All 237 mapped SSRs were selected based on their distribution among 25 chromosomes. From Supplementary Table 2, we found that the higher average PIC values of the chromosomes were for Chr10 (0.589), followed by Chr21 (0.465) and Chr04 (0.438). However, there was no obvious relationship between the amplification efficiency of markers and chromosomes. Figure 3 showed that the ratios of polymorphic primers of Xinjiang accessions on Chr21 and Chr10 were larger than those on other chromosomes. From the above results, it can be deduced that the polymorphic information is rich on Chr21 and Chr10. This can be of great help in discovering genes related to the traits of sea-island cotton.

To some extent, PIC is a measure to detect the polymorphism of primers, which can be used to estimate the value of allele variation of each primer pair. PIC value of primers shows a positive relationship with the ability to reveal allelic variation (Rohlf, 2000). As shown in Table 1, higher average PIC values of SSR repeat motifs were: compound, interrupted, trinucleotide, and dinucleotide. Qureshi et al. (2004) reported a positive correlation between polymorphism and repeat number. Guo et al. (2007) found that the level of polymorphism appeared to be related to re-

peat type. Zhang et al. (2008) found that the polymorphic rate of tetranucleotide repeats was higher than dinucleotide, trinucleotide, hexanucleotide, and pentanucleotide repeats. However, due to the limitation of different repeat motifs, except dinucleotide, the other five types of repeat motifs were fewer. Moreover, this study did not pick out SSRs of tetranucleotide repeat motifs. Therefore, the results reflected by this article had some limitations.

In this study, PIC analysis by SSR was carried out on 56 sea-island cotton accessions. The PIC values of SSR primers ranged from 0.035-0.862, and the average was 0.320. For SRAP markers, PIC values ranged from 0.035-0.896, with an average of 0.52 (Li et al., 2008). Obviously, the PIC value of SRAP was much higher than that of SSR. Moreover, the average polymorphic loci of SRAP was more than that of SSR (3.17 *vs* 1.66), indicating that SRAP was superior to SSR in the ability of detecting polymorphism, which was also confirmed by other researchers (Zhang et al., 2007; Tan et al., 2009).

The study of genetic diversity can help us understand the genetic structure and polymorphism of sea-island cotton, and provide a theoretical basis for studying the origin and classification of these accessions, as well as parent selection and variety protection. Li et al. (2008) and Chen et al. (2005) showed that the cultivars from Xinjiang were more similar to foreign accessions. In this study, the genetic relationship between cultivars from Xinjiang was much more complex: the basic cultivars were closer with the ones from the former Soviet Union, but the latest cultivars were stable. One possible reason may be that although the basic cultivars were developed from some foreign accessions, in the later breeding stage, Xinjiang cultivars mainly came from several basic germplasms (such as Junhai1, Xinhaimian and Tuhai2), and in the cultivation process, these cultivars produced other variations which gradually became a special genetic structure due to the unique climate condition of Xinjiang region (Wang and Diao, 2002).

The high similarity coefficient between cultivars showed that genetic diversity of these sea-island cotton accessions from Xinjiang was narrow, which was consistent with the results of He et al. (2002) and Li et al. (2009). That is to say, the breeding of Xinhai series is likely to face a bottleneck effect just as upland cotton does (Iqbal et al., 2001). Therefore, new breeding methods and the introduction of new germplasm are necessary. It is worth noting that the genetic similarities between Xinhai5 and Xinhai25 were far from Xinjiang varieties as well as foreign and other domestic sea-island cotton cultivars. According to the pedigree, Xinhai5 includes genes of Middle-Asia type, Egyptian type sea-island cotton and mallow, and Xinhai25 was bred from the hybrid of many high-generation varieties (Li et al., 2008). Another reason may be that genes were influenced by cultivation progress and environmental factors. Besides, Wu et al. (2010) studied the diversity of 36 cultivars and proved that the genetic relationship defined by molecular marker was basically consistent with the variety pedigree, but it could not be grouped only by pedigree or origins. The analysis of 56 sea-island cotton accessions in this study further confirmed this point, so it is important to combine genetic relationship by molecular markers and pedigree information to analyze genetic diversity.

