Development and characterization of 165 SNP markers in Mimosa bimucronata (Candolle) O. Kuntze

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Abstract

Mimosa bimucronata (Candolle) O. Kuntze, native to the American tropics, is the worst invasive tree species in China, where it is distributed in the southern coastal areas of Guangdong and Fujian provinces. It is characterized by rapid growth and waterlogging resistance, and was introduced in China for slope and bank protection. However, there is still a lack of effective molecular markers for its invasion management. In this study, we used a qubit dsDNA HS assay kit to extract DNA, and DNBSEQ-500 whole genome re-sequencing technology to obtain single nucleotide polymorphism (SNP) markers. In total, we developed and characterized 165 SNP markers. Observed (H_O) and expected heterozygosity (H_E) ranged from 0.0667 to 0.1000, and 0.1800 to 0.5000, respectively. The polymorphic information content (*PIC*) ranged from 0.1638 to 0.3750. These SNP markers will help to analyze the population genetic structure and assess the invasion risk for *M. bimucronata*.

Full Text

Mimosa bimucronata (Candolle) O. Kuntze, native to the American tropics, is the worst invasive tree species in China, and has been listed by China's State Council (https://www.gov.cn/) to be in the fourth batch of alien invasive species in China. *M. bimucronata* produces numerous propagules, leading to a wide propagation range, rapid growth, and strong reproductive ability (Zeng et al. 2013). It can therefore easily form a strong community in a short time and overcrowd local species, and has caused serious ecological harm in Guangdong, Fujian, and Hainan provinces. *M. bimucronata* can grow in a variety of soil conditions (Xu. 2014). It is heat, waterlogging, and drought-resistant with strong adaptability (Liu and Liu. 2011). It was first introduced to China as a slope and bank revetment plant. It can also be used as firewood, and to study degraded habitats or green space restoration in tropical areas (Lan et al. 2019).

Previous studies (e.g., Lan et al. 2019) have mostly focused on the morphological characteristics, distribution range, and ecological habits of *M. bimucronata*. So far, there is insufficient comprehensive research on the genetic variation of the main Chinese population of *M. bimucronata*, and limited information on its genome and effective molecular markers, which further hinders research on its management. Therefore, there is an urgent need to carry out genetic research on major *M. bimucronata* populations in China to better manage and protect surrounding natural resources and biodiversity. As a third-generation genetic marker (Liu et al. 2019; Qu et al. 2019), SNPs are the most convenient and rapid DNA markers in genetic research due to their wide genomic distribution and polymorphism (Gao et al. 2019; Li et al. 2018; Xu et al. 2021). SNP has the advantages of CO dominance, high polymorphism, strong representativeness, and wide distribution (Sun et al. 2021; Wang et al. 2022;). Therefore, DNBSEQ-500 whole-genome sequencing technology was used to develop and characterize SNPs to provide genetic markers for population genetic diversity and conservation genetics studies (Milano et al. 2014).

M. bimucronata specimens were collected from 15 populations from Zhangzhou City and Xiamen City in Fujian Province, China. Genomic DNA was extracted using a Qubit dsDNA HS assay kit according to the manufacturer's instructions. For Illumina sequencing, a rad-library with a 150 bp double-ended short insert was generated. To obtain SNP marker resources, DNBSEQ-500 whole-genome sequencing was used for double-terminal (150 bp) sequencing. A total of 6813 putative SNPs of *M. bimucronata* were identified.

A total of 6197 PCR amplification primer pairs were successfully created using the primer v3.0 to design primers. After filtering, high-quality PCR amplification primer pairs from the 165 SNP sites were obtained. According to the manufacturer's instructions (BGI, Shenzhen, China), 25 μL of PCR mixture was used for the reaction. The procedure for PCR amplification was as follows: initial denaturation at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, annealing for 30 s (annealing temperature of each primer pair in Table 1), 72 °C for 30 s, and a final extension at 72 °C for 7 min. The PCR products were extracted and purified using 1% agarose gel electrophoresis and sequenced using DNA nanosphere (DNB) core sequencing and joint probe anchoring polymerization (cPAS) technology (BGI, Shenzhen, China). GenAlEx 6.5 (Peakall and Smouse. 2006, 2012) was used to calculate the observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (*PIC*), and Hardy–Weinberg equilibrium (P_{HWE}) of the successfully developed SNPs.

