Molecular Mapping of the Major Resistance Quantitative Trait Locus *qHS2.09* with Simple Sequence Repeat and Single Nucleotide Polymorphism Markers in Maize

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ABSTRACT

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The major quantitative trait locus (QTL) *qHS2.09* plays an important role in resistance to head smut during maize breeding and production. In this study, a near-isogenic line (NIL), L34, which harbors the major QTL *qHS2.09* in bin 2.09, was developed using a resistant donor 'Mo17' in a susceptible genetic background 'Huangzao4'. Using 18,683 genome-wide polymorphic loci, this major QTL was finely mapped into an interval of ≈ 1.10 Mb, flanked by single nucleotide polymorphism (SNP) markers PZE-102187307 and PZE-102188421. Moreover, the favorable allele

from 'Mo17' for SNP PZE-102187611 in this interval that was most significantly associated with resistance to head smut ($P = 1.88 \times 10^{-10}$) and accounted for 39.7 to 44.4% of the phenotypic variance in an association panel consisting of 80 inbred lines. With combined linkage and association mapping, this major QTL was finally located between SNP PZE-102187486 and PZE-102188421 with an interval of ≈ 1.00 Mb. Based on the pedigrees of 'Mo17' and its derivatives widely used in temperate maize breeding programs, the favorable haplotype from 'Mo17' is shown to be the main source of resistance to head smut in these lines. Therefore, the SNPs closely linked to the major QTL *qHS2.09*, detected in both linkage and association mapping, and could be useful for marker-assisted selection in maize breeding programs.

Additional keywords: fine mapping, genome-wide association, *Zea mays*.

Head smut, caused by *Sporisorium reilianum* (Kühn) Clint, is a serious maize disease, particularly where maize is grown under low temperatures or at high latitude (5,24). The spring maize-growing region in northeastern China, where weather conditions and farming systems are conducive for disease development, is a major head smut epiphytotic region of the world (19). Due to the high costs and environmental concerns to chemical management agents, resistance breeding is a more efficient, economical, and sustainable means for controlling the disease (22).

Resistant germplasm and gene banks hold the keys to the successful breeding of improved crop cultivars with durable resistance to disease (15). To date, several resistant inbred lines and germplasms have been identified in field studies under natural and artificial inoculation conditions (11,16,33,34,36), and the Lancaster subgroup has been identified as the most important source of resistance to *S. reilianum* in China (36).

With the application of molecular markers in quantitative trait locus (QTL) analysis, a number of resistance loci to various diseases, including southern leaf blight (SLB), gray leaf spot (GLS), and northern leaf blight (NLB) (3,4,8,17,18,29,38,41,43) have been detected by maize linkage mapping. Moreover, diverse genetic resources have facilitated research into the disease resistance

genes in maize, including the *Ht* gene for NLB resistance (37), the *Rp* gene for rust resistance (31), the glutathione *S*-transferase gene for multiple disease resistance (39), etc. Resistance to head smut is a complex quantitative trait under polygenic inheritance (1). QTL mapping has located one major resistance QTL on chromosome 2, in addition to other minor QTL identified on all other chromosomes except for chromosome 7 (9,18,21,24). We previously reported a major QTL in bin 2.09 that explains up to 43.7% of the phenotypic variance and confers resistance to head smut in a population of 184 $F_{2:3}$ families derived from a cross between 'Mo17' and 'Huangzao4' (21). This major resistance QTL, designated *qHSR1*, was also revealed to be flanked by markers simple sequence repeats (SSR)148152 and STS661 in the 'Mo17' derivative inbred line 'Ji1037' (9). Therefore, it will be important to finely map this major QTL and to identify closely linked markers useful in molecular marker-assisted selection for head smut resistance in maize.

