Theor Appl Genet (2003) 106:961–970
DOI 10.1007/s00122-002-1160-5

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Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat

Received: 22 February 2002 / Accepted: 20 August 2002 / Published online: 13 December 2002

Abstract Fusarium head blight (FHB) caused by *Fusarium culmorum* is an economically important disease of wheat that may cause serious yield and quality losses under favorable climate conditions. The development of disease-resistant cultivars is the most effective control strategy. Worldwide, there is heavy reliance on the resistance pool originating from Asian wheats, but excellent field resistance has also been observed among European winter wheats. The objective of this study was to map and characterize quantitative traits loci (QTL) of resistance to FHB among European winter wheats. A population of 194 recombinant inbred lines (RILs) was genotyped from a cross between two winter wheats Renan (resistant)/Récital (susceptible) with microsatellites, AFLP and RFLP markers. RILs were assessed under field conditions For 3 years in one location. Nine QTLs were detected, and together they explained 30–45% of the variance, depending on the year. Three of the QTLs were stable over the 3 years. One stable QTL, *QFhs.inra.2b*, was mapped to chromosome 2B and two QTLs *QFhs.inra.5a2* and *QFhs.inra5a3*, to chromosome 5A; each of these QTLs explained 6.9–18.6% of the variance. Other QTLs were identified on chromosome 2A, 3A, 3B, 5D, and 6D, but these had a smaller effect on FHB resistance. One of the two QTLs on chromosome 5A was linked to gene *B1* controlling the presence of awns. Overlapping QTLs for FHB resistance were those for plant height or/and flowering time. Our results confirm that wheat chromosomes 2A, 3A, 3B, and 5A carry FHB resistance genes, and new resistance factors were identified on chromosome arms 2BS and 5AL. Markers flanking these QTLs should be useful tools for combining the resistance to FHB of Asian and European wheats to increase the resistance level of cultivars.

Keywords *Fusarium culmorum* · *Triticum aestivum* · Disease resistance · QTL mapping

Communicated by H.C. Becker

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Introduction

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum* or *F. culmorum*, is an economically important disease of cereals that occurs in humid and semi-humid wheat-growing areas (Parry et al. 1995; McMullen et al. 1997). FHB may cause serious losses when favorable climate conditions occur between the flowering and soft dough stage of kernel development (Parry et al. 1995; McMullen et al. 1997) by affecting yield and grain quality and contaminating grains with mycotoxins that are toxic to humans and animals. The presence of mycotoxins in infected grains futher exacerbates the economic losses caused by FHB (McMullen et al. 1997). Fungicide treatments and agriculture management practices reduce the damage, but significant yield and quality losses can still occur (Parry et al. 1995). Thus, the development of disease-resistant cultivars will probably be a key component in an effective strategy to control FHB.

The genetic basis of resistance appears to be nonspecific, because FHB induces the same ranking for resistance in most wheats reported to date (Van Eeuwijk et al. 1995; Miedaner 1997). Although no complete resistance or immunity to FHB has been observed among resistant wheat germplasm, resistant genotypes have been identified, and three origins of resistant germplasm are recognized: (1) spring wheat from Asia (Ning 7840, Sumai 3, Nobeokabozu); (2) spring wheat from Brazil

(Frontana); (3) winter wheat from Europe (Praa 8, Novokrumka) (Gilbert and Tekauz 2000). Resistance to FHB in wheat is quantitatively inherited, and environmental conditions greatly affect the expression of resistance (Snijders and Van Eeuwijk 1991; Bai and Shaner 1994). The number of genes involved in the resistance has been determined by monosomic analysis and studies employing substitution lines, and resistance is controlled by two to five major genes and several other genes with minor effects (Van Ginkel et al. 1995; Buerstmayr et al. 1997, 1999; Miedaner 1997; Yao et al. 1997; Grausgruber et al. 1998, 1999). Among genotypes originating from China and South America, less than five genes are thought to be involved. Resistance in Frontana is controlled by a minimum of three minor genes, and in Sumai 3, by two major genes with additive effects (Van Ginkel et al. 1995; Miedaner 1997; Grausgruber et al. 1999).

Worldwide, wheat breeding programs rely heavily on the FHB resistance pool from Asia (Sumai 3 and its derivatives), which is considered to be the most effective source of resistance. The best characterized resistance is that of Sumai 3, which has been widely used in crosses (Rudd et al. 2001). The extensive use of only a few sources of resistance may induce a selection pressure in the pathogens to erode the effectiveness of the genes involved. Moreover, new sources of resistance could improve the level of wheat resistance and prevent a potential breakdown of resistance. FHB-resistant genotypes have already been identified in winter wheat, and excellent field resistance have been observed in some European wheats (Snijders 1990a, b; Mesterhazy 1995). However, to date the resistance of European winter wheats is not well characterized compared to Asian germplasm.

In recent investigations on FHB resistance molecular markers have been used to estimate the number and location of genes involved in traits showing a complex inheritance. In barley, quantitative trait loci (QTLs) were used to assess gene action and interactions with plant organs, plant growth stages and environments at the individual QTL level (De la Penna et al. 1999; Zhu et al. 1999; Ma et al. 2000). Using Ning 7840 as a resistant wheat cultivar, Bai et al. (1999) reported a major QTL initially associated with the long arm of chromosome 7B. More recently, the same major QTL was reassigned to the chromosome 3BS using microsatellite mapping (Zhou et al. 2000). Using recombinant inbred lines (RILs) from the crosses Sumai 3/Stoa and ND2603/Butte86, Waldron et al. (1999) and Anderson et al. (2001) identified the same major QTL of resistance on chromosome 3BS.