Two hundred SNPs were confirmed using Sanger sequencing. The observed H_O and H_E ranged from 0.0667 to 0.9333, and from 0.18 to 0.5, respectively. The *PIC* ranged from 0.1167 to 0.3750 (Table 1). After Bonferroni correction, significant deviations from the Hardy–Weinberg equilibrium (P_{HWE}) were observed at 12 loci (P < 0.05) (Sole et al. 2006). The newly developed *M. bimucronata* SNP markers play a sigificant role in population genetic analysis, natural resource protection and management.

Declarations

Acknowledgements

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Author contributions

Chun-Jing Wang and Ji-Zhong Wan conceived and designed the experiments. Qian Wang wrote the manuscript. Chun-Jing Wang and Ji-Zhong Wan revised the manuscript. Qian Wang performed the experiments. Ji-Zhong Wan and Chun-Hui Zhang conducted the fieldwork. Qian Wang and Ji-Zhong Wan provided formal analysis. All authors read and approved the final manuscript.

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Data availability

The data and material that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors do not have any conflict of interest.

Ethical approval

This article does not contain any studies with human participants, animals or endanger plants performed by any of the authors.

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Table

Table 1 Summary of the 165 SNP markers in *Mimosa bimucronata*

Primer ID	Primer sequence (5'–3')	SNP type	SNP position	H_O	H_E	P_{HWE}	*PIC*
SNP00002	CTGATGGGCTTGCTTCAATCA TGGTGGGGTCATTCTCAAAGG	67279	C/T	0.8667	0.4911	0.007930	0.3705
SNP00068	CTCAGCCAACTCCCATCGTT TGGGTCCTGTCTTTGTTGCT	346278	A/G	0.8000	0.4800	0.027900	0.3648
SNP00079	TCAGGAGCACAACTTTGAGTCT AAACGGCAGACATCTCAGGA	135468	C/T	0.8000	0.4800	0.027900	0.3648
SNP00095	CATGCAGGAGAGTGCTTGGA GTGCCAGAACCAACCTCAGA	142099	C/A	1.0000	0.5000	0.000211*	0.3750
SNP00153	CGATCAATGCCTCCGAATGC AGATTCGAAAGTCTCTGCTTCCA	590580	T/A	1.0000	0.5000	0.000211*	0.3750
SNP00158	TGGCACAAGGCAGAAGTTGA ATGAGAGGGCGGAAGAGGAT	52369	T/A	1.0000	0.5000	0.000211*	0.3750
SNP00198	ATGCTAGGCTTCCCAATGCT CACAGCTCGTAGGCTTCTCC	69325	T/A	1.0000	0.5000	0.000211*	0.3750
SNP00204	TGGGGAAGCCACTGAATGTC GAAATGGCTGGACCGATGGA	51715	T/C	0.8667	0.4911	0.007930	0.3705
SNP00286	TGTCCGCTGGTTACTAACAACA GCCCAACACAAAGCCACAAA	25634	A/T	0.8000	0.4800	0.027900	0.3648
SNP00307	AAGTGGCGTCGGAACTCATT TCTCCAGCTCCCGATCATCA	73043	T/G	1.0000	0.5000	0.000211*	0.3750
SNP00326	GGGGAGAGTTCACCAACAAAC CCTGATTTGTGCAGCGGTTT	159494	C/T	1.0000	0.5000	0.000211*	0.3750
SNP00328	TCGAGCAAGCATCTGAACCA GGAGCTGCAAAGGTTGTGTG	29813	A/G	1.0000	0.5000	0.000211*	0.3750
SNP00372	AGCGTCTACTGGGTGCATTT CCAAAGGGGCTGGAAGATTT	38301	A/T	1.0000	0.5000	0.000211*	0.3750
SNP00376	CGTGGAGCGGAGACTATTCA AGGCACACCATTCTCTTCTCT	47539	C/T	1.0000	0.5000	0.000211*	0.3750
SNP00413	GCTGCAAACAGTCCCTCCAT GACGTGGGTTCCTTTTATGCA	216394	G/A	1.0000	0.5000	0.000211*	0.3750
SNP00416	GCACAGGGCACTTTCTGTTC GCAACACTTGGCCCCTCTTA	248194	G/C	1.0000	0.5000	0.000211*	0.3750
SNP00439	ACCGACCCATCTATGCAAAGG ATGCACAAACGCACACACAT	146684	G/A	0.7333	0.4644	0.085300	0.3566
SNP00460	GCCGCTGCTTTTCTGCTATC TCCCTCCCTCGCTTTCCATA	22803	G/C	0.8667	0.4911	0.007930	0.3705
SNP00506	GGCTACCACCGAAACAAAGC ACAGTTCCTACAAACATCCAATAGC	157908	A/G	1.0000	0.5000	0.000211*	0.3750
SNP00523	AATGGAGGTTGCTCGATCCC TCGGGTCCATGCTTCATCTC	11075	A/T	0.8667	0.4911	0.007930	0.3705
SNP00665	CAAAGGGGCTGCAAAGTGTC TGTATTACCGTGTCCGTGTGT	93381	C/A	0.8667	0.4911	0.007930	0.3705
SNP00681	CTTGGAGGCTTGTTGTAGGC GTGTTGCTACTTCTTTCCTTCCA	18562	T/C	0.8667	0.4911	0.007930	0.3705
SNP00691	ACCCAGAAGTCATGCCCATG CAACATCCCAAACGCCTTCG	1321	C/A	0.4667	0.3578	0.528000	0.2938

