

Development of high-density interspecific genetic maps for the identification of QTLs conferring resistance to *Valsa ceratosperma* in apple

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Abstract *Valsa* canker (*Valsa ceratosperma* (Tode ex Fr.) Maire) is one of the most destructive fungal diseases of apple, especially in Eastern Asia. In this study, the first high density genetic linkage map of *Malus asiatica* × *Malus domestica* was constructed by 640 simple sequence repeats (SSRs) and 490 single nucleotide polymorphisms (SNPs), which spanned 1497.5 cM with an average marker interval of 1.33 cM per marker. Quantitative trait loci (QTLs) for apple's resistance to *V. ceratosperma* isolates 03-8

and xc56 were identified using the linkage map. Lesion lengths were used as the phenotypic data for the QTL analysis, which were measured on 1-year-old shoots inoculated with conidia of the two isolates. One QTL for resistance to isolate 03-8 was mapped on LG 16, and one QTL for resistance to isolate xc56 was detected on LG 9. Our research not only promoted the further understanding of the genetic basis of apple's resistance to *Valsa* canker but also provided two molecular markers that might be used in future marker-assisted selection for resistance in apple breeding programs.

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Introduction

Valsa canker, caused by the ascomycetous fungus *Valsa ceratosperma* (Tode ex Fr.) Maire, is one of the most destructive fungal diseases of apple, which severely limits apple production especially in Eastern Asia (Hayova and Minter 1998; Sawamura et al. 1990; Wang et al. 2011). *V. ceratosperma* infection can induce cankers on the trunk, branches, and limbs of apple trees, resulting in a significant decrease in fruit set and yield (Cao et al. 2009; Sawamura et al. 1990). Screening for resistance genes and the breeding of resistant cultivars are highly important for the modern apple industry.

Almost all commercial apple cultivars (*Malus* × *domestica*) are susceptible to *Valsa* canker (Bessho et al. 2004). After inoculating *V. ceratosperma* onto shoots or branches, over 94.9% of *M.* × *domestica* cultivars were found to be susceptible or highly susceptible (Abe et al. 2007; Liu et al. 1990, 2011a). However, resistant sources to *Valsa* canker have been found in wild relatives, such as *M. asiatica* and *M. sieboldii* (Abe et al. 2007; Bessho et al. 2004). Therefore, it is necessary for apple breeders to introduce resistant wild *Malus* species into cultivated apples (Abe et al. 2000).

The mycelia of *V. ceratosperma* collected from one orchard are incompatible on oatmeal agar medium, and this incompatibility is generally related to genetic differences among fungal strains, indicating that apple trees in the field are usually under the threat of more than one genotype of *V. ceratosperma* (Kohn et al. 1991; Suzaki 2008). The resistance of the host varies when inoculated with different *V. ceratosperma* isolates. For example, when inoculated with isolate xc56, the disease severities of *M. domestica* ‘Blushing Golden’ and ‘Spur Huaguan’ are much lower than those observed when inoculated with isolate 03-8 (Liu et al. 2011a). Thus, when evaluating genetic determinants of apple resistance for *V. ceratosperma*, the results are likely to rely on the inoculation of multiple isolates.

Knowledge of apple’s genetic resistance to *Valsa* canker is the basis for screening resistance genes or

cultivating resistant varieties, which are important for breeding. After inoculation with *V. ceratosperma*, the lesion lengths of *Malus* progenies from different crosses show continuous variation, implying the quantitative nature of apple resistance to *Valsa* canker (Abe et al. 2000, 2011). Based on the estimation of variance components for apple *Valsa* canker resistance, variance of the parental effect (general combining ability) was much larger than that of parental interaction effect (specific combining ability) (about triple), which suggests that the additive genetic variance was the major source of the total genetic variance of resistance (Abe et al. 2011). However, no molecular markers or exact chromosomal locations have been reported for apple’s resistance to *Valsa* canker.

