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ORIGINAL PAPER

S. Paillard · T. Schnurbusch · R. Tiwari · M. Messmer · M. Winzeler · B. Keller · G. Schachermayr

QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.)

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Abstract Fusarium head blight (FHB) of wheat is a widespread and destructive disease which occurs in humid and semi-humid areas. FHB epidemics can cause serious yield and quality losses under favorable climatic conditions, but the major concern is the contamination of grains with mycotoxins. Resistance to FHB is quantitatively inherited and greatly influenced by the environment. Its evaluation is costly and time-consuming. The genetic basis of FHB resistance has mainly been studied in spring wheat. The objective of this study was to map quantitative trait loci (QTLs) for resistance to FHB in a population of 240 recombinant inbred lines (RILs) derived from a cross between the two Swiss winter wheat cultivars Arina (resistant) and Forno (susceptible). The RILs were genotyped with microsatellite and RFLP markers. The resulting genetic map comprises 380 loci and spans 3,086 cM. The 240 RILs were evaluated for resistance to FHB in six field trials over 3 years. Composite interval mapping (CIM) analyses carried out on FHB AUDPC (i.e. mean values across six environments) revealed eight QTLs which altogether explained 47% of the phenotypic variance. The three main QTLs were mapped on the long arms of chromosomes 6D (R^2=22%), 5B (R^2=14%) and 4A (R^2=10%). The QTL detected on 5B originated from the susceptible parent Forno. Other QTLs with smaller effects on FHB resistance were detected on chromosomes 2AL, 3AL, 3BL, 3DS and 5AL.

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S. Paillard · M. Winzeler
Swiss Federal Research Station
for Agroecology and Agriculture (FAL-Reckenholz),
Reckenholzstrasse 191, 8046 Zurich, Switzerland

T. Schnurbusch · B. Keller
Institute of Plant Biology,
University of Zurich,
Zollikerstrasse 107, 8008 Zurich, Switzerland

R. Tiwari
Directorate of Wheat Research,
Karnal, 132 001 Haryana, India

M. Messmer
Pharmaceutical Institute,
University of Basel,
Benkenstrasse 254, 4108 Witterswil, Switzerland

G. Schachermayr (✉)
Indo-Swiss Collaboration in Biotechnology (ISCB),
Institute of Biotechnology,
ETH-Hoenggerberg, 8093 Zurich, Switzerland
e-mail: schachermayr@biotech.biol.ethz.ch

Present address:
S. Paillard, Unité de Recherche en Génomique Végétale (URGV),
UMR INRA 1165, CNRS 8114,
Université d'Evry Val d'Essonne,
2 rue Gaston Crémieux, CP 5708, 91057 Evry Cedex, France

Introduction

Fusarium head blight (FHB) or head scab of wheat is a widespread and destructive disease which occurs in humid and semi-humid wheat-growing areas (Parry et al. 1995; McMullen et al. 1997). FHB can be caused by several *Fusarium* species, but the most frequent pathogen is *Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein.) Petch; synonym = *G. saubinatti*]. FHB epidemics may cause serious losses when favorable climatic conditions occur after flowering. The major threat associated with FHB is the mycotoxins which are produced by *Fusarium* species and accumulate in the infected grains. These toxins, mainly deoxynivalenol (DON), are becoming a major concern in grain used for human consumption and animal feed. Even if the effects of epidemics can be reduced by fungicide treatments and agricultural practices, yield losses and the contamination level of the grains remain important problems (Parry et al. 1995). The development of disease-resistant cultivars appears the most effective strategy against FHB. FHB symptoms and DON levels were shown to be strongly correlated (Bai et al. 2001; Miedaner et al. 2003). A selection for reduced head blight severity would both

lower the impact of the disease on yield and decrease the contamination of the kernels with mycotoxins.

Several studies show that the resistance to FHB is horizontal and non-species specific as well as non-strain specific (van Eeuwijk et al. 1995; Mesterhazy et al. 1999). FHB resistance in wheat is a quantitatively inherited trait (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Gervais et al. 2003) which is greatly influenced by the environment (Snijders and van Eeuwijk 1991; Bai and Shaner 1994, 1996). Evaluation of FHB resistance is time consuming, laborious and costly. A better understanding of the genetic basis of this resistance and the development of appropriate molecular markers could thus greatly improve the efficiency of breeding for this trait.

Resistance to FHB consists of at least two components (Schroeder and Christensen 1963): the resistance against initial infection (type I) and the resistance to pathogen spreading in infected tissue (type II). Three additional components of resistance have been proposed by Mesterhazy (1995): the resistance to kernel infection (type III), the yield tolerance (type IV) and the decomposition or non-accumulation of mycotoxins (type V). The studies realized so far mainly focused on type II resistance, by inoculating *Fusarium* spores directly into a floret (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002). This component of resistance was preferentially studied because it is less prone to environmental effects. In such studies, only a part of the variation for FHB resistance is investigated and it is of great interest to also take into account the effects of type I resistance.