In the investigation reported here we employed a population of RILs originating from a cross between a susceptible (Récital) and a resistant European winter wheat (Renan) for 3 years of experimentation under field conditions to map loci associated with *Fusarium culmorum* resistance. The stability of resistance over years and the relationships between resistance to FHB, plant height, and heading date were also studied.

Materials and methods

Plant material

A mapping population of 194 F_7 RILs was developed by single-seed descend from the cross between two European winter wheat cultivars, Renan and Récital. Renan is a scab-resistant cultivar derived from the cross Mironovskaia 808/Maris Huntsman//VPM Moisson 4/Courtot, and Récital is a susceptible cultivar derived from the cross Mexique 267/5/(81.12/Besostaya1//Heine VII/3/Nord/4/Tadorna)/9369.

The F_7 to F_9 generations of the RILs of both parents were evaluated in the field with two replicates during 1998, 1999, and 2000, at the INRA Experimental Station in Rennes. RILs were grown in hill plots of 20–30 plants under irrigation. Each plot was inoculated individually by spraying a spore suspension of *Fusarium culmorum* at anthesis. The field was irrigated for 20 min (6 mm/h) prior to inoculation.

The *F. culmorum* inoculum consisted of a mixture of field isolates that originated from naturally scabbed ears from the previous year and maintained by periodic transfer onto potato dextrose agar. The spores were mass-produced on sterile barley seeds that had been autoclaved twice. Following incubation at room temperature until sporulation, conidia were washed from colonized kernels, and the concentration was adjusted to 10^6 spores/ml using a hemacytometer. Inoculum was stored at –20 °C until inoculation.

Following inoculation, mean daily temperatures were recorded and thermal time (degree days = °C.d) was calculated. FHB severity was assessed at 350 °C.d and 450 °C.d after inoculation. The proportion of infected spikelets was recorded using a logarithmic scale from 1 (no disease symptoms) to 9 (spikes completely diseased) (Saur 1991; Ban and Suenaga 2000). Flowering time was recorded in all 3 years tested (1998, 1999, and 2000), and plant height was measured (to the tip of the spike, excluding awns) in 1999 and 2000.

Map construction

A genetic linkage map was constructed using 182 restriction fragment length polymorphism (RFLP), 258 microsatellites, and 68 amplified fragment length polymorphism FLP(A) markers. This map also included some storage protein loci and the locus B1 for awn presence. RFLP analysis was performed using the radioactive probe protocol of Landry et al. (1991). The polymerase chain reaction (PCR) for microsatellites and AFLP detection were performed using the methods described by Tixier et al. (1998) and Bert et al. (1999), respectively.

For each segregating marker, a Chi-square analysis ($\alpha = 0.01$) was performed to test for deviation from the expected segregation (ratio 1:1). Linkage analysis was performed with the package MAPMAKER/EXP ver. 3.1 (Lander and Bostein 1989; Lincoln et al. 1992). Recombination fractions were converted into map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). Linkage groups were assigned to chromosomes via comparison with the reference maps Courtot/Chinese Spring and Synthetic/Opata described by Cadalen et al. (1997) and Röder et al. (1998), respectively. The map Renan/Récital was composed of 34 linkage groups and covered 2,259.6 cM.

Trait analysis

Statistical analyses of traits were performed using the SAS statistics package (SAS Institute, Raleigh, N.C.). Replicate, genotype, year, and flowering time effects were estimated by analysis of variance (ANOVA). For each year, one-way ANOVA were performed using a generalized linear model (PROC GLM). Normality of each residual distribution was verified using the PROC UNIVARIATE procedure, and homogeneity of phenotypic variances between replicates and genotypes was verified using Bartlett's test. The Pear-

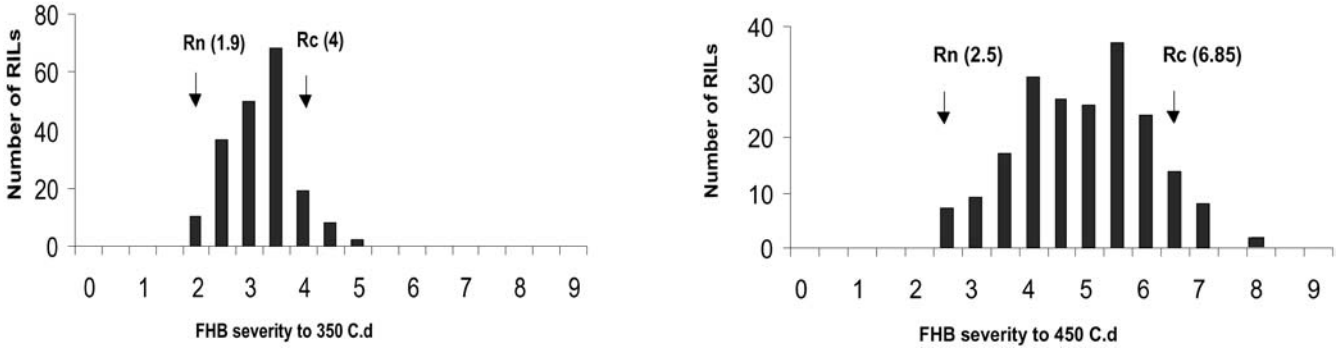

Fig. 1 Distribution of RILs derived from cross Renan/Récital for FHB severity at 350 °C.d and 450 °C.d after artificial inoculation with *Fusarium culmorum* for the mean over 3 years. *Rn* Renan, *Rc* Récital