SNP00716	TGCAGCCTGGAAGTGAAACA CCGGGTCATCAGCAATATTAGC	76703	T/C	0.8667	0.4911	0.007930	0.3705
SNP00744	GCGCACAGTAGCAGCAAATT CGAATCTCAAGGGCGTCTCT	147310	A/G	0.8667	0.4911	0.007930	0.3705
SNP00748	AACCATCTGGGAAGGCGAAG GCCACAGAAAACACCAAGCA	19133	A/G	0.8000	0.4800	0.027900	0.3648
SNP00793	TGCGGAACTCAAAGGTTTCCT TTCTGTCGCCCACAACACTT	2497	A/T	0.6000	0.4200	0.237000	0.3318
SNP00797	TGGGCGTTACAGCTTAGAACT CCACCTCAGCAAGCACAGAT	38616	G/C	0.7333	0.4644	0.085300	0.3566
SNP00828	AGCCACAGGAAAAGACAGGA CCGGAAGCAACTTCACATTCC	109549	T/C	0.8667	0.4911	0.007930	0.3705
SNP00836	TCACATCGTCACCTATTTCACA GCAGGGTGGTGGACAGTTAT	71767	A/G	0.8000	0.4800	0.027900	0.3648
SNP00845	TTCAGACGCCACTACCCTCT TCCTTGCGGAACAACCCTTT	12707	A/C	0.8000	0.4800	0.027900	0.3648
SNP00885	TGGACCCCAGAAAGTGTTGG CTGCTCTCTTGTGGGCTTCA	7724	A/C	0.8667	0.4911	0.007930	0.3705
SNP00895	GCCTGGATAGAGCAAAGCAT TCAGCAAGGCTACCAAGGAG	37386	C/T	0.8667	0.4911	0.007930	0.3705
SNP01044	AGTGCAGGCATAAGCTCAGT ACAGACTCTACGACCCTCACT	54766	T/G	0.8667	0.4911	0.007930	0.3705
SNP01050	TCCGAACCTGATCCCTTTGT TGGTGCAATGATGGAGTTGT	2525	T/G	0.6667	0.4800	0.289000	0.3648
SNP01058	ACTCCCTCCGGTCCTAAATAT TGGAGAGGTCAAGGTTACAAAA	154804	A/T	0.5333	0.3911	0.507000	0.3146
SNP01060	CATCTCGTCCATGCACCTGA ACCACTCCATTGCTTGTGTGA	13105	A/T	0.8667	0.4911	0.007930	0.3705
SNP01075	GCCTCGACACAGACAGCTTT GGCCCGAATTTTGCAGGTTT	38768	A/G	0.8667	0.4911	0.007930	0.3705
SNP01084	TGGGATTTGTGCGAATAGAGGA AAAGGAGTGTGCCCTTGAGG	154539	G/A	0.7333	0.4644	0.085300	0.3566
SNP01149	CGGAGGGAGTATTTGTTTACTTACT GGCAATATCTTATAGGTGAACTTGACA	20639	A/G	0.7333	0.4644	0.085300	0.3566
SNP01165	TGACCCTGCTGCCTTCAAAA TCGCAAATGACAATGGCAAGT	323303	T/G	0.4667	0.3578	0.528000	0.2938
SNP01308	TTGTGCGGCTACCACTACTG GGCGAGATTACCAGGACGAA	201011	C/A	0.8667	0.4911	0.007930	0.3705
SNP01457	ACACTCGATCACACACACACA GCGATGCCATGATTCGTCAC	25824	G/A	0.7333	0.4911	0.125000	0.3705
SNP01480	TGAGGACAACCCAATGGAAAA TCTTATGCAATACAAGAGGTCACA	76613	A/T	0.6667	0.4444	0.112000	0.3457
SNP01558	CGCACCAGTGATGGGAGAAA AAGACGTTGGCAGAGCAAGA	95570	T/C	0.8667	0.4911	0.007930	0.3705
SNP01565	GTGTGCCTGAGTCCATTGGA GAGGAGCGGTGTGATTTGGA	207242	A/G	0.8667	0.4911	0.007930	0.3705
SNP01631	TCCTCAGCAAGACCTCTTTCC TGCTGATCCTGTCGTCTCAT	64994	C/T	0.7333	0.4644	0.085300	0.3566

