

Short Communication

Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14

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Abstract

Identity of quantitative trait loci (QTL) governing resistance to fusarium head blight (FHB) initial infection (type I), spread (type II), kernel infection, and deoxynivalenol (DON) accumulation was characterized in Chinese wheat line W14. Ninety-six double-haploid lines derived from a cross of W14 × 'Pion2684' were evaluated for FHB resistance in two greenhouse and one field experiment. Two known major QTL were validated on chromosomes 3BS and 5AS in W14 using the composite interval mapping method. The 3BS QTL had a larger effect on resistance than the 5AS QTL in the greenhouse experiments, whereas, the 5AS QTL had a larger effect in the field experiment. These two QTL together explained 33%, 35%, and 31% of the total phenotypic variation for disease spread, kernel infection, and DON concentration in the greenhouse experiments, respectively. In the field experiment, the two QTL explained 34% and 26% of the total phenotypic variation for FHB incidence and severity, respectively. W14 has both QTL, which confer reduced initial infection, disease spread, kernel infection, and DON accumulation. Therefore, marker-assisted selection (MAS) for both QTL should be implemented in incorporating W14 resistance into adapted backgrounds. Flanking markers *Xbarc133* and *Xgwm493* on 3BS and *Xbarc117* and *Xbarc56* on 5AS are suggested for MAS.

Key words: *Triticum aestivum* — Fusarium head blight — microsatellite — QTL mapping

Fusarium head blight (FHB), commonly called scab, is one of the most destructive diseases of wheat, and causes significant reductions in grain yield and quality. Mapping of quantitative trait loci (QTL) associated with FHB resistance and application of marker-assisted selection (MAS) can be used to accelerate the development of FHB-resistant cultivars. Mapping efforts to date have identified a major QTL on wheat chromosome 3BS for resistance to disease spread in most Chinese and Japanese resistance sources, such as 'Sumai3' and related sources (Anderson et al. 2001, Zhou et al. 2002, Buerstmayr et al. 2003, Somers et al. 2003). In addition, a QTL on chromosome 5AS has been identified for resistance to initial infection in a 'Sumai3'-derived line (Buerstmayr et al. 2003) and in a Brazilian source 'Frontana' (Steiner et al. 2004). The objectives of the current study were to characterize FHB resistance in Chinese wheat line W14 in comparison to QTL identified in previous studies and to determine the extent of

allelic variation at the two known FHB QTL for resistance to initial infection (type I), spread (type II), kernel infection, and deoxynivalenol (DON) accumulation.

Materials and Methods

Mapping population and FHB assessment: One double-haploid (DH) population of wheat (*Triticum aestivum* L.) comprising 96 lines was developed using the wheat by maize hybridization method (Laurie and Bennett 1988) from a cross between W14 and Pioneer Brand '2684' ('Pion2684'). W14 was derived from a recurrent selection population comprising 20 parents, including 15 FHB-resistant cultivars, such as 'Sumai3', 'Ning7840', 'Zhen7495', 'Wangshuibai', 'Fanshanxiaomai', 'Shinchunaga', 'Frontana', 'Yangmai 4', etc. (Jiang 1997, personal communication). This line has improved FHB resistance compared to 'Sumai3' on the basis of lower observed disease spread, kernel infection and DON production (Chen et al. 2000, H. Buerstmayr, personal communication). 'Pion2684' is a FHB-susceptible soft red winter wheat cultivar.

The DH lines and parents were evaluated in two greenhouse (2001 and 2002) and one field (2004) experiment using the single-floret inoculation and spray inoculation methods (Chen et al. 2000), respectively. In the greenhouse experiments, four inoculated plants per line received overhead mist irrigation for 3 days at an interval of 45 s per 30 min. Fusarium head blight severity of one to three inoculated spikes per plant was evaluated on the 21st day after inoculation and was calculated using the formula: (number of infected spikelets/total number of spikelets) × 100. Fusarium head blight severity for each line was based on a grand mean of all inoculated spikes. The percentage of fusarium-damaged kernels (FDK) for each line was determined as the number of infected kernels subdivided by the total number of kernels in the inoculated and hand-threshed spikes. These seeds were then assessed for DON concentration (ppm) using a Shimadzu QP2010 GC/MS system (Shimadzu Corporation, Kyoto, Japan; Mirocha et al. 1998). Severity data were collected from two independent experiments (2001 and 2002), whereas FDK and DON content were evaluated only in the 2001 experiment. In the field experiment, the two parental and 96 DH lines were planted in single 1.5-m-long rows with two replications arranged in a completely randomized design. Each row was spray inoculated twice, once at 100% spike emergence and again at 50% anthesis, with an 80-ml conidial suspension (50 000 spores/ml) by using a CO₂-pressurized sprayer (R&D Sprayers, Opelousas, LA, USA). Overhead mist irrigation was applied at 1-h intervals from 8:00 to 9:00 A.M. and again from 4:00 to 5:00 P.M. daily for 3 weeks when natural

precipitation was absent. Ten spikes of each parental and DH line were evaluated on the 21st day after the second inoculation. Fusarium head blight incidence for each line was calculated as (number of infected spikes/10) × 100. Fusarium head blight severity for each infected spike was calculated as (number of infected spikelets/total number of spikelets) × 100. Fusarium head blight severity for each line was based on a grand mean of 10 infected spikes per line.