Compared to the UPGMA dendrogram of SRAPs (Li et al., 2008), 37 (Xinhai5) was also grouped alone; however, 56 (Xinhai25) could be clustered with the latest cultivars from Xinjiang based on SSRs. Both SRAPs and SSRs showed that the latest cultivars from Xinjiang were clustered together, and the most basic ones from Xinjiang were clustered with the foreign ones. Some disagreements appeared between SSRs and SRAPs; for example, 6 (Giza47), 9 (Baghmaramian), 13 (Syriamian), 30 (Shenli1), 41 (Xinhai9), and 51 (Xinhai19) were clustered in different groups. Previous research also compared genetic diversity by analyzing these two kinds of markers. Although the

results were different, the combined analysis of SSR and SRAP contained more abundant information (Zhang et al., 2007; Wang et al., 2008). As SSR and SRAP detect different variations of the genome, it is reasonable to obtain some different results. In order to obtain more comprehensive results, we combined the data of SSR and SRAP markers to get an integrated result as shown in Figure 4. In this Figure, the 56 accessions were divided into 4 groups: Xinhai5 and Xinhai25 were grouped, respectively, as group I and α; later varieties in Xinjiang are clustered together as group β, and the rest of the accessions were group χ. The results of UPGMA dendrogram based on SSRs+SRAPs (Figure 4) confirmed the necessity of combining SSR and SRAP markers.

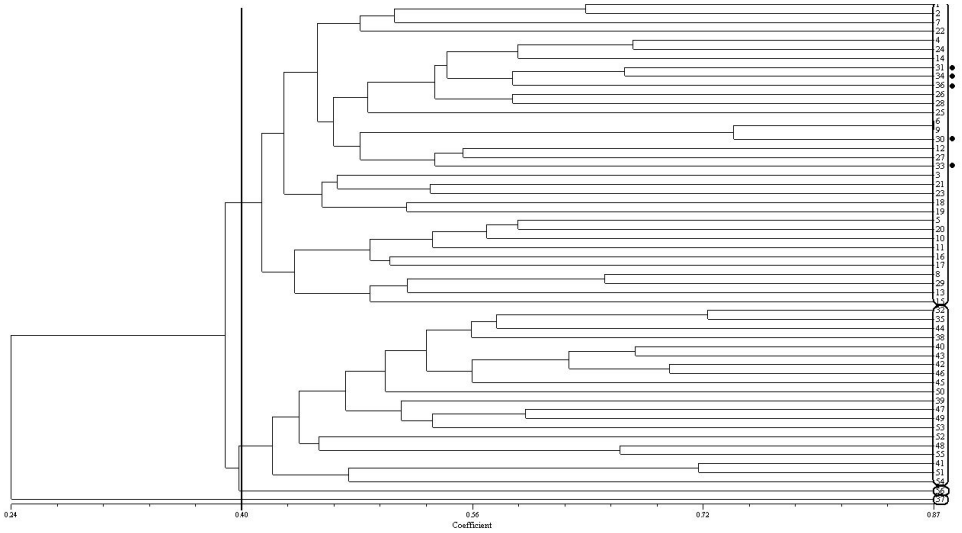

Figure 4. UPGMA dendrogram based on SSRs+SRAPs. Bold vertical line in the figure represents threshold lines. Oval boxes on the right represent different groups. The accessions are followed by a dot representing special individuals.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. SSR markers and the corresponding chromosome as well as the location of the linkage map of markers.

