Identification of QTL for resistance to head smut in Maize(Zea mays. L)

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Abstract

Head smut (HS) is one of the most devastating diseases in spring maize growing region. To provide theoretical and applied basics for breeding resistant maize to HS, quantitative trait loci (QTL) for HS resistance was identified in this study. The resistant QTL to HS was studied by the $F_{2:3}$ population derived from the cross T32 (highly resistant genotype) × HC (highly susceptible genotype). Analysis for each environment and joint analysis across three environments were used to identify QTL for the $F_{2:3}$ population. A significant difference in HS resistance was found between inbred lines 'T32' and 'HC'. A large genetic variation and transgressive segregation of the $F_{2:3}$ population were observed among three different sites, Guian (GA), Huaxi (HX), and Pingba (PB). Two stable QTL for resistance to HS were detected in different environments, located in the interval umc1177 to umc2224 on chromosome 1 and in the interval umc1006 to nc013 on chromosome 6. Both of these QTL can be used for further marker-assisted selection (MAS) breeding and theoretical study of maize resistance to HS.

Introduction

Maize head smut (HS) is a fungal disease caused by *Sporisorium reilianum* (Sánchez et al. 2011), a systemic disease of seedling infection. The pathogen spore remains in the soil or the harvested seeds and infects in the next year (Zhang et al. 2013). The pathogen mainly infects the germs at the initial stage of the seedling stage and doesn't infect others in the field (Potter 1914). HS in maize mainly ruins the panicle of maize. The symptom types of infected seedlings are shoot like, dwarf clump, yellow stripe, stem deformity, leaf abnormality, and top leaf curling (Little CR et al.2012). The infected plant's panicle would produce black powder spores and finally, become a black powder bag. In recent years, under the influence of extensive management and climate change, maize HS shows a trend of wild spread and aggravation (Ren Z Q et al. 2014). It has become one of the important diseases in the main production area of Heilongjiang province and even the spring maize area of China (Wang Z H et al. 2002; Zhao X R et al. 2012). The most effective way to control HS in maize is to breed and promote disease-resistant varieties (Jin Q M et al. 2003). The selection of resistant germplasm resources and the analysis of their HS resistance genetic mechanism have important roles in resistant breeding. In terms of screening and identification of germplasm resources, in total, more than 7000 germplasm resistant maize HS have been identified by scholars of all over the world (Song S Y et al. 2000; Gao J et al. 2006; Guo M K et al. 2007). The identification results showed that the most commonly used inbred lines or was highly susceptible to HS. That was, the resistant germplasm resources were seriously deficient (Wang L S et al. 2001; Wang Z H et al. 2004; Meng J et al. 2015).

Once the resistant source was identified, the marker-assisted selection (MAS) can be used to find markers closely linked to disease resistance sites, which is the basis of resource creation (Qian H T et al. 2007). Many researchers have carried out the quantitative trait loci (QTL) mapping research to further explore the resistance sites of HS in maize. Gao S H (2005) used the $F_{2:3}$ families from Mo17 × HZ4 and detected disease-resistant QTL in chromosome 1.02, 2.08 to 2.09, 2.09, 3.04. and 8.02. Chen Y S et al. (2008) developed an SSR marker SSR 148152 using the BC_2 population of HZS × J 1037. Shi H L et al. (2009) used two BC_3 populations of HZS × Mo17 and HZS × Q319 and developed a SCAR marker 5130 related to HS in maize resistance, and approved that SSR markers bn1g1893, umc1525, and UII1C2077 can effectively define the main effect QTL. Yong X L et al. (2015) found two resistant QTL on chromosomes 2 (q2.09 HR) and 5 (q5.03 HR) with the RIL population constructed by HZS × Q319. Lũbberstedt et al. (1999) used 220 F_3 families produced by the hybridization of two European maize inbred lines (D32×D145) and 11 QTL were located by the method of compound intervals mapping(CIM). Ten of the 11 QTL showed significant additive effects, and only the QTL on chromosome 3 showed significant dominant effects. Lu et al. (2009) used 100 recombinant inbred lines of Hi34 × Tzi17 as the mapping population; CIM detected only one QTL and four QTL were located on chromosomes 1, 2, 9, and 10. Due to different materials, environmental conditions and research methods, the research results were not completely consistent.