Genetic mapping and QTL analysis: Four sets of simple sequence repeat (SSR) primers were used in the current study: (1) Gwm primers from Röder et al. (1998); (2) BARC primers provided by P. Cregan, USDA-ARS, Beltsville, MD; (3) WMC primers from published sequences (Gupta et al. 2002) and (4) PSP primers (Bryan et al. 1997) provided by M. Gale, John Innes Centre, Norwich, UK. A total of 308 SSRs were screened for DNA polymorphism among parents (W14 and 'Pion2684') and two extreme bulks using bulked segregant analysis (BSA) analysis (Michelmore et al. 1991). Polymorphic primer pairs were used to characterize the DH population of 96 lines. Marker segregation data were used to construct linkage maps using MAPMAKER 3.0b (Lander et al. 1987). A logarithm of odd (LOD) threshold of 3.0 was used for grouping. The most likely marker orders were determined using the MAPMAKER 'ripple' command. Extraction of DNA, polymerase chain reaction amplification and SSR assays were conducted as previously described by Saghai-Marooof et al. (1994).

Analyses of variance of field and greenhouse data, and correlation and regression analysis were conducted using AGROBASE software (<http://Agronomix.mb.ca>). Quantitative trait loci analysis was performed using averages from individual experiments for FHB assessment data as well as a grand mean over experiments. Fusarium head blight incidence (%), FHB severity (%), FDK and DON concentration (ppm) were tested separately in the QTL analyses. Composite interval mapping (CIM, Zeng 1994) was applied using QTL CARTOGRAPHER 2.0 software (Basten et al. 2002). Significance threshold level was determined by a permutation test with 1000 permuted samples (Doerge and Churchill 1996). A genome-wide significance level $P \leq 0.01$ for the type I error with an LOD threshold of 1.8 was used as a criterion to indicate putative QTL positions. Genetic variance (R^2) explained by QTL was calculated by the software.

Results

Phenotypic analysis of parental lines and their DH progeny

The parents, W14 and 'Pion2684', consistently displayed significant differences in response to *Fusarium graminearum* infection for all the traits evaluated. Except for FHB incidence, distribution of DH lines was skewed towards the resistant parent W14 for all the traits evaluated (data not presented). High correlation coefficients were obtained for FHB severity between the two greenhouse experiments, and among FHB severity, FDK and DON in the 2001 greenhouse experiment ($r = 0.69-0.91$, $P < 0.0001$). A high coefficient of correlation was also observed between FHB incidence and FHB severity in the field experiment ($r = 0.78$, $P < 0.0001$); however, weaker correlations were obtained between disease data from single-floret inoculations (greenhouse) and spray inoculations (field) ($r = 0.27-0.37$, $P < 0.01$ to $P < 0.001$).

Bulked segregant analysis and genetic maps

Out of 308 primer pairs screened, 18 pairs detected polymorphism between parents and bulks in the DH population. When these SSRs were assessed in the entire population, 16 retained significance at $P \leq 0.05$. Five chromosomal regions (1B, 2B, 3B, 5A and 7A) showed association with FHB resistance on the basis of single-marker analysis. Additional markers flanking these 16 SSRs were used to construct the genetic maps of these linkage

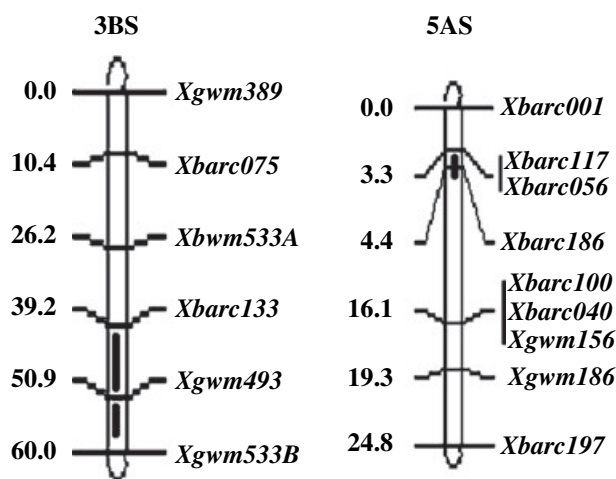


Fig. 1: Linkage maps of two major FHB QTL generated using a DH population of W14 × 'Pion2684'. Numbers at each marker represent the distance (in cM). The dark area indicates the QTL positions based on the CIM method

groups. A total of 39 SSRs were mapped in the DH population. Genetic maps of the linkage groups were constructed, and chromosomal identities were determined via comparison with the SSR maps of wheat published by Röder et al. (1998) and Shi et al. (2002). Genetic maps of 3BS and 5AS, which contain the major FHB QTL, are presented in Fig. 1.