Table 1 ANOVA of genotype, year and replication effect and proportion of phenotypic variation for FHB severity at 350 °C.d F350 and 450 °C.d F450 among recombinant inbred lines from a Renan/Récital cross

	F350					F450				
	df	MS	F	P	H^2	df	MS	F	P	H^2
Lines	193	1.83	6.06	0.0001		193	5.52	8.92	0.0001	
Years	2	106.16	352.67	0.0001		2	222.19	358.81	0.0001	
Lines × years	386	0.63	2.09	0.0001		386	1.58	2.56	0.0001	
Replications (in years)	3	1.07	3.52	0.0150		3	1.23	2.0	0.113	
error	572	0.305				573	0.61			
					0.66					0.72

son correlation coefficients between years were estimated for every trait on the adjusted means of the RILs using the PROC CORR procedure (SAS). Within each year, heritability was estimated from ANOVA using the formula: $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e^2/r)]$, and over the 3 years, using the formula: $h^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_{GE}^2/E) + (\sigma_e^2/rE)]$, with σ_G^2, the genetic variance: σ_{GE}^2, the genotype × environment interaction variance; σ_e^2, the residual variance; E, the number of environments, r, the number of replicates per line. Adjusted means from covariance analyses were used for QTL analysis. In all statistical tests, a probability level of $P < 0.05$ was employed.

QTL analysis

QTL detection was performed by interval mapping (IM) (Lander and Bostein 1989) and composite interval mapping (CIM) (Zeng 1993, 1994) using QTL CARTOGRAPHER software (Basten et al. 1997). A forward-backward stepwise regression was performed to choose cofactors before performing QTL detection by CIM. Ten cofactors with the highest F values were taken into account. A window size of 10 cM around the test interval was chosen for all analyses. Permutation tests were performed to estimate appropriate significant thresholds for both IM and CIM. After 1,000 permutations, LOD thresholds of 3.1 were chosen for IM and CIM. For each QTL, the position, the additive effect, and the percentage of phenotypic variation explained were estimated.

Epistatic interactions between 243 markers present in the framework map were analyzed by two-way ANOVA for all pairwise combinations of marker loci. Only interactions detected in the 3 years with $P < 0.0001$ were considered.

Results

FHB severity

There was significant variation among RILs for FHB severity at 350 °C.d and 450 °C.d. FHB severity in the RIL populations varied on average over the 3 years from 1.5 to 5.5 at 350 °C and from 2.5 to 7.7 at 450 °C.d (Fig. 1). Phenotypic means of the RILs population for disease rate at 450 °C in 1998 and 1999 were 5.1 and 5.2, whereas in 2000, the phenotypic mean was 3.8. This result was due to a lower FHB severity in 2000. In 2000, the FHB epidemic did not progress between 350 °C.d and 450 °C.d among susceptible lines. In 1998 and 1999, a distribution with two peaks was observed at 450 °C, but in 2000, a single peak for symptom distribution was observed (data not shown).

ANOVA showed genotype, year, and genotype × year interaction effects; block effects within years were not significant (Table 1). ANOVA also revealed a significant effect of the inoculation day in 1998 and 1999 (data not shown). In 2000, the flowering period was shorter and the probability levels for effect of inoculation day was lower (F = 0.60, P = 0.83) than in 1998 (F = 3.65, P = 0.0002) and 1999 (F = 7.32, P = 0.0001) due to the climatic conditions. Therefore, the inoculation day was employed as a covariate in two-way ANOVA.

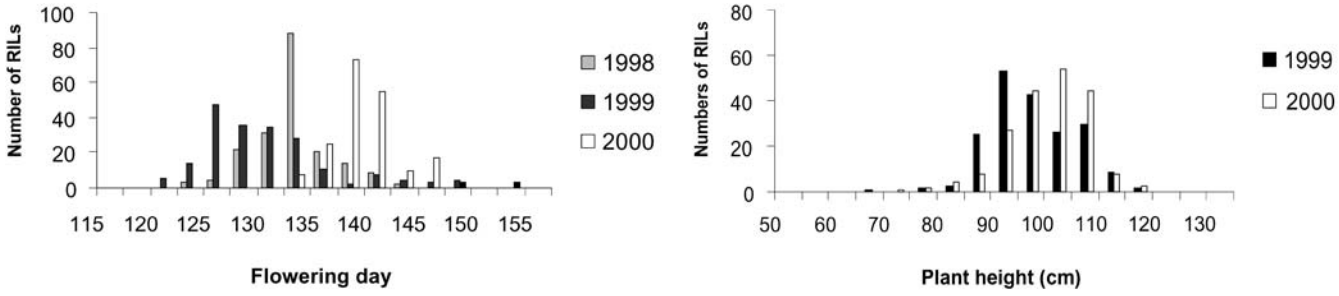

Fig. 2 Distribution of RILs derived from cross Renan/Récital for plant height in 1999 and 2000 and earliness in 1998, 1999, and 2000

Frequency distributions of RIL adjusted means showed a continuous genotypic variation of the trait (Fig. 1). Using the W statistics of Shapiro, distributions of residuals were not Gaussian, and data transformation did not restore the normality. As deviation was weak and variances were homogeneous, untransformed data were employed in the analyses.

Within-year heritabilities were high and ranged from 0.75 to 0.84 for disease scores at 350 and 450 °C.d, respectively. Over years, heritability values were 0.66 and 0.72 at 350 °C.d and 450 °C.d, respectively.