In this study, maize inbred line of T32 (highly resistant genotype) and HC (highly susceptible genotype), common research materials of maize breeding research materials in the southwest area, were used to construct mapping population F_2 and [illegible] p of F_2 was constructed by 145 SSR markers covering almost all

regions of maize's whole genome. Combined with the field identification results of three sites artificial inoculation pathogen in two years, QTL analysis of resistance to HS was carried out, it provides a new theoretical basis for MAS breeding of resistance to HS in maize.

Materials And Methods

Plant material

In this study, the mapping population included 184 $F_{2:3}$ families derived from randomly selected F_2 plants of the cross between excellent inbred lines T32 (highly resistant genotype) and HC (highly susceptible genotype), T32 and HC were selected from the inbreds on the analysis of resistance to HS (Tan et al. 2019). T32 is a selected line of tropical descent Thai germplasm Suwan1 C9, which is high resistance to HS and excellent comprehensive characters. It adapts widely to the southwest Mountainous area and is the parent of approval maize hybrids, which are widely used in the production of maize. HC is bred from Reid germplasm and susceptible to HS and other diseases, but it adapts to the central China and southwest region with high general combining ability. The F_2 individuals were produced between September 2017 and January 2018 in Hainan, the southernmost province of China. The $F_{2:3}$ families and their parents were planted in the spring of 2018 in Guian (GA) and the spring of 2019 in Huaxi (HX) and Pingba (PB), Guizhou, southwest of China.

Field experiment

$F_{2:3}$ families and their parents were planted in three environments: GA (29.13°N, 106.25°E; 1356 m altitude) in 2018, HX (26.43°N, 106.67°E; 1055 m altitude) and PB (26.22°N, 106.13°E; 1255 m altitude) in 2019. The phenotype of resistance to HS was evaluated at the milk-ripe stage after artificial inoculation of HS fungus (low temperature and low humidity). A completely randomized block design with two replications and 20 plants for each plot was conducted in both sites. All plants were self-pollinated, and 13 plants from the middle of each plot were used to evaluate the phenotype.Artificial inoculation and disease evaluation

The sori containing teliospores of *Sphacelotheca reiliana* were collected from the field in 2017 and stored in a dry and well-ventilated environment after drying in the shade. Before planting, spores were removed from the sori, filtered, and then mixed with soil at a ratio of 1:1000. The mixture of soil and teliospores were used to cover each maize kernel in the seedling tray when sowing seeds to conduct artificial inoculation. The plants were transplanted into the field when four or five leaves were visible (Ali and Baggett, 1990). Disease incidence of the plant was scored by examining the presence of sori in either ears or tassels as an indicator for susceptibility/resistance at the maturity stage.Statistical analysis of phenotypic dataThe statistical analysis of phenotypic data was performed using the IBM SPSS Statistical 20.0. The variances for genotype, environment, and interaction between genotype and environment were estimated using Microsoft Office Excel-based on the random model. The broad-sense heritability (H^2) was calculated as follows: $H^2 = \delta^2{}_G / (\delta^2{}_G + \frac{\delta^2{}_{GE}}{n} + \delta^2{}_E / nr)$, where δ^2_G represents the genetic variance, δ^2_{GE} represents the genotype and environment interaction variance, δ^2_E represents the error variance, and n and r are the numbers of environments and replications, respectively (Knapp et al. 1985).

Linkage map construction for the F_2 population

The genomic DNA was extracted from each plant's young leaf sample by using the cationic detergent cetyl-trimethyl ammonium bromide method (Chen and Ronald 1999). The genotype was identified using the 10% non-denaturing polyacrylamide gel electrophoresis. A total of 166 polymorphic markers between T32 and HC were used to develop the genetic map through the JoinMap version 4 (Jacobs et al. 1995) based on the publicly available SSR markers retrieved from the Maize Genetics and Genomics Database (http://www.maizegdb.org) (Portwood et al. 2019). The linkage map was constructed from 145 SSR markers, which covered almost the whole maize genome. The recombination frequency between linked loci was transformed into the genetic distance (centimorgans, cM) through the Kosambi's function.