Genetic linkage maps are indispensable tools for quantitative trait locus (QTL) analysis. Several linkage maps have been constructed using F₁ populations of *M. domestica* cultivars, such as ‘Prima’ × ‘Fiesta’ (Maliapaard et al. 1998), ‘Fiesta’ × ‘Discovery’ (Liebhard et al. 2002, 2003), ‘Ralls Janet’ × ‘Delicious’ (Igarashi et al. 2008), ‘Golden Delicious’ × ‘Jonathan’ (Zhang et al. 2012) and ‘Starking Delicious’ × ‘Jonathan’ (Moriya et al. 2013). In addition, some genetic maps for inter-specific populations have been developed, such as ‘Malling 9’ × ‘Robusta 5’ (Celton et al. 2009a), ‘Fiesta’ × ‘Totem’ (Fernández-Fernández et al. 2008) and ‘JM7’ × ‘Sanashi63’ (Moriya et al. 2012). The *M. asiatica* cultivars were once widely cultivated in China, and some of them are still commercially used (eFlorás 2013). Some *M. asiatica* accessions have great potential to be used as parents for breeding programs due to their moderate disease resistance and strong adaptability to the ambient environment (Abe et al. 2007; Liu et al. 2011b). However, no genetic linkage map has been developed for *M. asiatica*, not even between *M. asiatica* and *M. domestica*.

In this study, a dense genetic linkage map was constructed from 640 simple sequence repeats (SSRs) and 490 single nucleotide polymorphisms (SNPs) using the F₁ population of ‘Zisai Pearl’ × ‘Red Fuji’. Lesion lengths were used as phenotypic data, which were measured on 1-year-old shoots inoculated with the conidia of two *V. ceratosperma* isolates. The aim of this study was to identify QTLs for apple’s resistance to *V. ceratosperma*. The results will not only promote a further understanding of the genetic

basis of apple's resistance to *Valsa* canker but also provide molecular markers that may be used in future marker-assisted selection for resistance in apple breeding programs (Table 1).

Materials and methods

Plant and pathogen materials

An F₁ population derived from a cross between *M. asiatica* 'Zisai Pearl' and *M. domestica* 'Red Fuji' was used for phenotypic analysis, linkage map construction and QTL analysis. 'Zisai Pearl' is a Chinese domestic apple cultivar that exhibits good field resistance to *Valsa* canker compared with commercially popular *M. domestica* cultivars such as 'Red Fuji', 'Gala' and 'Golden Delicious' (Table S1). 'Red Fuji' is, however, susceptible to *Valsa* canker (Bessho et al. 1994; Liu et al. 2011a). The parents were crossed in 2007, and the hybrids (young seedlings from seeds) were planted in the orchard of China Agricultural University (Beijing, China) the following spring. The trees were grown on their own roots and planted at a density of 0.5 × 2.5 m², subjecting them to conventional fertilizer, irrigation and pest control.

Two *V. ceratosperma* isolates, 03-8 and xc56, were used as pathogens in this study. The 03-8 isolate was obtained from the Northwest Agriculture and Forestry University; xc56 was obtained from the Institute of Pomology, Chinese Academy of Agricultural Sciences. Isolate 03-8 was highly virulent, while isolate xc56 exhibited lower pathogenicity than 03-8 (Xing et al. 2013; Zang et al. 2007). The fungal mycelia were preserved in 30% glycerin at -80 °C and reactivated on potato dextrose agar (PDA) in darkness at 26 °C. The pathogenicity of the reactivated isolates was tested via an excised shoot assay using 1-year-old branches of the susceptible variety 'Red Fuji' (Bessho et al. 1994; Liu et al. 2011a).

Phenotypic analysis

To analyze the genetic variation and to estimate the heritability of resistance, a total of 740 and 743 individuals were used for inoculation with both isolate 03-8 and xc56 in March 2010 and 2011, respectively. Six dormancy-broken 1-year-old branches with identical diameters from each individual were collected and then cut to 30-cm lengths. Three branches per individual tree (as three replicates) were inoculated with isolate 03-8, and the other three branches were inoculated with isolate xc56.

Conidia of isolates 03-8 and xc56 were used as the inoculum. The fungal mycelia were cultured on PDA medium at 23 °C for 30 days to produce pycnidia. First, mature pycnidia were collected and ground in a sterilized mortar to release conidia. The mixture was transferred to a 50-mL centrifuge tube containing sterilized potato dextrose (PD) and centrifuged at 1800 rotations per minute for 5 min. Second, the supernatant-containing conidia was transferred into a sterilized conical flask and cultured at 200 rotations per minute in an incubator shaker at 26 °C for 18 h. Finally, the conidia suspension was adjusted to 2 × 10⁵ conidia/mL for inoculation.