Height and flowering date

The mean height over 3 years was 104 cm for Renan and 96 cm for Récital, thus the difference in height between Renan and Récital is small. Renan and Récital both carry the dwarfing gene *Rht1*, but they have different genetic backgrounds for height (Trottet, unpublished results). A segregation for plant height was observed. In the RIL population, plant height followed a normal distribution (Fig. 2) and varied from 69 to 116 cm in 1999 and from 72 to 117 cm in 2000.

We also observed a segregation for earliness; Récital was day-length insensitive, whereas Renan was sensitive. Flowering time followed a Gaussian curve (Fig. 2). Over the 3 years, Renan flowered on day 140 and Récital on day 132. For plant height and flowering date, heritability ranged between 0.88 and 0.92.

Relationship between traits

For FHB severity, correlations among years were positive and highly significant ($P < 0.0001$). For example, at 450 °C.d, correlations varied from 0.46 to 0.58 according to year. Correlations between FHB, plant height, and flowering were also observed. A negative correlation was found between FHB severity and plant height in 1999 ($r = –0.43$, $P = 0.0001$) and 2000 ($r = –0.30$, $P = 0.0001$), and between FHB severity and flowering time in 1998 ($r = –0.46$, $P = 0.0001$), 1999 ($r = –0.32$, $P = 0.0001$), and 2000 ($r = –0.21$, $P = 0.0001$).

QTL analysis

QTLs associated with FHB severity, plant height, and flowering time were identified in all 3 years and on the average of the 3 years. Results presented in this paper represent those detected with CIM. Nine QTLs with additive effect were associated with FHB resistance (Tables 2 and 3). QTLs were localized on chromosomes 2A, 2B, 3A, 3B, 5A, 5D, and 6D. The total phenotypic variance explained by these QTLs varied from 35% to 44% for scoring at 350 °C.d and from 30% to 45% for scoring 450 °C.d according to the year. In eight out of the nine QTLs detected, resistance was conferred by the alleles in the resistant parent Renan. Markers associated with an allele of Récital were located on chromosome 3A. QTL *QFhs.inra-3a* explained a small part of the variation and was detected only in 1998 and 1999. Three QTLs mapped on chromosomes 2B and 5A were consistent across the 3 years. On chromosome 5A, three distinct QTLs (*QFhs.inra-5a1*, *QFhs.inra-5a2*, *QFhs.inra-5a3*) were identified. QTLs *QFhs.inra-5a1* and *QFhs.inra-5a2* were separated approximately by 45 cM and QTLs *QFhs.inra-5a2* and *QFhs.inra-5a3* by 80 cM (Fig. 3) *QFhs.inra-5a1* is a minor QTL that explained a small part of resistance (3.6–5.4% of variance) and was mapped on the short arm of the chromosome 5A between markers *Xpsr0170a* and *Xgwm443*. *QFhs.inra-5a2* and *QFhs.inra-5a3* were mapped on the long arm of the chromosome 5A between markers *Xbcd0508* and *Xgwm271b*, and *Xgwm595* and *B1*, respectively. *QFhs.inra-5a2* and *QFhs.inra-5a3* were detected in all 3 years. *QFhs.inra-5a2* explained the largest part of the phenotypic variance, between 10.1% and 18.6% depending on the year. *QFhs.inra-5a3* explained 3.6–12.3% of the phenotypic variance and is associated with the phenotypic marker of awnedness, *B1*.

The expression of the *QFhs.inra-2b* QTL was also consistent over 3 years (Tables 2 and 3). *QFhs.inra-2b* was located in the *Xgwm388-Xgwm257*a interval on the short arm of the chromosome 2B and explained 6.9–10.8% of the phenotypic variation. *QFhs.inra-2b* was detected at 450 °C.d in 1998 and 2000 and at 350 °C.d in 1999. On the adjusted means averaged over 3 years, *QFhs.inra-2b* explained 12% of the phenotypic variation, and *QFhs.inra-5a2* and *QFhs.inra-5a3* explained 19.2% and 8.5%, respectively. The *QFhs.inra-3b* QTL, situated on the long arm of chromosome 3B, was identified in 1998, 1999, and 2000, although the as-

Table 2 Summary of QTLs for FHB resistance at 350 °C.d detected by CIM in the Renan/Récital population for 1998, 1999, 2000, and the mean over 3 years

Names	Closest markers[a]	Chromo-some	1998			1999			2000			Mean over 3 years		
			LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity
QFhs.inra-2a	*Xgwm311b*	2A	2.9	5.6	–0.211	2.3	4.7	–0.177				4.6	6.4	–0.163
QFhs.inra-2b	*Xgwm374*	2B				4.9	9.1	–0.236				5.5	8.5	–0.188
QFhs.inra-3a	*Xbcd0372*	3A	2.6	6.3	+0.230	2.4	5.4	+0.183				2.3	4	+0.125
QFhs.inra-3b	*Xtam61*	3B	2.4	4.6	–0.199									
	Xgwm383b	3B										3.0	5.4	–0.145
QFhs.inra-5a1	*Xgwm443*	5A	2.5	3.6	–0.176							4.3	6.2	–0.158
QFhs.inra-5a2	*Xgwm639b*	5A	7.3	18.6	–0.399	5.9	15.2	–0.302	3.3	10.2	–0.186	8.9	19.2	–0.273
QFhs.inra-5a3	*B1*	5A	2.7	3.6	–0.179	5.1	8.5	–0.230	7.6	12.3	–0.209	5.8	7.12	–0.171
QFhs.inra-5d	*Xcfd0029*	5D	4.4	7	–0.248									