QTL analysis

The chi-square (X^2) test was used to detect whether the SSR marker genotype separation ratio conforms to 1:2:1 and the population gene frequency conforms to 1:1. The inclusive composite interval mapping was performed using the QTL IciMapping to identify the QTL and estimate their effects (Jiankang, 2009; Li et al. 2008; Li et al. 2007). Parameters for forwarding regression analysis were set with window size and a walking speed of 10 and 1 cM, respectively. The significance threshold for the QTL detection was 1000 random permutations of the phenotypic data at the 5% level. The gene action mode of each significant QTL was estimated by the rate (A/D) of additive (A) and dominant (D) effects and classified into additive (A = 0–0.20), partial dominant (PD = 0.21–0.80), dominant (D = 0.81–1.20), and overdominant (OD > 1.20) (Edwards et al. 1987; Stuber et al. 1987; Tuberosa et al. 1998). QTL were named in the following way: Take GA*HS1a* for instance, 'GA' means the environment in which the QTL was identified (GA, HX, PB, and JA are abbreviated from Guian, Huaxi, Pingba, and Joint analysis, respectively); 'HS' means the name of the trait; the number '1' is the serial number of chromosome; 'a' presents the serial alpha code of the detected QTL.

Results

Phenotypic variation of HS in $F_{2:3}$ families and their parents

The *t* test for both parents within a site showed extremely significant differences in the HS resistance between the two parents (Table 1) ($P < 0.01$). The resistance to HS of T32 was significantly higher than that of HC. The incidence of HS in $F_{2:3}$ families showed continuous and approximately normal distributions across the three sites with low skewness and kurtosis, indicating that the HS resistance was the typical quantitative trait controlled by multiple genes (Table 1, Figure. 1). The transgressive segregation of resistance grade was observed in the population across the three sites. Besides, ANOVA indicated that the effects of genotypes ($F_{2:3}$ families), environments, and genotype/environment interactions showed a highly significant difference for HS resistance ($P < 0.01$) (Table 2), and then the heritability was higher by 50.75%.

linkage map

For the population, 145 polymorphic markers covering almost the whole maize genome were used to construct a linkage map for QTL mapping (Figure. 2, Table 3). The total map length was 1103.36 cM, and the average distance between markers was 7.61 cM. The average number of markers per chromosome was 14.5, ranging from 9 in chromosomes 5 and 6 to 23 in chromosome 1. A few alterations in the marker order of a chromosome were observed compared with the position presented by the IBM 2008 Neighbors Frame 6. These alterations may be due to the different materials used in the population.

QTL for resistance to HS detection in single environment analysis

In the population, twelve QTL for HS resistance were detected in $F_{2:3}$ families, which comprised three QTL at GA, five QTL at HX, and three QTL at PB (Table 4). Two loci were detected in different environments. The QTL-GAHS1a, the QTL-HXHS1a, and the QTL-PBHS1a were located in the common genetic region in the interval umc1177 to umc2224 (bins 1.01) on chromosome 1, which explained 11.89%, 12.21%, and 13.84 of the phenotypic variation at GA, HX, and PB, respectively. Additive effects of the three QTL for increasing trait value were contributed by T32, whose effect values were -0.22, -0.10, and -0.07 respectively. The QTL-GAHS6a, the QTL-HXHS6a, and QTL-PBHS6a were located in another common genetic region in the interval umc1006 to nc013 (bin6.02-6.05) on chromosome 6, which explained 16.02%, 12.91%, and 18.67 of the phenotypic variation at GA, HX, and PB, respectively. Additive effects of the three QTL for increasing trait value were also contributed by T32, whose effect values were -3.14, -3.19, and -3.51, respectively. For these twelve QTL, three QTL had additive effects, three QTL had partially dominant effects, and the others had an overdominant effect. The increasing effect of alleles came from both parents. The mode of gene action was additive, partial dominant, and over dominant.

QTL detection in joint analysis across three environments

Eighteen QTL were detected for resistance to HS in joint analysis across three sites (Table 5). Two common genetic regions detected in single environment analysis were also detected in joint analysis in the same marker intervals with relatively higher LOD values of 8.00 and 9.73. One QTL only at GA, two QTL only at HX, and One QTL only at PB detected in single environment analysis were also detected in joint analysis in the same marker intervals. QTL-HXHS1b detected in single environment analysis and QTL-JAHS1b detected in the joint analysis were located near the genetic region with the same marker (umc1306). Some QTL detected in joint analysis across three environments were not detected in single environment analysis. They probably have a minor effect and when we have more environments they can be detected.