SNP01659	GAGCAGTGGGACAGCTTAGG ACTAAACCAGCAAGGCATCCT	71838	T/C	0.6000	0.4200	0.237000	0.3318
SNP01663	CTCTTCGCTCCAACCTTCCC AGAGGCCGTTTGTTACATGAGA	82762	C/T	0.8667	0.4911	0.007930	0.3705
SNP01664	CTCTTCGCTCCAACCTTCCC AGAGGCCGTTTGTTACATGAGA	82768	C/T	0.8667	0.4911	0.007930	0.3705
SNP01804	ACAGGACCAATAGCACAAGCA TCCCTTTCCCTTTCCCTGTC	166312	A/G	0.8000	0.4800	0.027900	0.3648
SNP01982	TCACAAAGAAGTCTACTAGGGTATCT CGGTTTTGCCCCCGTAAATT	119624	T/C	0.8667	0.4911	0.007930	0.3705
SNP02011	GTACCCTACGTGGCAGACAG AGCTCAGCAAGAAGGACCAA	241155	T/C	0.4667	0.3578	0.528000	0.2938
SNP02017	TTGGACCGCCACGTATCAAA GGCAATTCTGGGAAGGAAGC	237568	A/T	0.8667	0.4911	0.007930	0.3705
SNP02025	CCCAGCCTATACCCGCTTTT CTGGGCTATTTCCACCCCTAC	16383	T/A	0.8000	0.4800	0.027900	0.3648
SNP02070	TGCAGATCCACGGACTTCAC TGATGCCTGTTGTGGTTGGT	85828	T/A	0.5333	0.3911	0.507000	0.3146
SNP02123	CCTCCTCCCTGTGGTAAACA ACTGCTTGGCTTTTAATCCATTCA	35332	T/A	0.5333	0.3911	0.507000	0.3146
SNP02224	GGGAGGAGTTTTGGGTGTGT GGGACAAAGACTTACATGAAAAGCA	23913	T/A	0.6000	0.4200	0.237000	0.3318
SNP02260	TGCAGTTCAGATATGTGGGAA CCCTCACTCCCCTTGTTTCC	129921	A/G	0.6000	0.4200	0.237000	0.3318
SNP02290	TCATCCTGACGGTGAGCATC TCACTAAAGGGTGGGCTTCG	71176	A/C	0.8000	0.4800	0.027900	0.3648
SNP02379	CTCAACACTGCCACCTCCAT CCCTCAAGTTGGGTCAAACT	4492	G/C	0.5333	0.3911	0.507000	0.3146
SNP02449	CTTGGTGGGGTTTGTGGAGT TCTGCTGCTTCAGAGTCTTGA	195847	G/A	0.8000	0.4800	0.027900	0.3648
SNP02521	AGCAAAAGCCCATAATACTCTTATTGA TTTCCCAATAGCCCTTCTGTT	99529	A/G	0.7333	0.4644	0.085300	0.3566
SNP02567	TGATGACGTTTCAGCTCCAGA GCCTTTGCTGTGGATGTTTTCT	172995	G/C	0.8667	0.4911	0.007930	0.3705
SNP02647	CAGGTTTCTTCCCTTTTCATTACCC CGCGACTAGCCTCTCCATTT	1817	A/G	0.6000	0.4200	0.237000	0.3318
SNP02662	TCTCCCAAGCTTCCGTGATG TGTCTCTTCTGTGGAGTCTCT	176771	A/G	0.6000	0.4200	0.237000	0.3318
SNP02688	TCTCCACTGCCACTGAAACC GCCTACTCCTGGTGCTGTTT	66695	T/C	0.8000	0.4800	0.027900	0.3648
SNP02707	TCCCAAACCCATCTATCAAGCT TTCTGCAAGGCTGGAAAGGT	9760	T/A	0.8000	0.4800	0.027900	0.3648
SNP02721	GGTGGAAGCCTCTCATCCAG AGGTGACTGAGAAATGCGATGT	275579	C/T	0.6667	0.4800	0.289000	0.3648
SNP02736	TGCATCTGTGGGTTGGTCTG ACTTGTGGTCGCATGGTGTA	104395	C/T	0.8667	0.4911	0.007930	0.3705
SNP02773	ACCCACCTTGGTTTTGTTCA	4871	T/C	0.6000	0.4200	0.237000	0.3318