[a] The most closely associated marker locus with resistance is indicated
[b] Lod is the log-likehood at the position
[c] R^2 is the percentage of phenotypic variation explained by the QTL

Table 3 Summary of QTLs of resistance to FHB at 450 °C.d detected by CIM in the Renan/Récital population for 1998, 1999, 2000, and the mean over 3 years

Names	Closest markers[a]	Chromo-some	1998			1999			2000			Mean over 3 years		
			LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity	LOD[b]	R^{2c}	Additivity
QFhs.inra-2a	*Xgwm382c*	2A	2.7	4.6	–0.336	2.2	6.1	–0.330				6.3	14.4	–0.407
QFhs.inra-2b	*Xgwm374*	2B	5.8	10.8	–0.502				3.8	6.9	–0.269	7.6	12	–0.377
QFhs.inra-3a	*Xbcd0372*	3A				4.4	8.11	+0.379				3.7	6.2	+0.264
QFhs.inra-3b	*Xtam61*	3B	2.2	3.4	–0.285									
	Xgwm131b	3B				2.0	3.8	–0.256						
	Xgwm383b	3B							4.0	7.5	–0.276	5.4	10.5	–0.34
QFhs.inra-5a1	*Xpsr0170a*	5A	3.1	5.4	–0.359				2.3	4.2	–0.207	3.8	5	–0.244
QFhs.inra-5a2	*Xgwm639b*	5A	3.0	10.1	–0.491	6.1	13.8	–0.491				6.6	14	–0.394
QFhs.inra-5a3	*B1*	5A	2.5	4	–0.317	5.8	8.7	–0.396	3.5	5	–0.237	6.3	8.5	–0.314
QFhs.inra-6d	*Xcfd0042*	6D	3.2	10.5	–0.495							2.7	6.6	–0.270

[a] The most closely associated marker locus with resistance is indicated
[b] Lod is the log-likehood at the position
[c] R^2 is the percentage of phenotypic variation explained by the QTL

