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RESEARCH PAPER

Mapping of QTLs Associated with Resistance to *Fusarium* Head Blight Using an "Immortalized F_2" Population

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Abstract: *Fusarium* head blight (FHB), caused by *Gibberella zeae* (Schw.) Petch, is a serious disease in many wheat (*Triticum aestivum* L.) growing regions worldwide. To study the inheritance of FHB resistance against fungal penetration (type I resistance), an "Immortalized F_2" (IF) population containing 198 lines was constructed by crossing recombinant inbred lines chosen with random permutation of 132 RILs. The 132 RILs were chosen from the RIL population derived from the cross between FHB-susceptible cultivar Nanda 2419 and FHB-resistant cultivar Wangshuibai. The population was then evaluated for the percentage of infected spikes (PIS) across 2 years, and 6 chromosome regions were detected as being associated with type I resistance through interval mapping, among which *Qfhi.nau-4B* and *Qfhi.nau-5A* with the resistance alleles originating from Wangshuibai and *Qfhi.nau-2B* with the resistance allele from Nanda 2419 were consistently detected. *Qfhi.nau-4B* and *Qfhi.nau-5A* had the largest effects among the detected QTLs and for the most part, both showed additive allelic effects. The former was also partially dominant. In addition, 4 pairs of significant interaction loci were identified. These results demonstrated that wheat scab resistance was under complex genetic control and also implied that early generation selection for type I resistance in scab resistance breeding was feasible with Wangshuibai as the parent.

Keywords: *Fusarium* head blight; Wangshuibai; "immortalized F_2" population; type I resistance

Fusarium head blight (FHB) or scab, caused mainly by *Fusarium graminearum* Schwabe [*Gibberella zeae* Schw. (Petch)], widely occurs in regions with a warm and humid climate, resulting in wheat (*Triticum aestivum* L.) yield loss up to 40% in epidemic years. Moreover, grains contaminated with *Fusaium*-produced mycotoxins are unsuitable for human and livestock consumption. To maintain wheat productivity and quality, development of FHB-resistant cultivars has been one of the major goals for wheat breeders.

To date, the mechanism underlying FHB resistance has been too complicated to be clear. Schroeder and Christensen [1] initially proposed 2 types of resistance with type I against initial infection and type II against fungal spread within the spike. Other types of resistance, including kernel resistance, yield tolerance, and low-accumulation of mycotoxins, were later suggested by Mesterhazy in 1995 [2]. Because of the weakness of definition or methodological difficulty in evaluation, these concepts have not been widely accepted [3]. FHB resistance is controlled by a few major genes and some minor genes, whose effects are greatly influenced by environments. With the availability of molecular markers, chromosome regions related to FHB resistance, mainly for type I and type II resistances, have been identified in certain resistance germplasm. In most cases, the major resistance QTLs have been mapped to chromosomes 3B, 6B, and 5A [4–6].

Additive genetic variation is the major factor affecting FHB resistance, but the dominance and epistatic effects are also important [7]. The significance of epistasis on FHB resistance has been verified in QTL mapping studies [8–10]. "Immortalized F_2" (IF) population, consisting of hybrid (F_1) individuals created by making crosses between a set of recombinant inbred lines (RILs), has been proposed, to dissect the individual genetic components influencing quantitative traits [11]. The IF population has a genetic structure resembling the F_2 population, and the genotypes

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within the population can be duplicated when necessary, with the advantages of permanent conservation such as the RIL and double haploid populations. The latter 2 types of populations cannot be used to measure dominance or the associated epistatic effects because of the genetic structure limitation. With an IF population, Hua et al. [11] have mapped QTLs with measurement of single-locus effects and all kinds of digenic epistatic interaction effects.

Using the RIL population derived from Nanda 2419 × Wangshuibai, QTLs associated with resistance against fungal penetration have been mapped [10]. In this article, these QTLs have been verified using the immortalized F_2 population constructed with these RILs and their genetic effects have been examined.

1 Materials and methods

1.1 IF population construction and field trial design

A molecular marker linkage map, covering over 4,223.1 cM of the wheat genome, has been constructed with the RIL population derived from the cross between an improved cultivar Nanda 2419 and a landrace Wangshuibai [12]. One hundred and thirty-six RILs selected from this population were divided into 2 groups by random permutations and the lines in one group were randomly paired with the lines in the other group to make crosses. Each of the 132 RILs was used only once in the crosses. This procedure was repeated thrice, resulting in a population of 198 F_1 lines.