Association mapping has been a powerful complement to classical mapping in bi-parental crosses as a strategy to detect quantitative trait loci underlying complex traits in plants. In *Arabidopsis*, the power of association studies for complex traits has been demonstrated using 107 phenotypes and 250,000 single nucleotide polymorphisms (SNPs) (2). In barley, the *HvbHLH1* gene that encodes a protein involved in anthocyanin pigmentation was finely mapped within a 140 kb interval in a genome-wide association study using 1536 SNPs derived from expressed sequence tags (10). In rice, six significant loci harboring candidate genes for grain shape (*GS3* and *GW5/qSW5*), seed color (*OsCI* and *Rc*), amylose content (*Wx*), and gelatinization temperature

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(*ALK*) were identified in an association study using ≈ 3.6 million SNPs in a panel of 517 rice landraces (14). In maize, association mapping in 350 tropical and subtropical maize inbreds with 1229 SNPs from 540 candidate genes for drought resistance identified allelic variations in three genes, which encoded a MADS-box (*PISTILLATA*) protein, a pyruvate dehydrogenase kinase, and an aldehyde oxidase involved in ABA and carbohydrate metabolism in floral tissues during drought stress (32). With 253 maize inbred lines, a glutathione *S*-transferase gene was found to be associated with multiple disease resistance to SLB, GLS, and NLB (39). Using a nested association mapping (NAM) population in maize, loci associated with effects on SLB (17), NLB (29) flowering time (7), and leaf architecture (35) have been detected using 1.6 million SNPs (12).

When combined, linkage mapping and genome-wide association have been even more powerful approaches to identify candidate QTLs for complex disease traits. For example, natural allelic variation for resistance to downy mildew has been detected in *Arabidopsis* (27) using such a combined approach. In maize, particularly, NAM has been used for disease resistance with a combined QTL mapping and association mapping approach (17,29). The objectives of this study were to (i) finely map the major QTL *qHS2.09* with SSR and SNP markers in five near-isogenic lines, (ii) perform a genome-wide association study of head smut resistance using 41,101 SNP markers in 80 Chinese elite maize inbred lines, and (iii) examine the favorable haplotype for head smut resistance in inbred line ‘Mo17’ and its derivatives. Knowledge of the genetic basis of head smut resistance will facilitate selection of resistance genes for this disease in maize breeding.

MATERIALS AND METHODS

Plant materials. In this study, five near-isogenic lines, L34, L52, L162, L163, and L170, were developed from the BC₄F₂ with the susceptible background (‘Huangzao4’) carrying the major resistance QTL from the resistant donor line (‘Mo17’) in bin 2.09 using SSR marker-assisted selection (Fig. 1). A total of 80 inbred lines representing two subgroups identified by population structure analysis (42), LAN (Lancaster Sure Crop), and SPT (lines derived from Si-ping-tou, a Chinese landrace) (Table 1) were used in this genome-wide association study. ‘Mo17’ and ‘Huangzao4’ were the key maize inbred lines in these two subgroups, respectively (42).

Evaluation of resistance against *S. reiliana*. Resistance responses of the maize inbred lines to *S. reiliana* infection were recorded after artificial inoculation in the field from August to September 2009 and 2010 at Harbin, Heilongjiang Province, China (45.8°N, 126.5°E). The lines were planted in randomized complete blocks with two replicates. Each plot consisted of two 5 m rows with 0.67 m between rows, which were over-planted and later thinned to 25 plants per row. Artificial inoculation was performed by sowing seed together with 0.1% *S. reiliana* inoculum. Sori containing teliospores of *S. reiliana* were collected from the field during the previous growing season and stored in cloth bags in a dry and well-ventilated environment. Normal agronomic practices were used in field management (40). At maturity, each plant was scored for *S. reiliana* sori when disease symptoms were fully expressed, and the mean of two replicates per plot (21) was subjected to further analysis as described below in genotype-phenotype association mapping.

Fig. 1. Fine mapping of the major quantitative trait locus *qHS2.09* associated with reduced disease incidence for head smut, with **A**, simple sequence repeats and **B**, single nucleotide polymorphism markers on chromosome 2. The gray bars indicate the chromosome regions from ‘Mo17’, and black bars indicate the regions from ‘Huangzao4’. Mean disease incidence for near-isogenic lines in 2010 is indicated by the number on the right of the bars. The names of near-isogenic lines are indicated on the left of the bars.