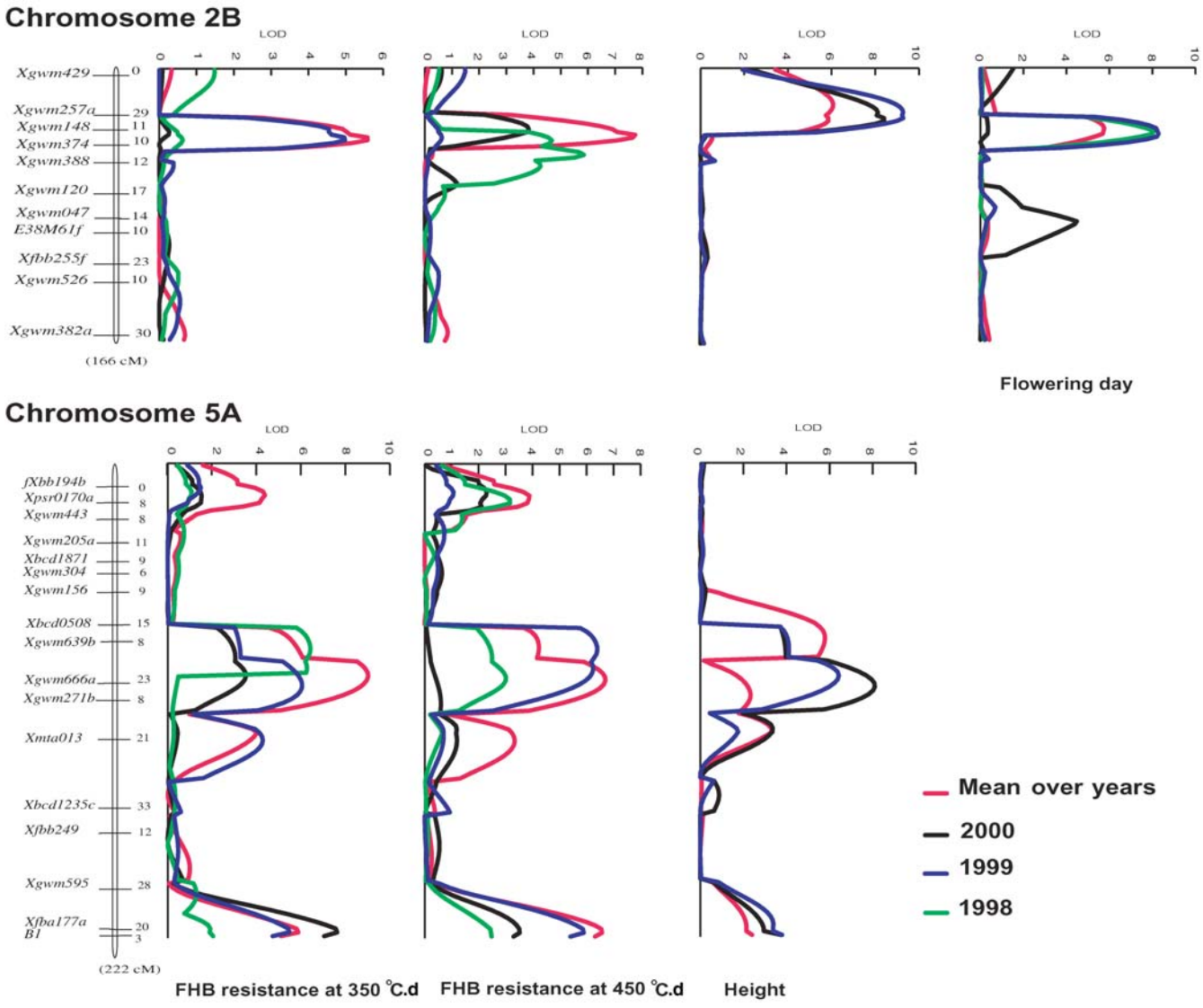

Fig. 3 LOD score scan, on chromosome 2B and 5A, for QTLs associated with resistance, plant height, and flowering date. The scan for QTLs for each year (1998, 1999, and 2000) and the mean over the three years are represented separately. The position and the names of molecular markers are shown on the chromosome along the *horizontal axis*. The LOD score scan was obtained by CIM using ten cofactors

sociation between the closest marker and the QTL depended on the year. *QFhs.inra-3b* was linked to a different marker each year between *Xtam61* and *Xgwm383b*. *QFhs.inra-3b* explained 3.4–7.5% of the phenotypic variation depending on the year, and exceeded the LOD threshold only in 2000 (LOD > 3.1). When the means were averaged over 3 years, *QFhs.inra-3b* explained 10.5% of the variation at 450 °C.d.

Other QTLs with minor effects were identified only in 1 or 2 of the years on chromosomes 2A, 5D, and 6D. QTLs *QFhs.inra-5d* and *QFhs.inra-6d* were detected only in 1998 at 350 °C.d and 450 °C.d, respectively. QTL

Table 4 QTLs for plant height detected by CIM in the Renan/Récital population for the mean over 2 years (1999 and 2000)

Names	Closest markers[a]	Chromosome	LOD[b]	R^{2c}	Additivity
QHt.inra-2b	*Xgwm429*	2B	5.8	15.4	+2.94
QHt.inra-4a	*Xfba243a*	4A	6.5	15	+2.9
QHt.inra-5a	*Xgwm639b*	5A	5.7	10.8	+2.43
QHt.inra-6d	*Xcfd0076*	6D	3.7	8.1	+2.07
QHt.inra-7a	*Xcdo0545*	7A	3.2	7.7	+3.74

[a] The most closely associated marker locus with resistance is indicated
[b] Lod is the log-likehood at the position
[c] R^2 is the percentage of phenotypic variation explained by the QTL

QFhs.inra-2a was inconsistent; it was detected in 1998 and 1999 and explained 4.6–6.1% of the variance. When the mean of the 3 years was employed, *QFhs.inra-2a* was significant (LOD > 3.1) and explained 14% of the phenotypic variation.

Table 5 QTLs of flowering time detected by CIM in the population Renan/Récital for the mean over 3 years (1998, 1999, and 2000)

Names	Closest markers[a]	Chromosome	LOD[b]	R^{2c}	Additivity
QEet.inra-2b	*Xgwm148*	2B	5.7	11.9	+1.81
QEet.inra-2d	*XksuE003b*	*2D*	2.7	6.5	+1.32
QEet.inra-7d	*Pch1*	7D	3.9	7.3	–1.38

[a] The most closely associated marker locus with resistance is indicated
[b] Lod is the log-likehood at the position
[c] R^2 is the percentage of phenotypic variation explained by the QTL

Analysis for digenic epistasy by two-way marker analysis showed a significant interaction between markers *Xfba118b* and *Xgwm611* on chromosomes 1A and 7B for the 3 years that explained 11–14% of the variance. The combined additive and epistatic effects accounted for 41–59% of the total variation depending on the year.

Six QTLs for plant height were detected (Table 4); four QTLs were detected over 2 years. QTLs *QHt.inra-2b* and *QHt.inra-5a*, on chromosomes 2B and 5A, respectively had a strong effect on plant height (Table 4). *QHt.inra-2b* explained 10.2–15.5% of the phenotypic variance and *QHt.inra-5a* 14.6–18.6%. QTL *QHt.inra-2b* was mapped in the *Xgwm257*a-*Xgwm429* interval on chromosome 2B, and *QHt.inra-5a* in the *Xgwm639b-Xgwm666a* interval on chromosome 5A. *QHt.inra-2b* and *QHt.inra-5a* overlapped with QTLs *QFhs.inra-2b* and *QFhs.inra-5a2* for FHB resistance (Fig. 3). Other stable QTLs were identified on chromosome 3A and 4A. The expression of *QHt.inra-4a* was consistent but explained 15% of the phenotypic variation on the average of the 2 years. *QHt.inra-3a* was detected in 2 years but has a minor effect. A year-specific QTL was also identified on chromosome 6D.

Three QTLs were identified for flowering time on chromosomes 2B, 2D, 7D (Table 5). These QTLs were observed over the 3 years. One major QTL, *QEet.inra-2b*, which was detected on chromosome 2B between markers *Xgwm257a* and *Xgwm374*, explained 14–17.1% of the phenotypic variance. *QEet.inra-2b* overlapped with the QTL of FHB resistance (*QFhs.inra-2b*) and plant height (*QHt.inra-2b*) (Fig. 3). *QEet.inra-2b* is conferred by the allele of Renan. Two other stable QTLs, *QEet.inra-2b* and *QEet.inra-7d*, mapped on chromosome 2Dand 7D, respectively, explained 4.5–17.4% of the variance phenotypic. QTL *QEet.inra-7d* was linked to the marker *Pch1*.