Discussion

In this study, 184 $F_{2:3}$ families were evaluated for resistance to HS in GA, HX, and PB. The disease incidence in the three environments was mostly between the parents, which conforms to the normal distribution rule, and the heritability was higher by 50.75%. This indicates that the resistance to HS is the character of quantitative inheritance with high heritability, which is consistent with the results of Zhang et al. (2002) and Bai Y F (2009). Environmental and genetic factors had a large effect on QTL detection. Different environments often result in a change of magnitude of significant QTL effect or direction of additive effects (Boer et al. 2007; Peng et al. 2011).

To increase the reliability and consistency of QTL detection for the resistance to HS of maize, the three environments were used in the present study. Under the three conditions, the resistance to HS was a quantitative trait controlled by several QTL, some of which were identified in different conditions and detected in a joint analysis. Two common genetic regions detected at different environments for the HS resistance, and other QTL were not stable across the three environments. Due to the different soil and climate conditions at three environments, some QTL accounted for a relatively large amount of variance at one site. In contrast, they were not detected at other sites. The QTL which was extremely stable in different sites is important for MAS programs, fine mapping, and map-based cloning in maize.

Some QTL for the resistance to HS has been reported in maize. Shi et al. (2005) detected two QTL for the resistance to HS, which were located on the two chromosomal regions in bin 1.02/3 (Lu and Brewbaker 1999) and in bin 2.09 (Chen et al. 2008). However, the support intervals of QTL for the resistance to HS given by Shi et al. (2005) do not overlap the map position in the present study. The different amount and positions of QTL detected in different studies indicated that HS resistance in maize is a complex quantitative trait, which is closely dependent on the material and the environment.

So far, a large number of pest-resistant genes or QTL have been located on chromosomes. Studies have shown that resistant QTL distribution was not randomly distributed, but clustered aggregation and QTL for resistance to different diseases were often clustered together (Palloix et al. 2009). There was a tightly linked resistance genes (QTL) group on chromosome 3 in the bin of 3.04 to 3.05, where resistant gene to sugarcane mosaic virus *Scmv2* in maize (Wu et al. 2007; Liu et al. 2009), resistant gene to stripe rust *Rp3* (Sanz-Alferez et al. 1995), resistant wheat streak mosaic virus gene *Wsm2* (McMullen et al. 1994), mosaic resistant gene *Mvl* in maize (Ming et al. 1997) and QTL for maize dwarf mosaic resistance (Wang et al. 2004b) have been found. Meanwhile, Balint-Kurti et al. (2007) and Belcher et al. (2012) detected QTL for resistance to southern leaf blight in maize in bin3.03 to 3.04, and it was proved that the fragment could significantly improve the resistance to southern leaf blight in maize.

In this study, QTL were located on chromosome 1 (1.01) and chromosome 6 (6.02 to 6.05), within or closed to the regions of the resistance gene group mapped. Chromosome 1 (1.01) also included resistant QTL to the European corn borer, *heliothis armigera* (Hübner), and northern leaf blight. There are various resistance genes near chromosome 6 (6.01), resistance gene *Scmv1* to sugarcane mosaic virus (Wu et al. 2007; Liu et al. 2009), resistance gene to southern leaf blight (Belcher et al. 2012), *Mdml* to the dwarf mosaic virus (Simcox.1995), *rhm1* to southern corn leaf blight (Zaithlin et al. 1993) and *Scm1* to sugarcane mosaic dominant (Melchinger et al. 1998). Therefore, the results of this study support the existence of clusters of pest-resistant genes on maize chromosomes. The function of the gene fragment should be further analyzed in the

At present, it is generally believed that resistance to maize HS is a quantitative trait and controlled by additive, dominant and epistatic effects. The additive effect plays a dominant role while the non-additive effect has a little function but stable inheritance of resistance. Lübberstedt et al. (1999) demonstrated that the inheritance of resistance to maize HS was mainly additive, but Ali et al. (1990) believed that the inheritance of resistance was related to the incidence of HS, when the incidence was high resistance was dominant. Otherwise, it was additive. Bernardo et al. (1992) found that additive effects play a decisive role in the inheritance of resistance, and the others are small. The resistant QTL detected in this study were mainly in the form of dominant inheritance, four QTL showed additive inheritance only in the joint analysis of the three environments, while the rest were all in the form of dominant inheritance. Therefore, the inheritance of resistance to maize HS can be an additive effect or a dominant effect due to different genetic backgrounds of the materials, but whether the inheritance of resistance changes with the change of incidence needs further study.