Three inoculation sites (5 mm in diameter) were located on each branch segment at 10-cm intervals. The sites were punched deep to the xylem using a steel puncher, and the bark tissue was removed. Twenty milliliters of freshly-prepared *V. ceratosperma* conidia suspension (2 × 10⁵ conidia/mL) was pipetted into each inoculation site and then covered with sterilized moist towels to maintain moisture. Finally, the inoculated branch segments were placed in an artificial climate chamber at 25 °C under saturated humidity for 15 days. At that time, the lesion lengths were measured using a ruler (Gao et al. 2012; Liu et al. 2011a).

To determine the heritability of the resistance to *V. ceratosperma*, the variance of the 2-year combined

Table 1 Estimates of heritability of the resistance to *Valsa ceratosperma* in the F₁ population of 'Zisai Pearl' × 'Red Fuji' (*Malus asiatica* × *M. domestica*)

Isolate	Population variance (S)	genetic variance (Sg)	Environmental variance (Se)	Heritability (%)
03-8	3485.6	2949.9	535.7	84.6
xc56	4021.9	3199.0	822.9	79.5

phenotypic data was computed. Theoretically, the phenotypic values were determined by genotype and environment. The phenotypic variance (S) was calculated as the total variance of the population. The environmental effect variance (Se) was measured by averaging the variances for the individuals inoculated at both years. The genetic variance (Sg) was then estimated by subtracting Se from S . Finally, the heritability of the genes was expressed as Sg/S .

DNA extraction

Two hundred and ninety-six individuals were randomly selected as the mapping population from the F_1 population of 'Zisai Pearl' \times 'Red Fuji'. Young leaves of each progeny along with the parents were collected at the spring season for genomic DNA extraction. DNA extraction was conducted using a method previously described by Maguire et al. (1994). After RNase digestion, the concentration and purity of DNA were detected by ultraviolet spectrophotometry. DNA samples were diluted with deionized water to a concentration of 50 ng/ μ L and then stored at -20 °C.

Simple sequence repeat (SSR) genotyping

As shown in Table S2, a total of 1350 SSRs were initially collected. Among them, 325 genomic SSRs (G-SSRs) from *Malus* were searched from the HIDRAS website (<http://www.hidras.unimi.it/>); 885 expressed sequence tag SSRs (EST-SSRs) and 47 BAC end sequence SSRs (BES-SSRs) were obtained from the Apple EST Project website (<http://titan.biotech.uiuc.edu/apple/apple.shtml>). Sixty G-SSRs and 15 EST-SSRs were from *Pyrus* (Fernández-Fernández et al. 2006; Nishitani et al. 2009; Oddou-Muratorio et al. 2001; Sawamura et al. 2004; Yamamoto et al. 2002a, b). Eighteen G-SSRs came from *Prunus* (Dirlewanger et al. 2002).

After amplification in the two parents, polymorphic SSRs were used for genotyping of the mapping population. SSR genotyping was implemented using a three-primer strategy (Patocchi et al. 2009), including a forward primer with an M13 tail (5'-TGTAACGACGGCCAGT-3') at the 5' end, a regular reverse primer, and a universal M13 primer labeled with either FAM, HEX, or TAMRA (Applied Biosystems, Foster City, CA). The SSRs were divided into several groups based on size, and a preliminary

amplification experiment was performed. According to the amplification efficiency of SSRs, amplification systems were optimized by adjusting the rate of primers or regrouping. The SSRs that could not amplify after adjustment were subjected to double or single amplification. The composition of different amplification systems is listed in Table S3. PCR amplification was performed as follows: 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 45 s at 56 °C, and 45 s at 72 °C, 10 cycles of 30 s at 94 °C, 45 s at 53 °C, and 45 s at 72 °C, and a final extension at 60 °C for 10 min. Nine PCR products derived from three triplex PCR reactions with different fluorescent labels were mixed, and the mixture was diluted 1:10 as follows: 1 μ L of PCR product was mixed with 8.5 μ L deionized formamide and 0.5 μ L 500-ROX ladder. The mixture products were detected on an ABI 3730xl DNA analyzer (Applied Biosystems), and the genotype data were collected and analyzed using GeneMapper v3.2 software (Applied Biosystems).