SSR assay. Genomic DNA of near-isogenic lines was extracted from the upper two leaves of 20-day-old plants from near-isogenic and parental lines ('Huangzao4' and 'Mo17') using the CTAB procedure (26). One hundred and twenty-five polymorphic simple sequence repeat (SSR) markers evenly distributed on 10 chromosomes, published in a previous study (9,21), were used for marker-assisted selection of the *qHS2.09* locus and its genetic background in subpopulations. Polymerase chain reaction (PCR) was performed using a PTC-200 Thermal Cycler (Bio-Rad). A total volume of 10 µl of reaction mixture composed of 10 ng of genomic DNA, 1× reaction buffer (Tiagen, Beijing, China), 2.0 mM MgCl₂, 1 U of *Taq* DNA polymerase (Tiagen, Beijing, China), 0.1 mM of dNTPs (Tiagen, Beijing, China), and 0.9 mM primers. PCR cycle conditions included an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55 or 58°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 7 min. Amplification products were resolved by electrophoresis on 10% polyacrylamide denaturing gels at 150 V for 2 h, and the bands were silver-stained, and photographically recorded for analysis.

Genotyping. SNP genotyping was performed using the MaizeSNP50 BeadChip processed by Emei Tongde (Beijing). The SNP content featured on this chip, using 56,110 evenly spaced SNPs to cover the entire maize genome according to the B73 genome reference sequence, was selected from several public and

private sources with 984 negative controls (Illumina, Inc.). DNA from these 80 maize inbred lines was extracted by a modified CTAB procedure according to a previous report (26), and prior to genotyping, DNA quality for each sample was carefully checked using electrophoresis and a spectrophotometer (Nanodrop 2000, Thermo Scientific). The SNP genotyping of these 80 lines was performed according to the Infinium HD assay ultra-protocol guide (Illumina, Inc.).

Linkage mapping. In the previous result, the major QTL *qHS2.09* was mapped into an interval between markers SSR148152 and STS661 in bin 2.09 (9,21). Five NILs harboring the major resistance QTL in bin 2.09 were generated using 125 SSR markers in a BC₄F₂ population. The line L34, 'Mo17', and 'Huangzao4' were analyzed using 56,110 high-density genome-wide SNP markers. The location of the major QTL *qHS2.09* was then narrowed into an interval between two outermost SNP markers, which were derived from the donor lines in bin 2.09. Compared with the physical position of SSR and SNP markers, this QTL was finally mapped.

Genotype-phenotype association mapping. SNP alleles were calculated using PowerMarker 3.25 (23). The population structure and kinship information of 80 lines for a mixed linear model (MLM) were input using the softwares STRUCTURE version 2.3 (30) and SPAGeDi (13), respectively, with 5,000 SNPs (minor allele frequencies, MAF ≥ 0.2). STRUCTURE was run to test K = 2 according to our previous study (42) three times with a burn-in

TABLE 1. Analysis of the pedigrees and disease incidences for 80 inbred lines in the association panel in 2009 and 2010