Several sources for FHB resistance have been identified worldwide, e.g. Sumai 3 (China), Nobeokabozu (Japan), Frontana and Encruzilhada (South America) in spring wheat germplasm, Arina and Praag-8 (Europe) in winter wheat germplasm (Snijders 1990, Ruckenbauer et al. 2001). No complete resistance or immunity to FHB has been observed among resistant wheat germplasm so far. Most of the genetic studies focused on the resistance of Sumai 3, which is considered to be the most efficient. In Sumai 3, two major QTLs on chromosomes 3B and 5A contribute to resistance (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002). In comparison, the genetic basis of FHB resistance is not well studied in European winter wheats. Two studies on QTLs for resistance to FHB in winter wheat have been published recently (Gervais et al. 2003; Shen et al. 2003). In a first study on FHB field resistance in the Renan × Récital cross (Gervais et al. 2003), nine QTLs were detected, out of which four were consistent across environments on chromosomes 2B, 3B and 5A. Shen et al. (2003) found QTLs for FHB type II resistance on chromosomes 1B, 3A, 3D and 5A in the winter wheat cross Patterson × Fundulea 201R. The QTLs located on chromosomes 1B and 3A were consistently found in three experiments.

The aim of the present study was to investigate QTLs for field resistance (combined type I and type II resistance) to FHB under field conditions in the winter wheat variety Arina. We used a population of recombinant inbred lines (RILs) derived from a cross between two Swiss winter wheat varieties. Arina is one of the sources for resistance in the European winter wheat gene pool (Ruckenbauer et al. 2001), Forno is a susceptible variety. FHB resistance assessment was carried out in six environments over 3 years. Heading date, flowering time and plant height assessments were also included in the study because the association of these morphological and developmental traits with FHB resistance is well known in wheat (Mesterhazy 1995; Miedaner 1997; Hilton et al. 1999; Buerstmayr et al. 2000).

Materials and methods

Plant material

We used a population of 240 F_5 RILs from a cross between Arina and Forno, two Swiss winter wheat varieties. The parents and the population have been described in Paillard et al. (2003). Forno is a short variety (103 cm) susceptible to FHB whereas Arina is a tall variety (120 cm) which has covered more than 40% of the Swiss wheat acreage since 1985 and shows a quantitative resistance to FHB.

Field experiments

A total of six field trials were conducted in 2000 (2000-I, 2000-II), 2001 (2001-I, 2001-II) and 2002 (2002-I, 2002-II) near Zurich, Switzerland, at the Swiss Federal Research Station for Agroecology and Agriculture (FAL-Reckenholz, 440 m above sea level, 10°C average temperature, 1,233 mm annual average precipitation). The locations were different each year. The 240 RILs were grown together with five replicated entries of the parental lines in a rectangular lattice design with three replications and nine genotypes per incomplete block. The material was sown in five-row plots (1.2 m long, 1.3 m wide, approximately 50 seeds per row). In each field trial, two out of the three replications were inoculated with spore suspensions of *F. graminearum*. The inoculum consisted of a mix of equal amounts of five different local strains multiplied individually. The final concentration of the mix was 5×10^5 conidia/ml in 0.1% Tween 20. Cultures were grown on potato dextrose agar (PDA 39 g l^{-1}) for 5–6 days at 18°C and under near ultra-violet light. Conidia were washed from the plates with 0.1% Tween 20. The final spore suspension was applied in the evenings with a motor driven back-pack sprayer, at a rate of 800 l/ha. Each field trial was inoculated three times to account for the variation in flowering time within the population. As flowering rate in the population was strongly influenced by the climatic conditions, the delay between two subsequent inoculations was not fixed and ranged between 2 to 4 days. Thus, each genotype was inoculated once at mid-anthesis, which corresponds to the optimal inoculation time. Disease severity was scored three to five times, with a 3–5 day range between two subsequent scorings, depending on the development of the disease in each field (except 2002-I, which was destroyed by a hail storm after the second scoring). In 2000, the disease severity was assessed by estimating the average number of infected spikelets on ten spikes randomly chosen in each plot for each scoring. In 2001 and 2002 subsequent scorings were made on ten spikes from each plot. The spikes were randomly chosen and labeled before the inoculation. For each scoring the number of infected spikelets was counted. The total number of spikelets was counted once. The percentage of diseased spikelets was calculated over the ten spikes as the number of infected spikelets divided by the total number of spikelets. The adjusted mean per genotype, calculated over the four experiments carried out in 2001 and 2002,

was used to estimate the average total number of spikelets for ten spikes for each genotype. These means were used to calculate the percentage of diseased spikelets for the 2000 experiments. Heading date (HD, days after 1 January), flowering time (FT, days after 1 January) and plant height (PH, to the top of the spike in cm) were recorded in each field trial.