Discussion

FHB assessment

This paper reports the mapping of QTLs associated with low FHB severity in the winter wheat cv. Renan. Using a spray method to inoculate spikes and scoring disease at 350 °C.d and 450 °C.d, we observed high heritability values for FHB resistance in Renan/Récital RILs. These results are consistent with previously genetic studies of FHB resistance (Bai and Shaner 1996; Anderson et al. 2001). Saur and Trottet (1992) and Singh et al. (1995) measured FHB resistance by visual observation and reported heritability estimates ranging from 0.66 to 0.93 under field conditions. Recently, high broad-sense heritabilities ($h^2 > 0.75$) for FHB resistance have been reported (Buerstmayr et al. 2000). The spray method proved to be very simple and convenient; however infection is known to vary in response to environmental conditions. In the field, to minimize environmental effects, we have evaluated RILs for resistance to FHB by artificial inoculation under irrigation. Following 3 years of experimentation and two replications within each year, an accurate evaluation of the population for the resistance to FHB under field conditions was obtained. This is the first report of QTL analysis for wheat resistance to FHB under field conditions. Previous genetic and molecular marker studies for FHB resistance in wheat focussed on type-II resistance because resistance to the spread of the pathogen is more stable than resistance to the primary infection (Bai and Shaner 1994). As a favorable environment is a key factor in the initiation and development of FHB infection (Bai and Shaner 1994), previous studies on QTL mapping focussed on the assessment of type-II resistance under controlled environment and greenhouse conditions (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001). In evaluating only type-II resistance (especially that derived from Sumai 3), the authors have simplified the evaluation of a complex disease system. However, other types of resistance also need to be identified and combined to adequately protect against severe FHB epidemics.

In the population Renan/Récital, variation of FHB symptoms was clearly quantitative, thereby confirming the polygenic control of this resistance in winter wheat. The distribution for FHB resistance characterized by two peaks, particularly at 450 °C.d, was clearly observed in crosses Sumai 3/Stoa and Ning/Clark (Bai et al. 1999; Waldron et al. 1999). Collectively, these results confirm that resistance to FHB is controlled by one or few major genes and several minor genes.

QTL analysis

The total variance explained by all QTLs detected in this study varied from 30% to 45% according to the year. Less than half of the total variance was explained. The evaluation of FHB resistance in wheat under field conditions in order to map QTLs resulted in greater environmental variation, and this reduced the percentage of the phenotypic variation that utilized for QTL detection. The unexplained variation in FHB resistance may be attributable to QTLs that remain undetected due to their minor effects. Alternatively, there may not be available markers

in regions associated with those QTLs since the map covers approximately only 85% of the wheat genome. Epistatic interaction between additive QTLs could also contribute to the unexplained variation. Several studies have reported that digenic epistasy may play an important role in unexplained variation in many mapping experiments (Lefebvre and Palloix 1996; Li et al. 1997; Lin et al. 2000). The importance of epistasy in FHB resistance is unclear. While some evidence of epistatic effects contributing to FHB resistance have been observed (Bai and Shaner 1994), several investigators have concluded that additive effects account for most of the genetic variance for FHB resistance and that epistasy might occur only in some crosses (Bai et al. 2000; Buerstmayr et al. 2000). Recently, Anderson et al. (2001) found no epistatic interactions in either Sumai 3/Stoa or ND2603/Butte 86 that explained more than 5% of the variation. In our study, one significant epistatic interaction was observed, and additive QTLs explained most of the variation.

By combining the results over the 3 years, we identified nine genomic regions containing putative quantitative trait loci associated with field resistance to FHB on chromosomes 2A, 2B, 3A, 3B, 5A, 5D, and 6D. Genes for FHB resistance in wheat have been reported to be located on many different chromosomes (Yao et al. 1997; Buerstmayr et al. 1999). While 18 of the 21 wheat chromosomes have been implicated in FHB resistance (Fedak et al. 1998), Buerstmayr et al. (1999) concluded that chromosomes 6D, 6B, 5A, 4D, and 7A were the ones most frequently associated with FHB resistance of wheat cultivars. The use of molecular markers to study Sumai 3 and its derivatives has improved our knowledge of the locations of FHB resistance genes; chromosomes 5A, 3B, and 6B are implicated in FHB resistance genes in a major way, and chromosomes 2A, 3A, and 7A to a lesser extent (Kolb et al. 2001). Recently, several QTL mapping studies on FHB resistance of Sumai 3 have been published. On a Sumai 3/Stoa cross, QTLs were identified on chromosomes 6BS and 3BS in Sumai 3 and on chromosomes 2AL and 4BL in Stoa (Waldron et al. 1999). Collectively, these studies (Chen et al. 2000; Zhou et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002) confirm that chromosome 3BS of Sumai 3 carries a gene with a major effect on FHB resistance. We have also identified QTLs on chromosomes 2A and 3B with a minor effect on FHB resistance in Renan. Using common markers between maps, we can confirm the QTL on chromosome 2AL to be the same as that of Waldron et al. (1999) and Anderson et al. (2000). The QTL identified on chromosome 3B in cv. Renan was located on the long arm and appears to be unrelated to the major effect gene in Sumai 3 on chromosome 3BS.