QTL in W14 for FHB resistance identified in greenhouse experiments

Quantitative trait loci analyses indicated that one major QTL on chromosome 3BS was significantly associated with resistance to disease spread, kernel infection, and DON content, and another minor QTL on chromosome 5AS was significantly associated with resistance to kernel infection and DON content. Chromosomal map positions of these two QTL were the same or in the flanking regions for all three disease assessment parameters (Table 1, Fig. 1). This explains the highly significant correlation coefficients among FHB severity, FDK and DON content ($r = 0.79-0.88$). Quantitative trait loci estimates (Table 1) indicate that the 3BS QTL has a greater effect than the 5AS QTL, and the two QTL together explained 33%, 35% and 31% of the total phenotypic variation for disease spread, kernel infection and DON concentration, respectively.

QTL in W14 for FHB resistance identified in field experiments

Two QTL on chromosomes 3BS and 5AS were also detected for FHB incidence and severity in the field experiment (Table 1). On the basis of QTL estimates provided by CIM, the 5AS QTL had a greater effect than 3BS, especially for FHB incidence, type I resistance. These two QTL together explained 34% and 26% of the total phenotypic variation for FHB incidence and severity, respectively (Table 1).

Discussion

Mapping efforts to date have targeted several QTL on wheat chromosomes 2D, 3A, 3B, 4B, 5A and 6B (Anderson et al. 2001, Zhou et al. 2002, Buerstmayr et al. 2003, Shen et al. 2003, Somers et al. 2003, Steiner et al. 2004). The current study confirmed only

Table 1: Quantitative trait loci estimates for various FHB parameters in greenhouse and field experiments in a DH population of W14 × 'Pion2684'. Quantitative trait loci analysis was done by CIM, and was declared significant at LOD ≥ 1.8 determined by a permutation test

Map interval	Position (left marker, cM)	Chromosome	LOD	R ² (%)
Mean FHB severity (greenhouse 2001 and 2002)				
<i>Xgwm493–Xgwm533B</i>	52.9	3BS	8.4	33
FDK (greenhouse 2001)				
<i>Xbarc133–Xgwm493</i>	45.2	3BS	7.7	29
<i>Xbarc117–Xbarc186</i>	3.3	5AS	1.9	6
DON (greenhouse 2001)				
<i>Xbarc133–Xgwm493</i>	45.2	3BS	6.1	23
<i>Xbarc117–Xbarc186</i>	3.6	5AS	2.7	8
FHB incidence (field 2004)				
<i>Xbarc133–Xgwm493</i>	41.3	3BS	2.8	10
<i>Xbarc117–Xbarc186</i>	3.6	5AS	6.5	24
FHB severity (field 2004)				
<i>Xgwm493–Xgwm533B</i>	54.7	3BS	2.9	10
<i>Xbarc117–Xbarc186</i>	3.6	5AS	4.4	16

the presence of major FHB resistance QTL on chromosomes 3BS and 5AS. Furthermore, an additive-by-additive epistasis between these two QTL was detected in the greenhouse experiments, but not in the field (data not presented).

The two W14 QTL were mapped to similar chromosomal positions on 3BS and 5AS in both greenhouse and field experiments. However, the 3BS QTL explained a greater proportion of the phenotypic variance for resistance to fungal spread in the greenhouse experiments than for FHB resistance in the spray-inoculated field experiment, while the opposite was observed for the 5AS QTL in the greenhouse vs. field experiments. This suggests that genetic control of type I resistance is likely different from that of type II resistance. It is also consistent with the moderate to low correlation values observed between FHB resistance data from single-floret-inoculated and spray-inoculated experiments ($r = 0.27–0.39$).

This study confirms previous reports that FHB resistance in many Chinese lines, such as W14, is governed by two major QTL on chromosomes 3BS and 5AS. The 3BS QTL was confirmed to have a major effect on type II resistance (Anderson et al. 2001, Zhou et al. 2002, Buerstmayr et al. 2003) and DON accumulation (Somers et al. 2003), whereas the 5AS QTL was confirmed to have a major effect on type I resistance (Buerstmayr et al. 2003, Steiner et al. 2004) and DON accumulation (Somers et al. 2003). Therefore, MAS for both QTL should be implemented to facilitate the development of cultivars having more overall effective FHB resistance via reduction of initial infection, FHB spread, kernel infection and DON production.

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