The IF population was planted in the Jiangsu Academy of Agricultural Sciences (JAAS) in 2005, and Jiangpu County (JP) in 2006, in a randomized complete block design, with 2 replicates. Each plot with 10 seeds had a single row 1.5 m long. Adjacent rows were spaced by 50 cm. Phenotyping and SSR marker analyses were used to exclude false F_1 lines.

1.2 Resistance evaluation

At anthesis, a mixed conidial suspension of 4 local virulent strains of *F. graminearum* strains (F4, F15, F17, and F34) was sprayed on the heads once a day. This procedure lasted for a week in the JAAS trial. In the JP trial, the inoculation was conducted by scattering FHB by wheat grains on the soil surface about 10 d before anthesis and it was repeated a week later. In addition, 2 sprays of mixed conidial suspension were applied. Fifteen days after inoculation, the number of spikes with visible FHB symptoms, in at least one of their florets, and the total spikes of each plot were scored. The percentage of infected spikelets (PIS) was used to represent type I resistance.

1.3 Statistical analysis

Analysis of variance (ANOVA) and correlation was performed using statistical software Data Desk v. 5.0 (Data Description, Ithaca, N.Y.). Each site–year combination was treated as an environment. Broad-sense heritability was calculated with the following formulae for a single environment and multiple environments, respectively:

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2) \quad \text{and}$$

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gl}^2 / n + \sigma_e^2 / nr)$$

where σ_g^2 is the genetic variance, σ_e^2 is the experimental error, σ_{gl}^2 is the variance of genotype × environment, r is the number of replications, and n is the number of environments.

1.4 QTL analysis

The genotype of each F_1 line in the IF population was deduced from the genotypes of parents. The genotype distribution of each marker locus was examined using the Chi-square test. The framework linkage map was constructed using Mapmaker Macintosh v2.0 [13], with a LOD of 3.0. Recombination fractions were converted into map distances (cM) using the Kosambi mapping function [14].

Before interval mapping analysis, the data from the JP trial were square root-transformed. A QTL scan was performed with Mapmaker/QTL Version 1.9 [15] through simple interval mapping (SIM) and composite interval mapping (CIM), where the QTL with the highest LOD score from SIM was fixed at the given peak position using the 'sequence' command for the second round of the whole map scan. The LOD score for declaring a QTL was 3.0 in SIM or 3.0 higher than that of the fixed QTL in CIM. A QTL scan was also performed in multi-environment models through SIM, with MQTL.v.1.0 [16]. QTL locations were inferred based on the peaks of the main effects and QTL × environment interactions and the statistic significance threshold were declared as TS, which is equivalent to 4.6 times that of the LOD scores.

1.5 Model of inheritance

Marker regression against the closest markers of individual QTLs was conducted under the free, additive, dominance, recessive genetic models, to test the inheritance mode of each QTL, using Map Manager QTXb20 [17]. The free model had separate regression coefficients for additive and dominance components. In the additive model, the dominant component was forced to be 0. The dominant model used a single coefficient combing both the additive and dominance components. The Nanda 2419 allele was regarded as the dominance in the dominant model. The recessive model was the same as the dominant model, but the Wangshuibai allele was regarded as dominance. The additive, dominance, and recessive models were constrained because the dominance component was not allowed to assume an independent value.

The significance of the model was measured with the likelihood ratio statistic (LRS) generated by the software, which was equivalent to 4.6 times that of LOD.

1.6 Digenic interactions

All pairs of marker loci were tested for interaction with Map Manager QTXb20 [17]. Significant epistatic interactions were claimed when the total effects of 2 tested loci had a *P*-value less than 1×10^{-5} and the interaction effect itself had a *P*-value less than 0.01.

2 Results

2.1 Phenotypic analysis

In the IF population, the PIS varied from 11.5% to 76.6% and 8.6% to 60.2% with an average of 39.1% and 27.3% for 2005JAAS and 2006JP, respectively. The population data of 2005JAAS displayed a near-normal distribution (Fig. 1). The 2006JP data displayed a skewed distribution toward the lower side and those shown in Fig. 1 had been square root transformed.

ANOVA with the single environment data indicated that the between-line variation in both trials was significant at $P = 0.001$. In the 2005 JAAS trial, the difference between the replicates was significant at the level of $P = 0.001$, but the correlation coefficient between them was as high as 0.745. The correlation coefficient between the 2 trials was 0.448, significant at $P = 0.001$. The broad-sense heritability of PIS was 0.76 in JAAS2005 and 0.62 in 2006JP, and 0.82 across the environments.

2.2 QTL mapping

The genotypes of 347 marker loci were examined in the IF population and the proportions of the 3 genotypes for each marker locus fit the segregation ratio of 1:2:1. A framework marker map covering 2,723.5 cM of the 21 wheat chromosomes was constructed. Six chromosome regions were detected through interval mapping for their association with type I resistance, distributing on chromosomes 2B, 3A, 4A, 4B, 5A, and 6B (Table 1).