Single nucleotide polymorphism (SNP) genotyping

The Illumina GoldenGate™ assay on the BeadStation system (Illumina Inc., San Diego, CA) at the W. M. Keck Center for Functional Genomics (University of Illinois at Urbana-Champaign) was used to analyze the SNP genotyping. A total of 1536 SNPs from the EST database of 14 apple genotypes, with the above 60-bp flanking sequence for primer design and the above 0.5 GoldenGate oligonucleotide pool assay (OPA) designability rank, were selected to constitute the SNP chip (Khan et al. 2012a). Genomic DNA (250 ng) from each sample (296 F_1 progeny and both parents) was used for SNP genotyping through the platform.

Based on the GenCall (GC) function of the BeadStudio package (Illumina, San Diego, CA), each SNP obtained a GC score corresponding to each seedling. The GC score for a SNP was calculated from the mean score of all 296 genotypes. The minimum threshold for GC score was 0.35 to declare a successful genotyping call for a SNP (Eckert et al. 2009; Jermstad et al. 2011). SNPs with mean GC scores <0.35 were considered to be unavailable and were removed from the analysis. SNPs with mean GC scores ≥ 0.35 were collected to compare sequence reads of all 296 F_1 progeny and both parents from the platform, and further procedures were performed.

Construction of the linkage map

The genetic linkage map was constructed using JoinMap version 4.1 (van Ooijen 2006). The segregation data of all SSRs and SNPs were tested for deviation from the expected Mendelian ratio (1:2:1) using the χ^2 test ($p < 0.01$). A logarithm of odds (LOD) of 4.0 was used to assign markers to different linkage groups. The Kosambi mapping function was used to calculate the genetic distance between molecular markers as described by Liebhart et al. (2003), and map distances were denoted in centi-Morgans (cM). The order of markers in a linkage group was confirmed with the Haldane mapping function. The genetic maps for each parent were independently constructed, and then the consensus maps were merged using the CP population model.

QTL mapping

MapQTL 6.0 software (van Ooijen 2009) was used to identify QTLs. First, the Kruskal–Wallis (KW) test was used to detect candidate QTLs. Then, interval mapping (IM) was used to identify QTLs. Finally, markers near the QTLs were searched for cofactors and performed under Multiple QTL model (MQM). After a 1000 permutation test, a LOD score of 2.8 was set as the genome-wide threshold to identify significant QTLs at a 95% confidence level. The linkage group ranges above the LOD threshold of 2.8 were identified as confidence intervals. The QTLs only confirmed by MQM are listed in the text.

Candidate gene prediction

According to the primers of the linked markers, NCBI BLAST was used to search the genomic database for Rosaceae (GDR) (https://www.rosaceae.org/tools/ncbi_blast) for physical positions of QTLs. The information on the apple genome was downloaded from the genome database for Rosaceae (GDR) (<http://www.rosaceae.org/>).

Results

Variation in lesion lengths among the F1 population

The symptoms of *Valsa* canker were observed, and the lesion length of each inoculation site were measured

(Fig. 1). The lesion lengths among progenies showed continuous variation in each year inoculated with each isolate (Gao et al. 2012), which suggested that the resistance of the F1 population to the two *Valsa ceratosperma* isolates was a quantitative trait controlled by multiple genes. The heritability of the resistance to *Valsa* canker was estimated as 84.6 and 79.5% for isolate 03-8 and xc56, respectively.

SNP and SSR genotyping and map construction

Out of the 1350 SSRs, 750 were polymorphic between ‘Zisai Pearl’ and ‘Red Fuji’, and of the 750 SSRs, 125 were discarded because they failed to amplify clearly or generated low-quality gel images. Finally, 625 SSRs, which generated 702 correct loci, were used to genotype the mapping population. These 625 SSRs were divided into $lm \times ll$ (26.6%), $nn \times np$ (20.5%), $hk \times hk$ (5.1%), $ef \times e.g.$ (23.8%), and $ab \times cd$ (23.9%). Among the 1536 SNPs, 546 exhibited mean GC scores ≥ 0.35 with the segregation of $lm \times ll$ (27.3%), $nn \times np$ (49.5%), $hk \times hk$ (22.7%), and $ef \times e.g.$ (0.5%).