Conclusions

We have successfully detected two stable QTL for resistance to HS in different environments. Both of these QTL can be used for further marker-assisted selection (MAS) breeding and theoretical study of maize resistance to HS.

Abbreviations

HS head smut

QTL Quantitative trait loci

MAS Marker-assisted selection

GA Guian

HX Huaxi

PB Pingba

JA Joint analysis

cM Centimorgans

CIM compound intervals mapping

Declarations

Data availability

The authors described the relevant data sources in the manuscript. The data generated or anzlyzed during this study are included in this manuscript and its supplementary material.

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Conflicts of interest

All the authors declared that they have no conflict of interest.

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Tables

Table 1 Phenotypic performance of the HS resistance for $F_{2:3}$ families and their parents in three environments

Site	Parents		$F_{2:3}$						
	T32（%）	HC（%）	Mean（%）	Range（%）	SD [a]	CV [b]（%）	Skewness	Kurtosis	W [c]
GA	0**	35	13.38	0-40	8.15	60.91	0.73	0.61	0.959
HX	0**	39	14.03	0-42	8.50	53.53	0.60	0.61	0.959
PB	0**	38	14.16	0-43	8.20	57.93	0.78	1.03	0.957

[a] standard deviation of phenotypic data, [b] coefficient of variation of phenotypic data, [c] Shapiro–Wilk statistic for the W test of normality, ** significance with $P < 0.01$

Table 2 ANOVA for disease incidence of the HS in three environments

Source	DF[a]	Stdev Square	Mean Square	F Value	P Value
Block	5	1104.608	220.921	4.411	0.0001
Inter-familial	158	33548.59	212.332	4.239	0.0001
Location	2	333.151	166.575	4.009	0.001
Family × environment	158	16520.924	104.563	2.516	0.0001
Error	790	39564.828	50.082		

[a] degree of freedom

Table 3 Linkage map information of SSR markers in the F_2 population

Chromosome	Markers	Cover the chromosome length(cM)	Average map distance (cM)
1	23	199.449	8.672
2	17	119.008	7.000
3	12	86.085	7.171
4	18	127.224	7.068
5	9	81.611	9.068
6	9	66.999	7.444
7	15	147.152	9.810
8	12	70.272	5.856
9	12	102.797	8.566
10	18	102.758	5.708

Table 4 QTL for the resistance to HS in single environment analysis in the $F_{2:3}$ population

Site	Chr	QTL name	Bins	Interval markers	Position	LOD Score	PVE	Est A	Est D	Gene action
GA	1	GAHS1a	1.01	umc1177-umc2224	17	2.46	11.89	-0.22	4.27	OD
	6	GAHS6a	6.02-6.05	umc1006-nc013	39	2.98	16.02	-3.14	-2.27	PD
	9	GAHS9a	9.01	phi033-umc1867	102	2.15	6.29	2.13	-0.77	PD
HX	1	HXHS1a	1.01	umc1177-umc2224	20	3.14	12.21	-0.10	5.31	OD
	1	HXHS1b	1.09-1.10	umc1306-umc2189	151	2.08	5.1	2.26	-1.79	PD
	3	HXHS3a	3.02-3.03	bnlg1325-bnlg1447	17	2.87	9.07	0.43	4.48	OD
	4	HXHS4a	4.01-4.03	umc2281-umc1757	110	2.23	5.62	2.14	1.77	D
	6	HXHS6a	6.02-6.05	umc1006-nc013	37	3.61	12.91	-3.19	-3.02	D
PB	1	PBHS1a	1.01	umc1177-umc2224	19.00	3.16	13.84	-0.07	5.22	OD
	3	PBHS3a	3.02-3.03	bnlg1325-bnlg1447	18.00	2.31	7.42	0.45	3.72	OD
	6	PBHS6a	6.02-6.05	umc1006-nc013	38.00	4.16	18.67	-3.51	-3.49	D