Inbred lines	Pedigrees	Subgroup ^a	Incidence (%)	
			2009	2010
Mo17	187-2/C103	LAN	1.3	0.8
Yu12	Derived from Mo17	LAN	2.6	0.8
Ji412	Derived from Mo17	LAN	9.6	4.6
Ji419	B68 ^{ht} /Mo17	LAN	8.9	2.3
Ji465	Improved Mo17	LAN	4.2	1.2
Ji842	Ji63/Mo17	LAN	2.5	1.9
J002	Ji63/Mo17	LAN	2.2	2.8
Huotanghuang	Huotangbai/Mo17 ^{ht}	LAN	2.4	1.1
Guan17	Mo17/Guan73	LAN	3.0	0.8
706fu	Improved C103	LAN	3.8	1.2
Zhong17	Zhong17	LAN	5.0	2.1
H3	H3	LAN	2.5	7.4
485	Ai331/C103	LAN	5.6	2.6
Si533	8112/416	LAN	9.6	1.4
Ji992	Mo17/Ji846	LAN	6.2	5.0
C416	Mo17/Dan360	LAN	4.6	1.9
D185	Mo17//B37/Mo17	LAN	1.2	0.8
JiA-034	Mo17/78599	LAN	4.8	4.6
ZaC546	C103	LAN	10.7	4.9
416	404/Mo17	LAN	6.0	3.9
Dan1324	Mo17//NN14B ^{ht2} /Mo17	LAN	2.2	2.5
Ji846	Ji63/Mo17	LAN	5.9	1.4
Ji477	Recycled from hybrid HC777	LAN	1.1	2.2
He344	Baitoushuang/Mo17	LAN	2.2	0.8
C649	Mo17/Jinhuanghou	LAN	3.9	2.5
Si-419	B68 ^{ht} /Mo17	LAN	1.4	0.8
M0113	1134/Mo17	LAN	3.0	6.3
4F1	Mo17 ⁶⁰ Co	LAN	3.8	1.5
Ji495	Mo17/L105//Mo17	LAN	2.2	0.8
J001	Ji846/Mo17/Ji846	LAN	0.8	2.5
Longkang11	Mo17/Zi330	LAN	4.0	1.1
D387	Mo17//L105/8112	LAN	6.0	8.5
Ji1037	Mo17//Suwan1/Mo17	LAN	4.0	2.6
Suixi701	He344/Mo17	LAN	3.0	2.5
5213	Improved Mo17	LAN	7.9	2.5
CN165	Recycled from hybrid P3268	LAN	1.4	0.8
Zao8-3	Zao8-3	LAN	29.8	42.4
HZ85	Recycled from hybrid 3385	LAN	22.2	33.3
Zhonghuang204	Recycled from Mo17	LAN	28.0	17.7

(Continued on following page)

^a LAN is the Lancaster subgroup, SPT is the subgroup from Si-ping-tou germplasm.

period of 500,000 and 500,000 replications. The MLM implemented in the program TASSEL 3.0 version (6) was used for association mapping with 41,101 SNPs ($MAF \geq 0.05$) in these 80 inbred lines.

RESULTS

Molecular mapping of the major resistance QTL *qHS2.09* with SSR markers. Using previous QTL mapping results, five NILs harboring the marker SSR148152 in bin 2.09 from ‘Mo17’ were generated by selecting for 125 polymorphic SSR markers in a BC₄F₂ population. The recovery rate for the genetic background of ‘Huangzao4’ in these five lines was more than 96.9%. Under field conditions using artificial inoculation in 2010, disease incidence in these five NILs was significantly decreased relative to that of ‘Huangzao4’, from 77.6 to 4.9%. Only one homologous introgression fragment was detected on chromosome 2 in L34, so this line was chosen for beadchip analysis (Fig. 1A).

Validation of the QTL *qHS2.09* with SNP markers. Using the MaizeSNP50 BeadChip, 18,683 genome-wide SNP loci between ‘Mo17’ and ‘Huangzao4’ were generated and 10 loci were found to be polymorphic between L34 and ‘Huangzao4’. Furthermore, two adjacent introgression fragments were shown to have been transmitted from ‘Mo17’ into L34. The major QTL for head smut resistance was finally narrowed into an interval of ≈ 1.10 Mb, flanked by SNPs PZE-102187307 and PZE-102188421 (Fig. 1B).

Genome-wide association study of resistance to head smut.