	AGCAATGCCAAAATGTCCAGA						
SNP02784	TGCTTGAGCGTGAAGGATGT TCTCACTGGTGTCATGTTGAA	56731	C/T	0.8667	0.4911	0.007930	0.3705
SNP02835	GCTCTCGGTGGTCACATGAA GGGCGACTATGGAAGGATCG	171853	G/A	0.4667	0.3578	0.528000	0.2938
SNP02891	GGGCAGTTGGTGTGTTGAAC ACATGGACTCCCTCATTGTCTG	94579	G/C	0.4667	0.3578	0.528000	0.2938
SNP02896	GGCCTTTGATGTGGGACAGA GTCCTCTTGCAATCTCCGTACA	50200	A/G	0.4667	0.3578	0.528000	0.2938
SNP02898	AATGGAGGTGGCAGAGAACC TGGAGATCATGTCGGGGTCT	55863	G/A	0.5333	0.3911	0.507000	0.3146
SNP02920	TGAACATACCCTGCGACCTC CCCATTTCCCATTTCAGATTCTCC	71242	A/C	0.8000	0.4800	0.027900	0.3648
SNP03001	TCGTCCTGTCCTGTCTCACA TCGGAAAGGGGAGGAAGTGA	152835	T/A	0.8000	0.4800	0.027900	0.3648
SNP03042	AGGATGCCGTTGTTGGTTGA CTGGCCCGCCCATTCTATTA	101393	G/A	0.8667	0.4911	0.007930	0.3705
SNP03049	AGGACGAGATGGAGTGTATGT AGCGAGCCCTAGAAGATCCT	83306	T/C	0.7333	0.4644	0.085300	0.3566
SNP03075	TGCAACAAGTCGTGCTTTGT TGGCCAAAATGGAAAGATGCA	7736	T/C	0.8000	0.4800	0.027900	0.3648
SNP03150	AGTGCTTACCCACCGTCTTG GTTGGATGCCAGTTCGGGTA	69627	C/T	0.8000	0.4800	0.027900	0.3648
SNP03168	TGGAGCTTGATATTCTCTCGAACT AGTTGCCTTGACAGTCCTGA	32849	T/C	0.8000	0.4800	0.027900	0.3648
SNP03191	GCTCGGCTCTCCATCCTTTT CTTGCTCAGACGCCTTTGGA	225091	T/G	0.7333	0.4644	0.085300	0.3566
SNP03193	GCTTGGACTTGGACACATGG AGGCAAACAGGTTCAATCATGT	29192	T/G	0.5333	0.3911	0.507000	0.3146
SNP03232	TCCAGAAGTTGAGCGTGCAT CCAAAGGTCCAACGGTTCCA	109100	A/G	0.4667	0.3578	0.528000	0.2938
SNP03345	ATTGGCCGAAGAGGTTGGAG GGAGAATGCCTTACCCTCCA	65869	A/G	0.8667	0.4911	0.007930	0.3705
SNP03402	ACTGTCACCCTCCCTCTCTT AGTACTCCCTCCAGTCTTACAT	138000	T/A	0.6000	0.4200	0.237000	0.3318
SNP03413	CAGAGCCTGGGGTTAGTTCG GTGGCTTTTCACACGCTTCC	39986	T/A	0.7333	0.4644	0.085300	0.3566
SNP03461	TCAGCAAGACAAATCTGGAGATCA GCCTCTGGTGGTTCAAGTGA	199506	T/G	0.8000	0.4800	0.027900	0.3648
SNP03504	CCCATGCCCTCCCTGTGATA TGCACGCACCAATCATTGTG	27997	A/G	0.6000	0.4200	0.237000	0.3318
SNP03530	CTTGACCCGAGCAGAAGGTT GGCTGCAATGACAACCACAG	376958	A/G	0.8667	0.4911	0.007930	0.3705
SNP03536	TCCACTCAACACCTGGTAGT TGTTGTCTCTCTGGACCTCCA	20600	A/G	0.6667	0.4444	0.112000	0.3457
SNP03581	AGTGTCCTGTCATCAATGCA AGAGCAGAAGAGACTTGTTTGA	146444	A/T	0.6000	0.4200	0.237000	0.3318