The valid 702 SSR loci and 546 SNPs were initially used to construct the linkage map. However, 118 SSR and SNP loci were excluded because they either failed to link with any linkage groups or conflicted with segregation patterns of neighboring markers. There were 45 individuals that showed multi-recombination (i.e., frequent recombination in the linkage region, which was against Mendelian genetic principles), and they were excluded from the map population (Ma et al. 2016; Sun et al. 2015; Zhang et al. 2012). Finally, a consensus linkage map, which included 1130 markers distributed on 17 linkage groups, was constructed using 251 individuals (Fig. S1, Table 2). Among the 1130 markers, 238 markers (21.1%) underwent segregation distortion, and they were mapped to LG 5 (70.1 to 118.7 cM), LG 10 (8.7 to 45.8 cM), LG 12 (13.5 to 40.3 cM), and LG 16 (15.9 to 75.9 cM) of the consensus linkage map.

The consensus linkage map spanned 1497.5 cM, with an average marker interval of 1.33 cM per marker, and the genetic length of each linkage group ranged from 63.5 cM to 125.3 cM (Table 2). The number of markers on each linkage group ranged from 35 to 93, with an average of 66.5. Among the 17 linkage groups, linkage group 15 had the longest genetic distance (125.3 cM) and

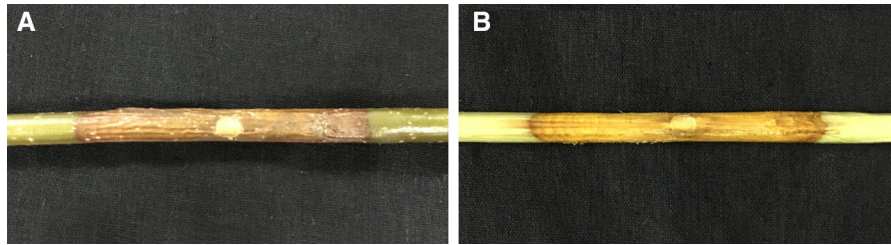


Fig. 1 The symptoms of *Valsa* canker (*Valsa ceratosperma*) near the inoculation site on the branches of progenies from ‘Zisai Pearl’ × ‘Red Fuji’ were observed at day 15 after inoculation

in vitro. **a** The lesion in the bark was crumpled, cankered and red-brown in color. **b** The lesion was orange in color and with a clear boundary after removing the epidermis. (Color figure online)

Table 2 The number, type, and density of markers on the consensus linkage map of ‘Zisai Pearl’ × ‘Red Fuji’ (*Malus asiatica* × *M. domestica*)

LG	Number of markers			Length (cM)	Average marker interval (cM)
	SSR	SNP	Total		
1	23	33	56	67.7	1.21
2	46	25	71	113.3	1.60
3	33	50	83	90.6	1.09
4	42	38	80	86.6	1.08
5	61	30	91	118.7	1.30
6	26	29	55	86.8	1.58
7	30	31	61	74.6	1.22
8	34	29	63	77.2	1.23
9	42	27	69	91.1	1.32
10	49	26	75	97.9	1.31
11	41	33	74	102.1	1.38
12	24	34	58	70.7	1.22
13	38	17	55	86.3	1.57
14	34	12	46	69.2	1.50
15	67	26	93	125.3	1.35
16	32	33	65	75.9	1.17
17	18	17	35	63.5	1.81
Total	640	490	1130	1497.5	1.33

contained the most number of markers (93). Linkage group 17 had the shortest genetic distance (63.5 cM) and contained the fewest number of markers (35), and it had the lowest average marker interval of 1.81 cM per marker. In addition, linkage group 4, which included 80 markers, was the densest linkage group, with 1.08 cM per marker. For the ‘Zisai Pearl’ and ‘Red Fuji’ map, 753 and 790 markers were mapped on the 17 linkage groups, respectively. The total genetic lengths of the linkage maps of ‘Zisai Pearl’ and ‘Red Fuji’ were 1313.6 and 1457.5 cM, and the average densities were 1.74 and 1.84 cM per marker, respectively (Table S4).