Disease incidence for 80 inbred lines in 2009 and 2010 was significantly correlated ($P < 1.00 \times 10^{-4}$), and the continuous distribution of disease incidence indicated quantitative inheritance of resistance to head smut (Table 1). Differences in mean disease incidence have been detected, with the lower level, 8.7%, in the Lancaster subgroup, and 40.1% in the Si-ping-tou subgroup, which suggested that the resistance genes originated from the Lancaster subgroup, especially the derivatives of ‘Mo17’ (Table 1). Ten SNPs across 10 chromosomes were significantly associated with disease incidence ($P \leq 1.00 \times 10^{-4}$) in 2009 and 2010, and among these, SNP PZE-102187611 in bin 2.09 was most significant. Three QTLs (SNP $P \leq 1.00 \times 10^{-4}$) conferring resistance to head smut, designated *qHS1.03*, *qHS2.09*, and *qHS4.03*, were detected on chromosomes 1, 2, and 4, respectively, in this association panel (Fig. 2; Table 2). The QTL *qHS2.09* within this region containing four closely linked SNP loci was the major locus associated with response to *S. reilian*a infection. With combined linkage and association mapping, the *qHS2.09* was finally mapped into an interval of ≈ 1.00 Mb between SNP PZE-102187486 and PZE-102188421. The QTL *qHS1.03*, associated with SNP PZE-101045549, was detected in 2009 and 2010, and *qHS4.03*, associated with SNP SYNGENTA6546, was detected only in 2010 (Fig. 2; Table 2).

SNP PZE-102187687, which has three genotypes (AA/CC/--), and located adjacent to SNP PZE-102187611, was not significantly associated with head smut resistance (Table 2). However,

TABLE 1. (Continued from preceding page)

Inbred lines	Pedigrees	Subgroup ^a	Incidence (%)	
			2009	2010
CA091	505/o2/7091//7091	LAN	25.7	14.9
Zao49	He344/Taixi138	LAN	63.8	53.5
Liaobai371	Recycled from hybrid	LAN	89.7	68.0
Huangzao4	Tangsiplingtou	SPT	78.4	63.3
Wenhuang	Huangzao4/Wenqing1331	SPT	82.2	65.2
Danhuang02	LvDa Red Cob Group	SPT	89.1	74.9
CN962	Recycled from Huangzao4	SPT	53.8	46.1
Luyuan133	Qi721/Huangzao4	SPT	20.5	12.5
196	Huangzao4/Dan340	SPT	78.1	56.7
H201	Dan340/Ye515//Huangzao4	SPT	14.1	13.3
7379-2	Jingyu1	SPT	72.4	48.6
H152	Huangzao4/Mo17//F28/150	SPT	68.8	44.8
PI143	Aiyuan311/Lu9801//Chang7-2	SPT	87.6	72.2
Tangsiplingtou	Sipington germplasm	SPT	55.4	30.5
PI42	Huangzao4/LU9801//Chang7-2	SPT	78.8	62.4
J9206	444/Danhuang02	SPT	42.0	38.0
Sizhisi	Huangzao4/Zi334//Huangzao4	SPT	23.5	15.4
H21	Huangzao4/H84	SPT	74.6	48.8
Chang7-2	Huangzao4/Wei59//S901	SPT	80.0	69.3
Jing7	Huangzao4/Luoxi3	SPT	62.5	47.6
Huangyesi	Yejihong/Huangzao4//Dunzihuang	SPT	39.2	34.5
444	Huangzao4/A619 ^{ht}	SPT	46.7	36.5
K12	Huangzao4/Weichun	SPT	57.3	31.5
Ye502	Huangzao4/Dan340	SPT	76.8	65.8
Ye515	Huafeng100/C103//Huangzao4	SPT	40.7	38.5
Dhuang212	D729/Huangzao4	SPT	88.4	68.8
LX9801	Ye502/H21	SPT	89.8	51.2
Huang428-3	Huangzao4/E28	SPT	48.6	42.4
Si287	444/255	SPT	17.6	13.8
Si279	Huangzao4/428	SPT	84.4	51.5
Ji853	Huangzao4/Zi330//Huangzao4	SPT	32.4	20.9
374	Huangzao4/Qun105//Duohuang15	SPT	8.1	3.8
Te70	He344/422	SPT	9.7	4.2
B467	434/444	SPT	2.5	1.1
Tianya4	Wu109/Huangzao4	SPT	7.6	4.8
Suixi605	8941/444	SPT	4.2	2.3
434	XL66/Hua94	SPT	6.7	5.9
Wu314	Huangzao4//Wu302D/Huangbaolie	SPT	6.9	9.6
Ji35	Huangzao4/Jiduo142	SPT	4.7	11.0
L34	Huangzao4/Mo17	SPT	2.3	1.9
L270	Huangzao4/Qi319	SPT	2.5	2.9

the mean disease incidence for 17 lines carrying the ‘Huangzao4’ genotype at the SNP PZE-102187687 locus was 56.1%, which was much higher than that for other lines in this association panel (Table 3), with an average of 14.9%.