SNP03590	GTCTTCGACAACATGCAACTCT ACACCAAAGTCAAGCTGGAGT	84509	T/G	0.8667	0.4911	0.007930	0.3705
SNP03649	GACTTTGGACCCTGCACAGA ACGCAGCAACCTCGACTTAA	68757	A/G	0.6667	0.4800	0.289000	0.3648
SNP03729	AGTGAAGCCGTTGAGAGCAA GCAGATCACAAGCCAAACCA	90838	T/G	0.6000	0.4200	0.237000	0.3318
SNP03759	TGCTGCCCTGTCCAAATACT GCTGCTGACCCGATTAGAGG	40141	A/G	0.8667	0.4911	0.007930	0.3705
SNP03770	TCAGCCTAGCATGAAGACCT GCGCACAACCATTCTCTTCC	3757	C/T	0.8000	0.4978	0.045100	0.3739
SNP03778	AGAAGTTTGAATACCAAGGCAAAC GTTTACTTATTTAGGAGTTGGAAGTTGA	60047	C/A	0.0667	0.1800	0.103000	0.1638
SNP03919	TGGGTGGTGAAAGAGAGAAGG GGGGAAGCTGAGAGGAGAGA	142282	T/A	0.4667	0.3578	0.528000	0.2938
SNP04002	GCCAGAGGCTAGATCAGTGT TCCAGTTCTGTGGAGGGGAA	11618	T/C	0.8667	0.4911	0.007930	0.3705
SNP04025	TGCCTACCTATTCCCTTTTCTTCA AGTGTGAGGGCAAGACATGG	301766	C/T	0.7333	0.4644	0.085300	0.3566
SNP04070	TCCTGTTGGTTGTCGCCTTT GCCACTGGTCCTCTAATGCT	32095	A/C	0.4667	0.3578	0.528000	0.2938
SNP04205	CAGTTGAGAGCACGCCAGAT CCTCCTCCACCTTGTCTGAC	261746	C/T	0.6667	0.4444	0.112000	0.3457
SNP04263	TTTTGGATGGGCTGCTAGGG GTCGCCAGTTTCCCTGAAGA	21648	A/C	0.8000	0.4800	0.027900	0.3648
SNP04282	CGCCCGTGCAGTGTTGATAT CCTTGTTCCATAGGCAGAACC	47530	A/G	0.0667	0.1800	0.103000	0.1638
SNP04378	CATTGGCACACTCAGGGGAT GCTAGAGGCAGCAAGACCTT	7980	A/C	0.8667	0.4911	0.007930	0.3705
SNP04480	TCCATTTGAGGGCTTCTTCCC GAAGTGAACCGGCATGGAGA	94837	A/G	0.7333	0.4644	0.085300	0.3566
SNP04492	ACCCAGTATCAAGTGACGATCA TGGATAATAATGCTGTAACTCATTGGT	102964	C/T	0.8000	0.4800	0.027900	0.3648
SNP04497	GACCCTAGTGCAGAGCGAAA TGCAAAGAGGCTGTCACCTT	110199	G/A	0.8667	0.4911	0.007930	0.3705
SNP04707	CTGGCTCTTGCTCCTTCCAA GGGTGCCAAATGTGAGGAGA	177210	T/C	0.8667	0.4911	0.007930	0.3705
SNP04711	AGCCGACCCTCTACTTACAGT TGTGGATTTGGACTGTTCTCTT	30484	A/G	0.6000	0.4200	0.237000	0.3318
SNP04763	TCCATCACACCTCCAAGTCC ACATTGGGCTCTGGGTTTGG	168230	T/C	0.4667	0.3578	0.528000	0.2938
SNP04809	TGGAAAGCCAAAATGCACATGA ACCAACAGCAGCAACAACAA	336763	A/G	0.5333	0.3911	0.507000	0.3146
SNP04944	GGGGAGACACTGGCAAGATT ATTCCCTGCCCTTCTGGTTG	43679	T/A	0.7333	0.4644	0.085300	0.3566
SNP04964	CCGGAAAGAGTGGGCAAGAA TTCTCGTCAGACTTAAACCTAACT	401	A/G	0.7333	0.4644	0.085300	0.3566
SNP04975	GCACCTAGCACTTTCATGAGA TCCTCCCTCAAAACTTCAAGCA	57298	T/C	0.7333	0.4644	0.085300	0.3566