Identifying QTLs for resistance to *Valsa ceratosperma*

QTL analysis was carried out for resistance to isolates 03-8 and xc56 of *Valsa ceratosperma* using the Kruskal–Wallis test, interval mapping and multiple QTL model with a genome-wide LOD threshold of 2.8. As a result, a total of two QTLs, *Vc.03-8.f16* and *Vc.xc56.z9*, were identified (Fig. 2; Table 3). One QTL was detected for the resistance to isolate 03-8, and the other QTL for the resistance to isolate xc56. In interval mapping (IM), the significant interval of *Vc.03-8.f16* spanned the region from 80.1 to 80.2 cM of ‘Red Fuji’ LG16, and *Vc.xc56.z9* ranged from 48.8

to 84.3 cM of ‘Zisai Pearl’ LG9. While in multiple QTL model (MQM), the confidence interval of *Vc.03-8.f16* ranged from 72.1 cM to 72.3 cM of ‘Red Fuji’ LG 16, and *Vc.xc56.z9* was mapped on 53.6 cM to 55.9 cM of ‘Zisai Pearl’ LG 9. MQM was considered more efficient and accurate for mapping multiple QTLs, which used for further analysis (Jansen 1993, 1994). *Vc.03-8.f16* was mapped to LG 16 of the male parent ‘Red Fuji’ in 2011 with a peak LOD score of 2.9, accounting for 6.3% of the phenotypic variance. *Vc.xc56.z9* was detected on LG 9 of the maternal parent ‘Zisai Pearl’ in 2010 with a peak LOD score of 3.3, accounting for 7.4% of the phenotypic variance. In the Kruskal–Wallis analyses, the *Vc.03-8.f16* was supported with significant ($p < 0.05$) values 5.2 of the K statistic; *Vc.xc56.z9* was supported with significant ($p < 0.0005$) values 13.7.

Candidate gene prediction

Because the confidence intervals of the two QTLs were narrow, no gene located in the regions. Putative candidate genes were searched near the QTLs based

on gene function. On chromosome 16, two allene oxide cyclase (AOC) genes, MDP0000180004 and MDP0000239834, were approximately 238,738 and 242,599 bp away from *Vc.03-8.f16*; The RPM1 gene MDP0000759303 was located approximately 47,581 bp away from *Vc.03-8.f16*. Lastly, the ethylene-responsive transcription factor 118 (ERF118) gene MDP0000759299 was 32,141 bp away from *Vc.03-8.f16*.

Discussion

SSRs and SNPs have been widely used to construct genetic linkage maps in apple (Han et al. 2011; Ma et al. 2016; Sun et al. 2015; Zhang et al. 2012). In this study, a novel high density genetic linkage map was constructed with 640 SSRs and 490 SNPs using an interspecific population between *M. asiatica* and *M. domestica*. Compared with seven previously reported apple linkage maps, 91.1% markers in this study were mapped on the same linkage groups (Celton et al. 2009a, b; Han et al. 2011; Liebhard et al. 2002, 2003;

Fig. 2 QTLs for the resistance to *Valsa ceratosperma* detected in the ‘Zisai Pearl’ × ‘Red Fuji’ (*Malus asiatica* × *M. domestica*) mapping population. The confidence interval of QTLs was indicated by an *open box* for 2010 and a *black filled box* for 2011, respectively. Z 9: Linkage group 9 of ‘Zisai Pearl’; F 16: Linkage group 16 of ‘Red Fuji’. The ranges above the LOD threshold of 2.8 were identified as confidence intervals