Note：A：additive；PD：partial dominance；D：dominance；OD：over dominance

Table 5 QTL for the resistance to HS in joint analysis across three environments in the $F_{2:3}$ population

Chr	QTL name	Bins	Interval markers	Position	LOD Score (A)	LOD Score (A by E)	PVE	Est A	Est D	Gene action
1	JAHS1a	1.01	umc1177-umc2224	22.00	8.00	0.02	7.10	-0.19	3.67	OD
1	JAHS1b	1.07	umc1278-umc1147	119.00	3.32	0.31	2.92	1.54	-1.30	D
1	JAHS1c	1.09	bnlg1331-umc1306	150.00	4.26	0.54	3.78	1.75	-0.80	PD
1	JAHS1d	1.10-1.11	bnlg1347a-phi064	162.00	2.50	0.39	2.28	1.15	-1.25	D
2	JAHS2a	2.02-2.03	bnlg125-bnlg1537	32.00	3.61	0.17	2.92	-0.03	2.37	OD
2	JAHS2b	2.06-2.07	umc1108-umc1536	115.00	2.36	0.08	1.97	-1.29	0.81	PD
2	JAHS2c	2.08	umc1464-phi090	118.00	2.87	0.07	2.38	-1.50	0.64	PD
3	JAHS3a	3.02-3.03	bnlg1325-bnlg1447	19.00	6.13	0.07	4.94	0.19	3.00	OD
4	JAHS4a	4.07	umc2038-bnlg1784	52.00	4.40	0.14	3.75	1.82	-0.22	A
4	JAHS4b	4.01-4.03	umc2281-umc1757	85.00	5.12	0.08	4.27	1.93	1.04	PD
5	JAHS5a	5.03	umc2294-umc1557a	1.00	3.66	0.12	3.06	-1.90	0.14	A
5	JAHS5b	5.07-5.08	umc1072-umc1225	74.00	3.34	0.11	2.54	0.02	2.18	OD
6	JAHS6a	6.02-6.05	umc1006-nc013	34.00	9.73	0.14	7.93	-2.47	-1.44	PD
7	JAHS7a	7.02	umc1409-umc1585	52.00	2.81	0.28	2.23	-1.52	-0.42	PD
7	JAHS7b	7.05	umc1671-umc1154	134.00	2.60	0.16	2.18	-0.68	-1.93	OD
9	JAHS9a	9.03-9.04	bnlg1270-umc2338	49.00	2.06	0.15	1.63	-1.32	-0.31	PD
9	JAHS9b	9.01	phi033-umc1867	102.00	4.49	0.28	3.68	1.75	-0.90	PD
10	JAHS10a	10.07	umc2351-umc1196	19.00	2.69	0.12	2.30	1.49	0.27	A
10	JAHS10b	10.01-10.02	umc1432-umc1291	102.00	2.60	0.01	2.21	-1.49	-0.14	A