Chromosome	Marker	Map	Chromosome	Marker	Map
Chr1	BNL2440	1,2,6,7	Chr14	BNL1059	1,2,3,5,6,7
Chr1	BNL2921	1,2,3,5,6,7	Chr14	BNL2882	1,3,4,5,6,7
Chr1	BNL3580	2,3,5,6,7	Chr14	BNL3034	1,3,4,5,7
Chr1	BNL3888	1,2,3,5,6,7	Chr14	BNL3932	1,2,3,6,7
Chr1	CIR018	3,5,6	Chr14	CIR084	2,6,7
Chr1	MGHES10	2,3,7	Chr14	CIR295	2,3,6
Chr2	BNL1434	2,3,4,6,7	Chr15	BNL1350	2,3,4,6,7
Chr2	BNL1897	3,4,5,6,7	Chr15	BNL1667	2,3,4,5,6,7
Chr2	BNL3512	3,4,7	Chr15	BNL3902	2,3,4,5,6,7
Chr2	BNL3971	1,2,3,4,5,6,7	Chr15	BNL4082	2,3,5,6,7
Chr2	CIR401	3,6,7	Chr15	BNL786	4,5,6,7
Chr3	BNL3259	2,5,6,7	Chr15	CIR110	2,6,7
Chr3	BNL3441	3,4,5,6,7	Chr15	CIR158	2,3,6
Chr3	CIR133	3,6,7	Chr15	JESPR180	3,5,7
Chr3	CIR202	3,6,7	Chr15	JESPR298	3,5,7
Chr3	CIR228	3,6,7	Chr15	MUCS141	3,4,7
Chr3	JESPR231	3,5,7	Chr15	MUCS152	3,4,7
Chr3	MUSS162	3,4,5	Chr16	BNL2986*	2,3,4,5,6,7
Chr4	BNL2572	1,2,5,6,7	Chr16	BNL3008	1,2,3,4,5,6,7
Chr4	BNL3994	2,3,6	Chr16	BNL580	2,3,4,7
Chr4	BNL4047	2,3,5,6,7	Chr16	CIR107	2,3,6,7
Chr4	BNL530	3,5,6	Chr16	CIR175	3,6,7
Chr4	CIR122	5,6,7	Chr16	JESPR102	2,3,5,7
Chr5	BNL2448	1,2,3,5,6,7	Chr17	BNL2471	1,2,3,5,7
Chr5	BNL3992	1,3,4,5,6,7	Chr17	BNL3955	1,2,5,6
Chr5	BNL3995	2,4,5,6,7	Chr17	BNL834	5,6,7
Chr5	BNL542	5,6,7	Chr17	CIR038	3,6,7
Chr5	CIR034	2,6,7	Chr17	CIR375	3,6,7
Chr5	CIR185	3,6,7	Chr18	BNL1721	1,2,3,4,5,6,7
Chr5	CIR224	2,3,6	Chr18	BNL193	3,5,6,7
Chr5	CIR294	3,5,6,7	Chr18	BNL3280	1,2,3,4,5,6,7
Chr5	NAU1137	3,5,7	Chr18	BNL3558	2,3,5,6,7
Chr5	NAU922	4,5,7	Chr18	CIR099	2,5,6,7
Chr6	BNL1064	1,2,3,5,6,7	Chr18	CIR301	2,3,6,7
Chr6	BNL2884	2,3,5,6,7	Chr18	JESPR153	2,3,4
Chr6	BNL3650	3,4,5,6,7	Chr19	BNL1671	2,3,5,6,7
Chr6	CIR203	5,6,7	Chr19	BNL1690	2,3,5,6,7
Chr6	CIR280	3,5,7	Chr19	BNL2656	2,3,6,7
Chr6	JESPR273	3,5,7	Chr19	BNL3452	1,2,3,4,5,6,7
Chr6	NAU1272	4,5,6	Chr19	BNL3535	2,3,5,6,7
Chr6	TMB0154	2,3,5,7	Chr19	BNL390	2,3,5,6
Chr7	BNL1597	1,2,3,6,7	Chr19	BNL3903	1,2,3,5
Chr7	BNL3871	2,3,7	Chr19	BNL852	2,3,5,6,7
Chr7	CIR141	3,4,5,6	Chr19	CIR139	2,3,6,7
Chr7	CIR262	2,3,6	Chr19	CIR222	2,6,7
Chr8	BNL1017	2,3,4,6,7	Chr19	JESPR218	2,4,5,7
Chr8	BNL3257	1,2,3,5,6,7	Chr19	JESPR236	2,4,5,7
Chr8	BNL3627	1,2,3,4,5,6,7	Chr19	TMB0131	2,3,5,7
Chr8	BNL3792	1,2,3,4,5,6,7	Chr20	BNL1145	1,2,3,6,7
Chr8	BNL387	2,3,7	Chr20	BNL119	2,3,4,5,6,7
Chr8	CIR209	2,3,6	Chr20	BNL169	3,4,5,6
Chr8	CIR244	2,3,6,7	Chr20	BNL2553	1,2,5,6
Chr8	CIR343	3,4,5,6,7	Chr20	BNL2570	2,3,5,6,7
Chr8	CIR376	2,6,7	Chr20	BNL3646	2,,5,6
Chr8	JESPR66	2,3,4	Chr20	BNL3660	2,3,7
Chr8	MUCS148	3,4,7	Chr20	BNL3948	2,3,5,6,7
Chr8	NAU1164	3,5,7	Chr20	BNL3993	2,3,6,7
Chr9	BNL1030	1,3,5,6,7	Chr20	CIR043	2,3,6,7
Chr9	BNL1317	3,4,5,6,7	Chr20	CIR063	2,3,6,7