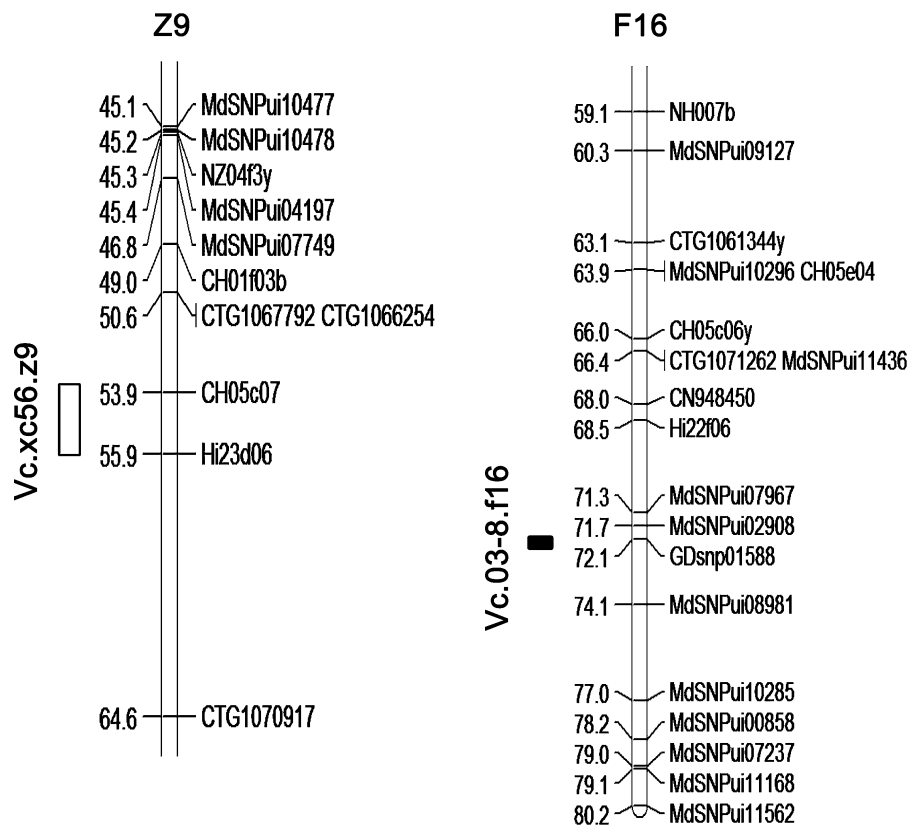


Table 3 Summary data for QTLs related to the resistance to *Valsa ceratosperma* in the ‘Zisai Pearl’ × ‘Red Fuji’ (*Malus asiatica* × *M. domestica*) population

Isolate	Year	QTL ^a	Parent	Linkage group	Peak (cM)	Interval ^b (cM)	LOD score	Expl (%)	QTL associated markers	K ^c
03-8	2011	<i>Vc.03-8.fl16</i>	Red Fuji	16	72.1	72.1–72.3	2.9	6.3	GDsnp01588	5.2**
xc56	2010	<i>Vc.xc56.z9</i>	Zisai Pearl	9	53.9	53.6–55.9	3.3	7.4	CH05c07	13.7*****

^a QTLs were named by the following three parts: *Valsa ceratosperma*, isolate 03-8 or xc56 and linkage group on male or female map

^b QTLs were identified by interval mapping (IM) and multiple QTL model (MQM), successively. The data presented in the table was from MQM. In interval mapping (IM), the significant interval of *Vc.03-8.fl16* spanned the region from 80.1 to 80.2 cM of ‘Red Fuji’ LG16, and *Vc.xc56.z9* ranged from 48.8 to 84.3 cM of ‘Zisai Pearl’ LG9

^c K: the highest value of the K statistic obtained in the Kruskal–Wallis (KW) test. The significance level of *Vc.03-8.fl16* and *Vc.xc56.z9* was 0.05 and 0.0005, which was indicated by ** and ***** , respectively

Silfverberg-Dilworth et al. 2006; Zhang et al. 2012). However, 24 of the 640 SSRs were located on different linkage groups, which is inconsistent with previous maps (Table S5). For the 490 SNPs, 5 SNPs (GDsnp01916, MdSNPui00311, MdSNPui02219, MdSNPui05507 and MdSNPui09716) were mapped on the different linkage groups from previous reports (Table S5) (Khan et al. 2012b, 2014). These results implied that our linkage map was likely to be accurate and the inconsistency mentioned above might be caused by structural variations in the apple genome (Khan et al. 2012b; Han et al. 2011).