Chromosome 5A in cv. Renan seems to play a predominant role in FHB resistance. Two consistent QTLs were mapped on chromosome 5AL, with one of the two QTLs explaining the majority of the phenotypic variance. Chromosome 5A has been implicated in FHB resistance in Sumai 3 using monosomic analysis (Yu 1982), substitution lines (Grausgruber et al. 1998, 1999), and in crosses with Sumai 3 and Saikai 165 (Ban and Suenaga 2000). In a Hungarian breeding line containing the two resistance sources of Nobeokabozu and Sumai 3, chromosomes 3B and 5A were also found to enhance resistance (Buerstmayr et al. 1999). Recently, a minor QTL has been identified on chromosome 5AS in the cross CM-82036/Remus (Buerstmayr et al. 2002).

QTL stability

QTL analysis on the adjusted means over the 3 years followed by analyses within each year was useful to assess the stability of QTLs across environments. In the present work, QTL stability over years was moderate. Of the nine QTLs detected, three QTLs on chromosome 2B and 5A, associated with low FHB severity, were detected in all years and seem to be essential for resistance expression in cv. Renan. The other QTLs mapped were expressed only during 1 or 2 years and had a minor effect on the resistance. Moreover, we observed a year effect on the expression of QTLs *QFhs.inra-2b*, *QFhs.inra-5a1* and *QFhs.inra-5a2*. This QTL instability could be due to variations in the infestation level in the field. This appeared to be true in 2000, when disease pressure was lower than in 1998 and in 1999. Consequently, in 2000, only consistent QTLs were identified with lower effects. A heavy disease pressure appears to be essential in resistance for QTL mapping for FHB.

Recent studies on mapping QTLs to FHB resistance in barley with respect to QTL stability in different environments over 2 or 3 years (De la Penna et al. 1999; Zhu et al. 1999; Ma et al. 2000) identified few stable QTLs of FHB resistance in all of the environments tested, however, a stable QTL was identified on chromosome 2HS (Ma et al. 2000).

Characters associated to FHB resistance

The QTL located on the long arm of the chromosome 5A was linked to the gene *B1* for the presence of awns. The linkage between FHB resistance and awnedness was first reported by Snijders (1990a) in winter wheat infected with *F. culmorum*. Recently, (Ban and Suenaga 2000) demonstrated that one of the two major genes in Sumai 3 is linked in repulsion to the *B1* gene. While the presence of awns has been shown to enhance the development of disease (Mesterhazy 1995), our results confirmed a linkage between one resistance factor and the presence of awns. However, the linkage appears easily broken, and the development of FHB resistant cultivars is easily attainable (Ban and Suenaga 2000; Buerstmayr et al. 2000).

QTLs for height and flowering time were also mapped to analyze their potential role on the level of FHB resistance. Stable QTLs on chromosomes 2B and 5A overlapped with QTLs of plant height or/and flowering time and support the negative correlation observed

between FHB severity and plant height or flowering date. Negative correlations between visual FHB symptoms and developmental traits have been observed in wheat (Mesterhazy 1995; Miedaner 1997; Buerstmayr et al. 2000) and in barley (De la Penna et al. 1999; Zhu et al. 1999; Ma et al. 2000). Co-localizations were also found between QTLs of resistance to FHB, plant height, earliness, and characters of spike architecture in barley (De la Penna et al. 1999; Zhu et al. 1999; Ma et al. 2000). These co-localizations could indicate that linkages may exist between genes for FHB resistance and height or flowering date or, alternatively, from pleiotropic effects among genes. The photoperiod response genes *PpdA1, PpdA2*, and *PpdA3* located on chromosomes 2A, 2B, and 2D, respectively, and a major QTL for photoperiod response on chromosome 2BS have been also mapped in the cross Courtot/Chinese Spring (Sourdille et al. 2000). Chromosome 5A of wheat is also known to carry a number of genes affecting adaptability and productivity (Kato et al. 2000), and a study with 120 recombinant substitution lines has shown that *Vrn-A1* gene on chromosome 5AL has an effect on plant height (Kato et al. 1999). In our case, a part of the resistance observed may also be due to plant morphology or earliness, which could be passive resistance factors. Additional experiments will be necessary to establish whether linkage or pleiotropy, or an effect in the microenvironment of the plant, are involved.

Conclusion

We report here for the first time QTL mapping of resistance to FHB in winter wheat cultivars under field conditions. Despite the inherently variable environment with field evaluation of FHB, stable QTLs were identified over 3 years of experimentation. One epistatic interaction was identified, but this was of a lower magnitude than the additive effects. Using molecular markers, the role of the chromosome 5A in FHB resistance of wheat and the link between one factor of resistance and the gene *B1* was confirmed. We also identified co-localization between QTLs of resistance and height and flowering date. However, the development of FHB-resistant cultivars independent of plant height, flowering date, and awnedness should be possible. A great variability for these traits among resistant genotypes to FHB worldwide has been described.

Comparing the resistance in cv. Renan with the Asian FHB resistance pool, we confirmed that chromosomes 2A, 3A, 3B, and 5A carry FHB resistance genes. In particular, new resistance factors in Renan on chromosome arms 2BS and 5AL were mapped. Additional field trials will be necessary to confirm mapped loci and to define QTL stability across different locations and genetic backgrounds. As FHB resistance is generally considered durable, combining resistance genes of Asian and European winter wheat pool using molecular markers, may be an interesting way to increase the resistance levels of cultivars.

Acknowledgements We thank D. Gaudet (Agriculture and Agri-foods Canada, Lethbridge Research Center, Alberta, Canada) and J. Jahier for their helpful comments on the manuscript. We also thank Y. Saintouen for helping with the seed multiplication and experiment preparation. This work was supported by the Genoplante project.

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