Haplotypes for resistance to head smut. In this genome-wide association study, four SNP loci, PZE-102187486, PZE-102187611, PZA00672.8, and PZE-102188421, were significantly ($P < 0.0001$) associated with disease incidence in the association panel, and 11 haplotypes harboring these four loci and SNP PZE-102187687 were generated in ‘Mo17’, ‘Huangzao4’, and their derivatives (Table 3). In the association panel, 30 inbred lines were derivatives of ‘Mo17’, and 26 inbred lines were derived from ‘Huangzao4’. The data showed that the mean disease incidence was 3.2% for 28 of these lines and was much lower than 39.8% in ‘Zhong204’ and ‘H152’ in the 30 derivatives of ‘Mo17’ (Table 1; Table 3). The favorable haplotype from ‘Mo17’ provided the major resistance to head smut in this panel.

DISCUSSION

Under natural field evaluations of head smut infection, Lu and Brewbaker (24) identified four QTLs on chromosomes 1, 3, 9, and 10, in 100 recombinant inbred lines derived from ‘Hi34’ and ‘TZil7’. Lübberstedt et al. (18) found three QTLs in the filed trails in France, and eight QTLs in China, using 220 F_{2:3} families from a cross between two European elite inbred lines, ‘D32’ and ‘D145’. Environmental and genetic background effects caused some inconsistencies in estimation of the position and magnitude of the QTLs. Therefore, the QTL identified for head smut resistance in this study should be revalidated under artificial inoculation conditions.

Using artificial inoculation, Li et al. (21) identified five QTLs, one each on chromosomes 1, 3, and 8, and two on chromosome 2, accounting for 5.0 to 43.7% of the phenotypic variance across four environments in 184 F_{2:3} families derived from a

Fig. 2. Genome-wide association analysis of resistance to head smut in maize with mixed linear model in **A**, 2009 and **B**, 2010. The single nucleotide polymorphisms (SNPs) significantly ($P < 0.0001$) associated with head smut resistance are indicated by arrows. Value of $-\log_{10}(P)$ was indicated by the y axis. Position of the SNPs in **B** in 2.09 is indicated by the gray vertical line.

TABLE 2. Three quantitative trait loci (QTLs) for head smut resistance using a genome-wide association study in 2009 and 2010

QTL	Single nucleotide polymorphism	Bin	Physical position	Genotype	P value		R ² (%)	
					2009	2010	2009	2010
<i>qHS1.03</i>	PZE-101045549	1.03	31,582,444	AA/GG	4.85E-05	1.00E-04	19.8	18.7
<i>qHS2.09</i>	PZE-102187486	2.09	231,382,602	AA/CC/-- ^a	3.64E-05	4.65E-04	20.5	16.0
	PZE-102187611	2.09	231,660,578	AA/GG	9.39E-09	6.56E-08	44.4	39.7
	PZE-102187687	2.09	231,712,426	AA/CC/--	8.70E-01	9.92E-01	9.12E-04	3.60E-06
	PZA00672.8	2.09	232,004,713	AA/GG/--	3.21E-07	7.99E-06	37.5	29.3
	PZE-102188421	2.09	232,381,945	AA/GG	4.21E-07	1.69E-06	32.6	29.8
<i>qHS4.03</i>	SYNGENTA6546	4.03	19,329,412	AA/GG	6.78E-04	5.32E-05	13.4	20.3

^a -- is the deletion in the SNP locus.