In addition, five SSRs were previously reported as single locus markers, and in this study, mapped on two linkage groups. Hi03b03 was mapped on LG 12 of the ‘Fiesta’ linkage map, while mapped on LG 12 and LG 17 of the ‘Zisai Pearl’ map (Silfverberg-Dilworth et al. 2006). Moreover, CTG1069215 was detected on LG 15 of the ‘Co-op 17’ and ‘Co-op 16’ linkage maps, while they were detected on LG 15 and LG 8 of the ‘Zisai Pearl’ map in this study (Han et al. 2011).

In this study, the first genetic linkage map of *M. asiatica* × *M. domestica* was constructed with 1130 markers. Two hundred and thirty-eight of the 1130 markers on this genetic map exhibited segregation distortion (21.1%). For genetic linkage maps of *M. domestica* cultivars, the rates of segregation distortion markers were also found to be approximately 21% (Kenis and Keulemans 2007; Maliepaard et al. 1998; Liebhard et al. 2003; Zhang et al. 2012). Segregation distortion reflected the heritability of different chromosomal segments. The segregation distortion markers were located on LG 5, LG 10, LG 12 and LG 16 of this map. Similar clustered distributions of segregation

distortion markers were also reported on other genetic linkage maps of apple (Maliepaard et al. 1998; Liebhard et al. 2003; Zhang et al. 2012). This may be due to self-incompatibility or to the presence of a lethal gene (Kasha and Kao 1970; Savolainen et al. 1992).

Due to self-incompatibility, a long juvenile period, and high heterozygosity, apple breeding is difficult and time consuming. Marker assisted selection (MAS) is an efficient method to save time and labor by identifying young seedlings with specific traits (Longhi et al. 2014). *Valsa* canker, called the cancer of apple trees, is one of the most destructive diseases in apple, especially in Eastern Asia. For the first time, two QTLs, *Vc.03-8.fl16* and *Vc.xc56.z9*, for *Valsa* canker resistance were mapped on LG16 and LG9, respectively. However, the phenotypic variances explained by the two QTLs were both below 10.0%, which implied that the QTLs did not have great potential in efficient marker-assisted selection. QTLs with low variance explanation (sometimes called minor QTL) might be attributed to the multi-genic quantitative trait peculiarity of the resistance to *Valsa* canker (Gao et al. 2012). The quantitative nature of the resistance to *Valsa* canker was also reported in 23 full-sib apple families (Abe et al. 2011). Based on the estimation of variance components for apple *Valsa* canker resistance, variance of the parental effect (general combining ability) was about three times larger than that of parental interaction effect (specific combining ability), which suggested that multi-genic additive genetic variance was the major source of the total genetic variance of resistance (Abe et al. 2011). Additionally, the heritability (79.5–84.6%) of the

resistance to *Valsa* canker was not high enough. Environmental effects could also represent another cause of the low variance of the two QTLs (Jansen et al. 1995).

Valsa ceratosperma, *V. malicola* and *V. personii* can cause *Valsa* canker of apple, but *V. ceratosperma* is the most dominant pathogenic species in Asia (Wang et al. 2011, 2014). For *V. ceratosperma*, different isolates showed different population structures and population colors (Suzaki 2008; Zang et al. 2007). It was observed that the same *Malus* species exhibited different resistances when inoculated with different isolates. For example, when inoculated with an unknown isolate, *M. baccata* was determined to be a highly resistant species (Liu et al. 1990), but when inoculated with isolates 03-8 and xc56, most *M. baccata* ecotypes were susceptible (Liu et al. 2011a; Xing et al. 2013). Two isolates of *Valsa ceratosperma*, 03-8 and xc56, were used as inocula in our research, and the QTLs for the two isolates were inconsistent. The results suggested that the genetic resistance of the population mentioned above to the two isolates was different, and it might be attributed to the genetic variation between isolates (Cui et al. 2015; Zhuang et al. 2011).

It is important to identify genes that participate in apple's resistance to *Valsa* canker. Through transcriptomics analyses of apple bark infected with isolate 03-8, jasmonic acid and salicylic acid signaling were revealed to be the primary phytohormone pathways of apple against *Valsa* canker (Ke et al. 2014; Yin et al. 2016). The pathogenesis-related protein RPM1 is known to provide resistance to plants after recognizing pathogens (Grant et al. 1995). In this study, two AOC genes, RPM1 and one ethylene-responsive gene were identified linked to the confidence intervals of the QTLs. Special attention should be paid to these genes in future experiments.

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