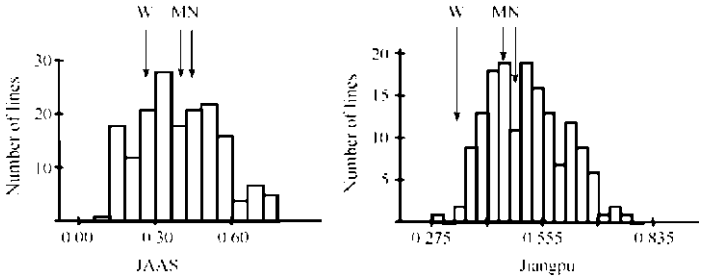

Fig. 1 PIS distribution in IF population
W: Wangshuibai; N: Nanda 2419; M: mean of the population.
Data from Jiangpu site are transformed by applying sqr(*x*) function.

Table 1 QTLs associated with percentage of infected spikes

Environment	Method of QTL detection	QTL	Interval	Source of resistance allele	Length (cM)	Peak position (cM)	*LOD*	R^2 (%)
2005JAAS	SIM	*Qfhi.nau-3A*	*Xmag615–Xmag896*	Wangshuibai	12.0	10.0	4.0	12.0
		Qfhi.nau-4B	*Xgwm149–Xwmc349*	Wangshuibai	7.8	1.0	4.2	11.5
		Qfhi.nau-5A	*Xwmc96–Xwmc446**	Wangshuibai	4.0	2.0	6.2	16.2
	CIM	*Qfhi.nau-2B*	*Xs1021m–Xgwm47.4*	Nanda 2419	6.7	4.0	3.2	8.9
2006JP	SIM	*Qfhi.nau-4A*	*Xwmc501.2–Xmag3886*	Nanda 2419	14.5	0.0	3.7	16.9
		Qfhi.nau-4B	*Xwmc413–Xmag1682*	Wangshuibai	24.5	13.0	3.8	20.3
		Qfhi.nau-5A	*Xwmc96–Xwmc446**	Wangshuibai	4.0	0.0	6.0	16.4
	CIM	*Qfhi.nau-2B*	*Xmag4281–Xaf2*	Nanda 2419	6.2	0.0	3.1	8.0
		Qfhi.nau-6B	*Xgwm219.2–Xbarc134*	Wangshuibai	18.7	5.0	3.3	14.7
Multiple environment	SIM	*Qfhi.nau-4B*	*Xgwm149–Xwmc349*	Wangshuibai	7.8	1.0	6.3	15.1
		Qfhi.nau-5A	*Xwmc96–Xwmc446*	Wangshuibai	4.0	1.0	11.0	27.1

Quantitative trait loci (QTLs) that overlap in the one-log support confidence intervals are assigned the same symbol. The position is represented by the left boundary locus of the interval plus a genetic distance (cM) proximal to it. The LOD and R^2 values for the QTLs identified by CIM are derived from the corresponding values of the two-QTL model minus the corresponding values of the fixed one.
CIM: composite interval mapping. SIM: simple interval mapping. * The fixed QTL position for each CIM.

The resistance alleles of all the detected QTLs, with the exception of *Qfhi.nau-2B* and *Qfhi.nau-4A,* originated from Wangshuibai. *Qfhi.nau-5A* had the largest effect, explaining 16.2% and 16.4% of the phenotypic variances in the 2 trials. Based on both single environment mapping and mutiple-environment mapping, the *Qfhi.nau-5A* peak was located within the *Xwmc96–Xwmc446* interval closely linked to *Xwmc96* (Table 1). The LRS values of the additive model were similar to those of the free model and were much higher than those of the other 2 models, suggesting that *Qfhi.nau-5A* contributed to scab resistance mainly with an additive effect (Table 2).

The QTL for FHB resistance on chromosome 4B was mapped to 2 adjacent intervals with the 2 sets of data, all having the second largest LOD scores in both trials (Table 1). Their one-log confidence interval was overlapped. In the multiple model, the QTL peak was closely linked to *Xgwm149*. This QTL also functioned additively, but with considerable dominance effects (Table 2). Similar to *Qfhi.nau-4B*, the 2 mapped *Qfhi.nau-2B* intervals overlapped in their one-log confidence intervals. *Qfhi.nau-3A*, *Qfhi.nau-4A*, and *Qfhi.nau-6B* were detected only in one of the 2 trials.