cross between resistant ‘Mo17’ and susceptible ‘Huangzao4’. The major QTL in bin 2.09 could explain up to 43.7% of the phenotypic variance in that panel. Chen et al. (9) found four QTLs on four chromosomal regions (bins 1.02/3, 2.08/9, 6.07, and 10.03/4) in the BC₁ population derived from the highly resistant donor parent, ‘Ji1037’, and the highly susceptible recurrent parent, ‘Huangzao4’. A major resistance QTL, *qHSR1* in bin 2.08/9, was detected and flanked by the markers SSR148152 and STS661. ‘Ji1037’ was originally derived from a cross between ‘Mo17’ and tropical germplasm (Table 1). The major QTL in bin 2.09 was probably transmitted from the same resistant donor, which was supported by the occurrence of a consistent

haplotype between ‘Ji1037’ and ‘Mo17’ in this major QTL region (Table 3).

Differences in mean head smut incidence among six subgroups have been observed, with the highest level, 56.4%, in the Si-ping-tou group, and the lowest level, 1.8%, in the Lancaster group (36). Similarly, relatively low disease incidence was observed in 37 Lancaster subgroup lines (Table 1), and one major resistance QTL, *qHS2.09*, was detected across two environments in this association panel (Table 2; Fig. 2). Twenty-four derivatives of ‘Mo17’ carrying the favorable haplotype from ‘Mo17’ showed reduced disease incidence relative to 39.8% in ‘Zhong204’ and ‘H152’, with an average of 3.3% (Table 3). Therefore, the Lancaster germ-

TABLE 3. Analysis of diverse haplotypes generated with five closely linked single nucleotide polymorphisms (SNPs) in ‘Mo17’, ‘Huangzao4’, and their derivatives

Haplotype	Mean of incidence(%)	Inbred lines (M/H) ^a	SNP genotypes				
			PZE-102187486	PZE-102187611	PZE-102187687	PZA00672.8	PZE-102188421
a	4.9	Mo17(M)	CC	AA	AA	GG	AA
		Yu12(M)	CC	AA	AA	GG	AA
		J002(M)	CC	AA	AA	GG	AA
		Guan17(M)	CC	AA	AA	GG	AA
		Huotanghuang(M)	CC	AA	AA	GG	AA
		C416(M)	CC	AA	AA	GG	AA
		416(M)	CC	AA	AA	GG	AA
		Dan1324(M)	CC	AA	AA	GG	AA
		Ji846(M)	CC	AA	AA	GG	AA
		He344(M)	CC	AA	AA	GG	AA
		D185(M)	CC	AA	AA	GG	AA
		4F1(M)	CC	AA	AA	GG	AA
		J001(M)	CC	AA	AA	GG	AA
		Suixi701(M)	CC	AA	AA	GG	AA
		Ji992(M)	CC	AA	AA	GG	AA
		Ji1037(M)	CC	AA	AA	GG	AA
		D387(M)	CC	AA	AA	GG	AA
		M0113(M)	CC	AA	AA	GG	AA
		5213(M)	CC	AA	AA	GG	AA
		Si-419(M)	CC	AA	AA	GG	AA
		Ji412(M)	CC	AA	AA	GG	AA
		Ji419(M)	CC	AA	AA	GG	AA
		Ji465(M)	CC	AA	AA	GG	AA
		Ji495(M)	CC	AA	AA	GG	AA
		Longkang11(M)	CC	AA	AA	GG	AA
		L34(H/M)	CC	AA	AA	GG	AA
		Luyuan133(H)	CC	AA	AA	GG	AA
		H201(H)	CC	AA	AA	GG	AA
		Ji853(H)	CC	AA	AA	GG	AA
		374(H)	CC	AA	AA	GG	AA
		Tianya4(H)	CC	AA	AA	GG	AA
b	55.6	Huangzao4(H)	AA	GG	--	AA	GG
		Wenhuan(H)	AA	GG	--	AA	GG
		CN962(H)	AA	GG	--	AA	GG
		196(H)	AA	GG	--	AA	GG
		Huangyesi(H)	AA	GG	--	AA	GG
		K12(H)	AA	GG	--	AA	GG
		Dhuang212(H)	AA	GG	--	AA	GG
		Zhonghuang204(M)	AA	GG	--	AA	GG
		Huang428-3(H)	AA	GG	--	--	GG
		Si279(H)	AA	GG	--	--	GG
c	55.9	Sizhisi(H)	AA	GG	--	--	GG
		Chang7-2(H)	AA	GG	--	--	GG
		Ye502(H)	AA	GG	--	--	GG
		H152(H/M)	AA	GG	--	--	GG
		444(H)	AA	GG	--	GG	AA
		PI42(H)	CC	GG	--	AA	AA
d	41.6	H21(H)	CC	AA	--	GG	AA
e	70.6	JiA-034(M)	CC	AA	CC	GG	AA
f	61.7	Ji842(M)	CC	AA	CC	GG	AA
g	4.5	Wu314(H)	CC	AA	CC	GG	AA
		L270(H)	CC	AA	CC	GG	AA
h	55.1	Jing7(H)	CC	GG	CC	GG	GG
i	7.8	Ji35(H)	--	AA	AA	GG	AA
j	39.6	Ye515(H)	--	GG	AA	GG	AA
k	3.2	C649(M)	AA	AA	AA	GG	AA