Note：A：additive；PD：partial dominance；D：dominance；OD：over dominance

Figures

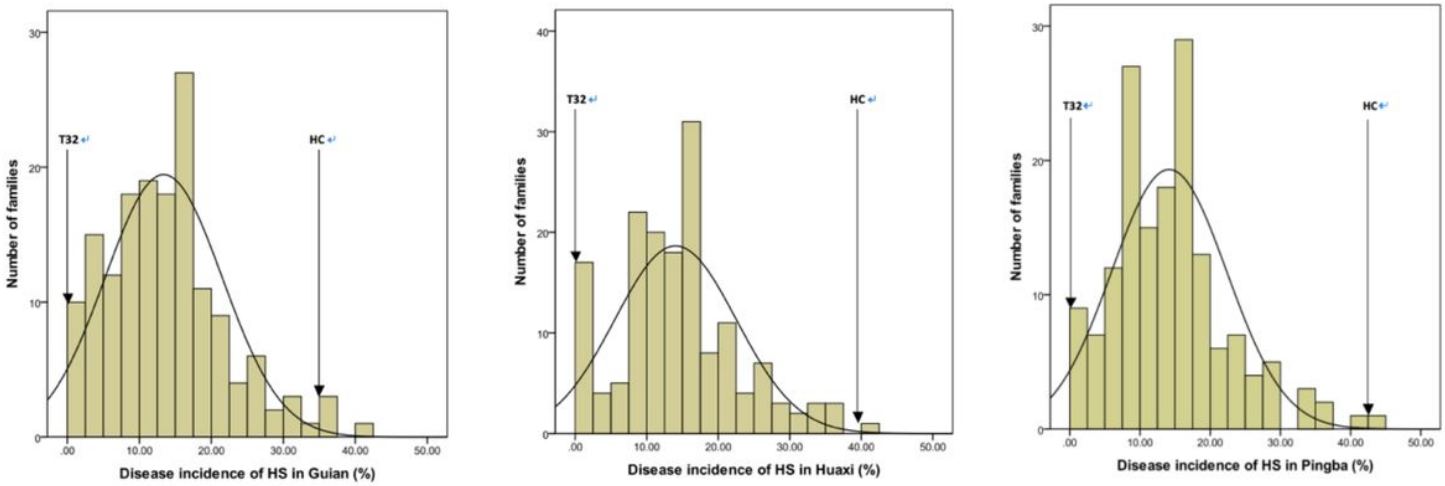

Figure 1

Frequency distribution of the disease incidence in the F2:3 population

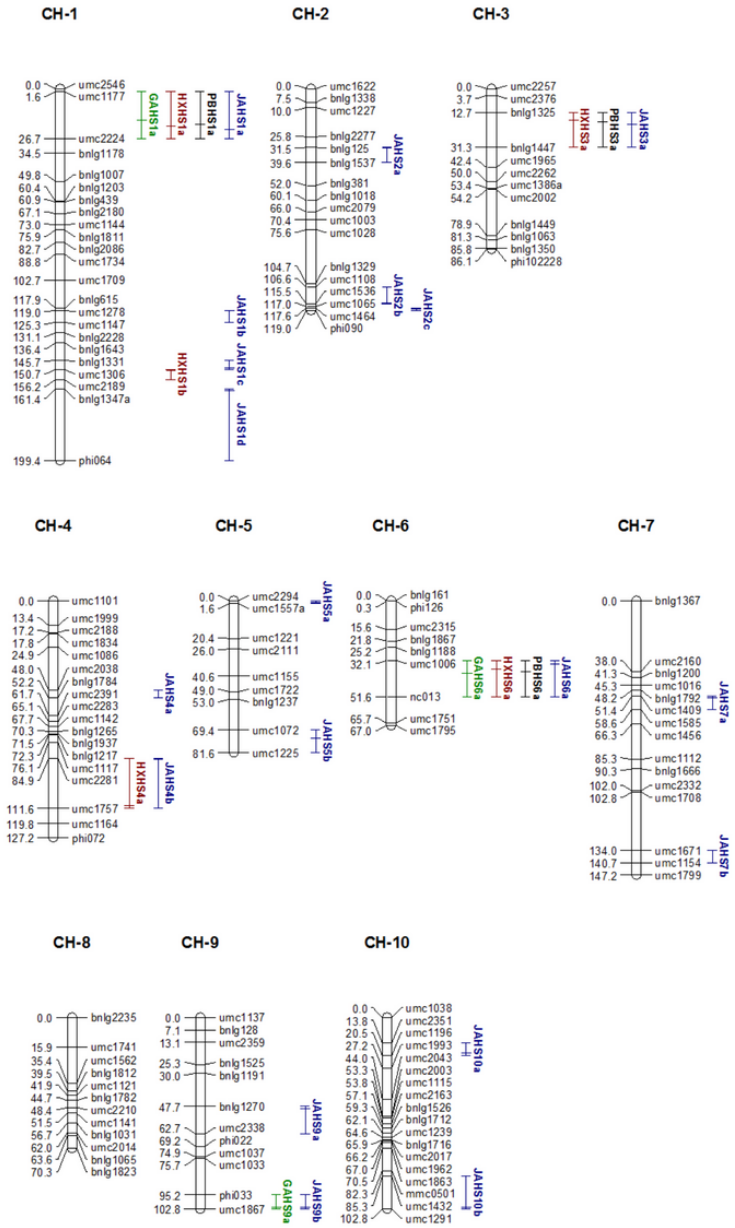

Figure 2

Distribution of QTL for head smut resistance on linkage map in the F2:3 population. The line segment indicates the marker intervals of QTL, and the node on the line segment indicates the position of the QTL on the linkage map.