SNP04986	AGTGGATGAGAAACAAGGAGATGA TGCCTGGATAGCAAACATGGT	8117	G/A	0.8667	0.4911	0.007930	0.3705
SNP05028	ATGGCATTGTAGCTCCTCAG CTCGGCAGACACCATTCACA	49725	C/G	0.8667	0.4911	0.007930	0.3705
SNP05095	AGGCCCCAAGGGTATTTTGG ATGTGTGTGTGTGTGTGTGT	519	C/G	0.8667	0.4911	0.007930	0.3705
SNP05118	TGGTGGGCAGCAAATCATTC GTGGACCCATATGCGAGGAA	7377	C/T	0.8000	0.4800	0.027900	0.3648
SNP05186	CGTGCCCTTCTTCAACTCCT AACATGCAGATGTAGCGTAGT	142873	A/G	0.8000	0.4800	0.027900	0.3648
SNP05223	GCTCCAGGACAGCATTCGAT GCAAGTAGTCCACCACTGACA	15961	G/T	0.8667	0.4911	0.007930	0.3705
SNP05227	ACCCTGATCCCTCTCACGTT GCCGTGGATACTTTTCATGCA	434	T/C	0.8667	0.4911	0.007930	0.3705
SNP05240	ACTTGATTCCCGTGCAGTGA ACTCGTTCTCTCCCCCTGAA	108334	G/C	0.8000	0.4978	0.045100	0.3739
SNP05256	CTCATGTTGTGAAAATGGGGAGT TGTGCCTCATTAAAACCTCACT	24524	C/T	0.8000	0.4800	0.027900	0.3648
SNP05301	TGATGCTGGATTCGCACTCA TTTGGCTCAGTCCCACTAGC	335950	A/G	0.6667	0.4444	0.112000	0.3457
SNP05310	TCTGTCGTTTCCTCATAAATCAATGT GGGGCTGTGCTCTGACTTTA	11156	A/G	0.8000	0.4800	0.027900	0.3648
SNP05353	TGGGGTGCCACTTTGGTATC AGTAGCTCGGGTTCCTCCAA	49781	T/A	0.8667	0.4911	0.007930	0.3705
SNP05378	AAACTTCCGGCCTGAGAGTC TATGACCCTGGAGCCCATCA	435048	A/G	0.6000	0.4200	0.237000	0.3318
SNP05430	TCAGTGTTGGAGGTGCAGAC CCGGTGAGGTGACAAATTGC	85759	C/T	0.8667	0.4911	0.007930	0.3705
SNP05494	TGGCCAGTTGCTCCTCAATT GGCTCCCTCAATACCCTTGG	86003	T/C	0.6667	0.4444	0.112000	0.3457
SNP05611	AACTTCATGCCGGTCACACT GGCTCTCCACAATGTCCTTGA	276643	A/T	0.7333	0.4911	0.125000	0.3705
SNP05655	CAGCTCCACCTGACTTGTGT GTTCCTCCCCACAGGTTTCC	159965	T/C	0.8667	0.4911	0.007930	0.3705
SNP05704	TGGCGAAGGACATCACTCAC TGTTCACGCACAGTCGCTTA	87665	A/G	0.5333	0.3911	0.507000	0.3146
SNP05755	TGACCTTGGGTGTGTGTGTG ACCCATCGAGTTCAGATTAGTCA	167993	A/G	0.7333	0.4644	0.085300	0.3566
SNP05793	TGGTGTGGTGGTTTTGTTGG CTAGCTGGGCAGGTGACAAA	378884	T/A	0.0667	0.1800	0.103000	0.1638
SNP05799	ACCGACCCTGATTCTTGAACT CGCTGCATCATCAGTCCATG	13892	A/T	0.8667	0.4911	0.007930	0.3705
SNP05828	GCAGAAGCATCGTAAGTTTGC TGAAGCCAAAATGCAGGTAACA	326	A/G	0.5333	0.3911	0.507000	0.3146
SNP05843	AAGGGCGAGTTCTGTTCTGG AGCTTCGACCATCTCCTCCT	324552	T/C	0.7333	0.4644	0.085300	0.3566
SNP05992	TTCCCGGTCGTGTTTTCCAT ACATGACTGCCCTGAAGGTT	168300	C/T	0.6667	0.4444	0.112000	0.3457