2.3 Digenic interaction analysis

Four significant digenic interactions were detected only with the JAAS data. Among them, the *Xwmc722.2-4D/ Xwmc446-5A* pair had the highest interaction LRS score (Table 3). *Xwmc446-5A*, similar to *Xwmc96-5A* in the other digenic interactions, was the marker linked to the major QTL *Qfhi.nau-5A* that was involved in all the 4 loci. The other involved in the loci, except for *Xgwm149*, were not associated with PIS alone.

3 Discussion

Using the IF population deriving from Nanda 2419 × Wangshuibai recombinant inbred lines, 6 chromosome regions were detected for their association with type I resistance, among which *Qfhi.nau-4A*, *Qfhi.nau-4B*, and *Qfhi.nau-5A* had been detected using the RIL population [10].

This study and the previous investigations have shown that *Qfhi.nau-4B* and *Qfhi.nau-5A* are 2 major QTLs contributing to type I resistance. The *Qfhi.nau-4B* interval has been identified in Wuhan-1 for its association with both incidence and disease spread resistance [18]. Liu et al. [19] detected a type II resistance QTL in cultivar Ernie in the same region. However, in the Nanda 2419 × Wangshuibai population, *Qfhi.nau-4B* was only related to type I resistance. *Qfhi.nau-5A* shared the same interval with the type I resistance QTLs in both CM82036 [20] and DH181 [21] and this chromosome region also conferred type II resistance in CM82036 [4], Fundulea 201R [22], Frontana [9], and Ernie [19], with relatively smaller effects.

However, the allelic relationship of either the 4B QTL or 5A QTL between different germplasms is still unknown. The *Qfhi.nau-2B* interval has been associated with both type II [23] and type I resistance in RIL population derived from Nanda 2419 × Wangshuibai (unpublished data), and with type II resistance in Ning 7840 and Ernie [19, 24]. *Qfhi.nau-6B*, mapped to the terminal region of chromosome 6BS, is different from *Fhb2* region [25]. The *Qfhi.nau-4A* interval is adjacent to the 4A QTL that has been mapped using a different population derived from Wangshuibai [5]. No FHB resistance QTL near the distal end of 3AL, where *Qfhi.nau-3A* mapped, has been reported before.

Both *Qfhi.nau-5A* and *Qfhi.nau-4B* influenced FHB resis-

Table 2 Additive and dominance model test of *Qfhi.nau-4B* and *Qfhi.nau-5A* using the average of the two-year data

QTL	Tested locus	Likelihood ratio statistic				$A^{1)}$	$D^{2)}$	$d/a^{3)}$
		Free model	Additive model	Dominance model	Recessive model			
Qfhi.nau-4B	*Xgwm149*	21.5	18.7	5.9	19.3	0.06	–0.03	–0.50
Qfhi.nau-5A	*Xwmc96*	35.8	35.7	21.3	24.2	0.07	–0.00	0.00

1) Additive effect = half of the difference between the mean PIS of Nanda 2419 and Wangshuibai genotypes.

2) Dominance = the difference between the heterozygous genotype values and the means of the 2 homozygous genotypes.

3) Ratio of the estimated dominance to the additive effect, which falls between 0 and 1, is regarded as partial dominance.

Table 3 Epistatic interactions detected using the data from the trial at Jiangsu Academy of Agricultural Sciences in 2005

Locus 1	Locus 2	Total LRS *	Interaction LRS	Main effect LRS of locus 1	Main effect LRS of locus 2
Xmag3319-2B	*Xwmc96-5A*	48.4	14.8	5.7	26.3
Xwmc54-3B	*Xwmc96-5A*	47.1	15.6	1.8	26.3
Xgwm149-4B	*Xwmc96-5A*	58.3	17.4	19	26.3
Xwmc722.2-4D	*Xwmc446-5A*	46.7	21.3	4.6	24.4

* Threshold for total LRS is 44.2. LRS: likelihood ratio statistic.

tance mainly by additive actions, and *Qfhi.nau-4B* also had a partial dominance effect. This result supported previous findings through conventional quantitative genetic analysis [7]. The additive effects of QTLs for FHB resistance have been reported in many mapping studies [4, 6, 21, 26], but the dominance effects are rarely investigated. We found that epistasis was involved in the resistance against initial penetration. Therefore, more attention should the paid to epistasis when dissecting the genetic basis of FHB resistance.

4 Conclusions

QTLs controlling FHB resistance in this study acted additively for most part, but epistasis should not be ignored. As *Qfhi.nau-4B* and *Qfhi.nau-5A* had the largest effects on type I FHB resistance, they could be easily included in conventional resistance breeding programs and marker-assisted selection.

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