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Supplementary Table 1. Continued.

Chromosome	Marker	Map	Chromosome	Marker	Map
Chr9	BNL1414	2,3,5,6,7	Chr20	CIR121	2,3,6,7
Chr9	BNL1423	1,6,7	Chr20	JESPR235	3,4,7
Chr9	BNL2847	2,5,6,7	Chr21	BNL1551	5,6,7
Chr9	BNL3582	1,2,5,6,7	Chr21	BNL3171	1,2,3,5,6,7
Chr9	BNL3626	1,5,7	Chr21	BNL3279	3,5,6
Chr9	CIR019	2,5,7	Chr21	BNL3449	2,3,5,7
Chr9	JESPR208	2,3,5,7	Chr21	CIR068	2,3,5,6,7
Chr9	JESPR290	2,3,7	Chr21	JESPR251	2,3,5
Chr9	MGHES70	2,3,7	Chr23	BNL1672	2,3,5,6,7
Chr10	BNL1161	1,2,3,5,6	Chr23	BNL2690	2,3,7
Chr10	BNL1665	2,3,4,5,7	Chr23	BNL3511	2,3,5,6,7
Chr10	BNL2705	2,3,4,7	Chr23	BNL686	2,3,5,6,7
Chr10	BNL3563	4,5,6,7	Chr23	CIR060	2,3,5,6
Chr10	BNL3790	2,3,7	Chr23	CIR286	3,5,6,7
Chr10	BNL3895	2,3,4,5,6,7	Chr23	JESPR110	2,5,7
Chr10	CIR171	3,6,7	Chr23	JESPR151	2,3,5
Chr10	TMB0307	2,3,5,7	Chr23	JESPR274	3,5,7
Chr10	TMB0325	2,3,5	Chr24	BNL1521	2,3,5,6,7
Chr11	BNL1034	1,2,3,4,6,7	Chr24	BNL1646	5,6,7
Chr11	BNL1151	1,3,4,5,6,7	Chr24	BNL252	3,6,7
Chr11	BNL1404	2,3,5,6	Chr24	BNL2597	2,5,7
Chr11	BNL1681	2,5,6	Chr24	BNL2961	1,2,6,7
Chr11	BNL2589	1,2,3,5,6,7	Chr24	BNL3474	2,5,6,7
Chr11	BNL2632	1,2,3,5,6,7	Chr24	BNL3860	1,2,5,6,7
Chr11	BNL2805	2,3,6,7	Chr24	CIR026	3,5,7
Chr11	BNL3411	1,2,5,6,7	Chr24	CIR413	2,5,6
Chr11	BNL3431	1,2,3,5,6,7	Chr24	JESPR157*	2,3,5,7
Chr11	BNL3442	1,2,3,5,6,7	Chr24	TMB0555	2,3,5
Chr11	BNL3592	2,3,4,5,6,7	Chr25	BNL1047	2,5,6,7
Chr11	BNL4094	2,3,5,6,7	Chr25	BNL1153	1,3,5,6,7
Chr11	BNL625	3,4,5,6,7	Chr25	BNL1417	4,6,7
Chr11	CIR003	4,6,7	Chr25	BNL1440	1,2,3,5,6,7
Chr11	CIR254	3,6,7	Chr25	BNL3103	2,3,4,5,6,7
Chr11	JESPR135	3,4,5,7	Chr25	BNL3190	2,3,4
Chr11	JESPR296	3,5,7	Chr25	BNL3264	2,3,5,7
Chr12	BNL1045	2,4,7	Chr25	BNL3806	2,3,5,6,7
Chr12	BNL1673	3,5,6,7	Chr25	CIR109	2,3,6,7
Chr12	BNL1707	2,5,6,7	Chr25	CIR150	2,3,5,6
Chr12	BNL2709	3,4,7	Chr25	CIR298	3,6,7
Chr12	BNL2967*	2,3,6	Chr25	CIR407	2,3,5,6,7
Chr12	BNL3261	2,5,6,7	Chr25	JESPR215	2,3,5,7
Chr12	BNL3599	3,5,6,7	Chr25	JESPR224	2,4,5
Chr12	BNL3835	3,6,7	Chr25	JESPR227	2,3,5,7
Chr12	BNL4059	1,2,3,5,6,7	Chr25	JESPR229	2,3,4,5
Chr12	CIR081	3,5,6	Chr25	TMB0313	2,5,7
Chr12	CIR293	3,6,7	Chr25	TMB0436	2,5,7
Chr12	CIR362	3,5,6,7	Chr26	BNL1669	2,3,5,6,7
Chr12	NAU1237	4,5,7	Chr26	BNL2495	1,2,3,5,6,7
Chr12	NAU1301	3,5,7	Chr26	BNL2725	2,3,4,7
Chr13	BNL1421	4,5,6,7	Chr26	BNL3368	1,2,3,7
Chr13	BNL1438	2,3,5,6,7	Chr26	BNL341	2,3,4,5,6,7
Chr13	BNL1495	4,5,6,7	Chr26	BNL3482	2,5,6,7
Chr13	BNL2449	3,5,6,7	Chr26	BNL3510	2,3,4,5,6,7
Chr13	BNL2652	1,2,5,6	Chr26	BNL3537	2,5,6,7
Chr13	BNL4029	3,5,7	Chr26	BNL3816	2,4,5
Chr13	BNL409	3,5,6	Chr26	CIR032	2,6,7
Chr13	CIR054	2,3,5,6	Chr26	CIR085	2,5,6
Chr13	CIR096	2,4,6	Chr26	MUSS303	3,4,5
Chr13	CIR342	5,6,7	Chr26	NAU1231	4,5,7
Chr13	CIR406	2,3,6,7			