SNP06056	TGCCCCATGTCACGAAGAAA GCACCTGAAGTCACCTCTGG	153004	C/T	0.8667	0.4911	0.007930	0.3705
SNP06063	AGCATTTGGGGTAGCAGCAT GTGGGGATGACAAGGAGCAT	135580	T/A	0.7333	0.4644	0.085300	0.3566
SNP06082	AGATGGCTTTTCCCGGATGA GGGGTCGTAGTCCACTTACC	40859	T/C	0.7333	0.4644	0.085300	0.3566
SNP06083	AGCAGCTTCAATGGCAGTCT ATGGCTCTCCCTCGACTACA	108410	C/G	0.4667	0.3578	0.528000	0.2938
SNP06093	TGTCATGCGTGCTGAACTCT TCCTGATGAGCTAACCCTACA	86858	T/C	0.7333	0.4644	0.085300	0.3566
SNP06110	ACGGCACCTTTCTTTCTCCA TGGCTGGGGCTGAATGAATT	130768	A/C	0.4667	0.3578	0.528000	0.2938
SNP06152	GGGTCTGGCTAAAGTTGTTTGC AACGCCCCAATAGTCCCAAG	62784	A/C	0.6000	0.4200	0.237000	0.3318
SNP06167	GCCTCCCCTTCCTCAATCAC GGATGAACGATGCCGGTACT	31917	T/C	0.8667	0.4911	0.007930	0.3705
SNP06212	AGGAGATGGTGTTCTTCCTAAGT TGTTGGTTGGTTAATTGGTCACA	574	C/A	0.8667	0.4911	0.007930	0.3705
SNP06213	AGGAGATGGTGTTCTTCCTAAGT TGTTGGTTGGTTAATTGGTCACA	579	G/T	0.7333	0.4644	0.085300	0.3566
SNP06252	TGGGAAGCTGGGCAAAATCA CAGACTGTGTCGGCAGCTAA	438659	A/G	0.8667	0.4911	0.007930	0.3705
SNP06293	CCAGGAATGGCTGCATTTGC AGAGCAATCCTTGACCCTTCA	22241	T/C	0.6000	0.4200	0.237000	0.3318
SNP06431	AACTCCTTTGCCACTGACCT AAGCCAACGTGCATACCTGA	42799	C/T	0.8000	0.4800	0.027900	0.3648
SNP06441	TGGGGCATTGGATGAAGAGG TGGGCTTGCTTTTCCCTAGG	3302	T/C	0.8667	0.4911	0.007930	0.3705
SNP06470	GCAACTTCACCCTCACTCTCA GGTGGTGGCAGAAATGGAGA	33373	G/C	0.8000	0.4800	0.027900	0.3648
SNP06503	AGGAATCACACGCACCTGAG GGCGAAATGGTTTGTATGTCTTG	15598	A/G	0.8667	0.4911	0.007930	0.3705
SNP06565	GGAAGGGAGTTGACTGAATGC ACTCGCCCTTTCACATCTCA	75141	G/T	0.4667	0.3578	0.528000	0.2938
SNP06609	TCCTGGAGAGATCTTTGGAGGT ACCAGCAGCATCAATCCTTT	279518	T/G	0.8000	0.4800	0.027900	0.3648
SNP06680	GCAGGCATGGACGATCTTCA ACGACCATCTTGAACCAGTCC	107729	G/A	0.8667	0.4911	0.007930	0.3705
SNP06681	CGGTTGCCCTATGCTTGAGA TCCCGCTGTCATCAATCTTGT	134787	T/C	0.8000	0.4800	0.027900	0.3648
SNP06727	GCAATGTGGGGAATCGGAGA TCGATACGCAGACTCCCTCT	41119	C/T	0.8000	0.4800	0.027900	0.3648
SNP06757	TGACATGAGGCTGCGATCTC ACCGACGAAAGGTGACGATT	13008	G/A	0.8000	0.4800	0.027900	0.3648
SNP06797	GTGCAGCCATTGAGCAAGAA CCTTCCCACACTGTCCAACA	45342	C/T	0.6667	0.4444	0.112000	0.3457

H_O observed heterozygosity, H_E expected heterozygosity, P_{HWE} the P value of the departures from Hardy–Weinberg equilibrium.

*Significantly deviated from Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.05/165$).