^a M is ‘Mo17’ inbred line or its derivatives, H is ‘Huangzao4’ inbred line or its derivatives.

plasm group, including 'Mo17' and its derivatives, was an important source of resistance to head smut, and the QTL *qHS2.09* was the major resistance locus in this subgroup. Selection for SNP PZE-102187611, closely linked to the resistance QTL in bin 2.09, could be undertaken to improve resistance to head smut in maize using 'Mo17' germplasm.

Maize is an ideal candidate system compared to self-crossing species for the application of association studies, because linkage disequilibrium decays more rapidly in maize (28). For instance, in rice, the *GW2* gene that controls grain width and weight, has not been detected in association mapping with a panel of 517 rice landraces (14). But two homologs of this gene, *ZmGW2-CHR4* and *ZmGW2-CHR5*, were identified with significant associations with kernel width and other three yield-related traits in maize (kernel length, kernel thickness, and one-hundred kernel weight) (20). So far, numerous small-effect QTLs for flowering time, SLB resistance, NLB resistance, and leaf architecture have been identified in a maize NAM population (7,17,29,35). Drought resistance QTLs were identified in an association panel with 350 tropical and subtropical maize inbred lines (32). In the present study, two subgroups including Lancaster and Si-ping-tou, were used for a genome-wide association study. Three QTLs for head smut resistance were detected in this association panel, including *qHS1.03* and *qHS2.09* that were relatively stable across two environments. This result confirms previous mapping of head smut resistance in an $F_{2.3}$ population of 'Mo17' $\times$ 'Huangzao4' (21), and a population derived from a cross of 'Ji1037' $\times$ 'Huangzao4' (9). Furthermore, *qHS2.09* was finely mapped into an interval of ≈ 1.10 Mb, flanked by SNP PZE-102187307 and PZE-102188421 (Fig. 1B). It is interesting that the SNP PZE-102187611, the locus most significantly ($P = 9.39 \text{ E-}9$) associated with disease incidence in the *qHS2.09* region, was also located in the introgression fragment of line L34 (Figs. 1 and 2). Then, the QTL *qHS2.09* was located between SNP PZE-102187486 and PZE-102188421 by combined linkage and association mapping within an interval of ≈ 1.00 Mb. A similar approach using joint linkage and association mapping has been explored to detect QTL underlying drought tolerance in maize (25). Now that many maize re-sequencing efforts have resulted in the generation of millions of SNPs useful for association, the high density of SNP markers will permit evaluation of potential candidate genes, detection of major QTL, and the definition of favorable alleles for many traits.

Together, these data show joint linkage and association mapping to be a powerful approach for detection of QTL underlying complex traits in maize. Lancaster germplasm has been one of the most important sources for head smut resistance in maize, and a favorable haplotype and markers identified in this study will facilitate the future selection of resistance genes among the segregating progenies for head smut resistance.

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