Map* = 1 (Rong et al., 2004); 2 (He et al., 2007); 3 (Zhang et al., 2008); 4 (Frelichowski Jr. et al., 2006); 5 (Guo et al., 2007); 6 (Nguyen et al., 2004); 7 (Yu et al., 2007).

Supplementary Table 2. Amplification of different markers on chromosome.

Chromosome	Number of markers	Polymorphic rate (%)	Average amplified loci	Range of PIC value	Mean PIC value
Chr1	7	85.7	2.17	0.035-0.761	0.415
Chr2	4	75	1.00	0.035-0.462	0.180
Chr3	7	100	1.43	0.035-0.726	0.295
Chr4	5	100	1.80	0.035-0.748	0.438
Chr5	10	100	1.40	0.035-0.623	0.264
Chr6	8	50	1.75	0.035-0.734	0.405
Chr7	4	100	1.52	0.035-0.408	0.128
Chr8	12	91.8	1.91	0.035-0.816	0.451
Chr9	11	100	1.80	0.035-0.845	0.264
Chr10	9	100	2.56	0.035-0.855	0.589
Chr11	17	100	1.41	0.035-0.777	0.257
Chr12	15	93.4	1.36	0.035-0.796	0.266
Chr13	11	81.8	1.78	0.035-0.851	0.274
Chr14	8	87.5	1.29	0.035-0.614	0.374
Chr15	10	100	1.73	0.035-0.762	0.364
Chr16	5	100	1.40	0.035-0.628	0.346
Chr17	5	100	1.60	0.035-0.734	0.284
Chr18	7	85.7	1.33	0.035-0.611	0.318
Chr19	13	92.3	1.92	0.035-0.783	0.404
Chr20	13	92.3	1.75	0.035-0.742	0.429
Chr21	6	100	2.00	0.069-0.672	0.465
Chr23	9	100	1.56	0.035-0.783	0.361
Chr24	11	81.8	1.78	0.035-0.818	0.407
Chr25	17	88.3	1.53	0.035-0.788	0.314
Chr26	13	92.3	1.67	0.035-0.778	0.346

PIC = polymorphism information content.