Genetic map

The detailed genetic map of the population was published by Paillard et al. (2003). The 240 RILs were additionally genotyped with four SSR primer pairs (barc88, barc140, barc273 and barc1121). Two loci were added to chromosome 5B (*Xbarc88* and *Xbarc140*) but the gap on the long arm of 5B could not be closed. The gap between *Xcfd47* and *Xgwm732* on the long arm of chromosome 6D could be closed by adding two loci (*Xbarc273* and *Xbarc1121*). Linkage analyses were carried out as described by Paillard et al. (2003).

Statistical analysis

Trait analysis

For the five environments 2000-I, 2000-II, 2001-I, 2001-II and 2002-II, two from the three to five FHB scorings (percentage of diseased spikelets) were chosen to calculate the area under disease progress curve (AUDPC) (Campbell and Madden 1990; Jeger and Viljanen-Rollinson 2001). The criteria for the selection of the two scorings were: (1) high heritabilities, (2) they had to be subsequent to each other and (3) the mean, minimum and maximum disease severity were at the same levels in the different environments. The two available scorings in environment 2002-I were used to calculate the FHB AUDPC. For each environment, the delay between the two considered scorings was 3 (2001-I, 2001-II, 2002-II) or 4 days (2000-I, 2000-II, 2002-I). The first scoring had been done 18 (2000-I, 2002-I), 21 (2000-II, 2002-II), 25 (2001-I) or 29 (2001-II) days after the first inoculation in the corresponding environment.

Lattice analysis of single environments was performed with the program PLABSTAT (version 2 M, Utz 1995). The segregation of the 240 RILs for the different traits was tested for normality using the UNIVARIATE procedure (SAS Institute, Raleigh, N.C.). Analyses of variance of single environments and across the six environments were performed using a general linear model (SAS GLM) with genotype and environment effects considered as random. The Spearman correlation coefficients between traits were estimated on the adjusted means of the RILs using the CORR procedure (SAS). The heritability within each environment was estimated using the formula $h^2=\sigma_G^2/[\sigma_G^2+(\sigma_E^2/r)]$ and across the six environments, with 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 and r, the number of replications.

QTL analysis

The effect of individual markers for each trait (FHB resistance, HD, FT, PH) was tested by analysis of variance using the SAS GLM procedure (SAS). The effect of a marker was considered significant when the P value was smaller than 0.0001. This threshold was obtained by dividing 0.05 by the number of markers on the map (385), and corresponds therefore to a very conservative test.

Simple interval mapping (SIM) and composite interval mapping (CIM) were carried out using PLABQTL (Utz and Melchinger 2000). For SIM as well as CIM, the LOD thresholds were determined by a permutation test: after 1,000 permutations, LOD thresholds of 2.5 and 3 (type I error level α=10%) were chosen for SIM and CIM, respectively. Due to the low level of heterozygosity of the population, only additive effects were estimated, as well as digenic QTL × QTL interactions. For CIM, cofactors were automatically selected with F-to-enter and F-to-drop thresholds set to 10. The effect of sampling on QTL estimation was tested by a five-fold and a 100-fold cross validation with the "cross-validate" option of PLABQTL. QTLs (position and effects) were estimated with 4/5 of the genotypes, and a validation was performed with the other 1/5. SIM results were consistent with CIM results. Therefore only CIM results are presented in this paper as this represents the most powerful method.

As high negative correlations were found between FHB resistance and HD/FT as well as PH, we corrected FHB AUDPC data for these traits. HD was preferred to FT because the correlations were higher between FHB resistance and HD, and because of higher heritabilities for this trait compared to FT. For the single marker analysis, HD and PH were added as covariates in the analysis performed using SAS GLM procedure: therefore the significance of individual markers was directly adjusted for these two traits. For interval mapping, HD and PH were added as covariates to the analyses of variance that produced the adjusted means used for QTL analyses.

Results

FHB resistance

The mean values for the scorings chosen for calculation for the 2000 and 2001 experiments and for experiment 2002-II ranged from 17.8 (2000-I) to 25.2% (2000-II) of diseased spikelets for the first scoring and from 29.4 (2000-I) to 40.1% (2002-II) for the second scoring. In environment 2002-I, the mean values for the first and second scorings were 7% and 28.3%, respectively. The RILs showed a continuous distribution for FHB AUDPC for the adjusted means across environments (Fig. 1) as well as for single environments (data not shown). The parental lines exhibited contrasting phenotypes for FHB AUDPC, with an over-year mean AUDPC of 33.2 for Arina and 176.5 for Forno. The Shapiro-Wilk test rejected the hypothesis of normality (P<0.0001). The distribution was skewed towards the resistant parent, with more lines exceeding parental susceptibility (17 lines) than parental resistance (8 lines). ANOVA revealed that all effects (genotype, environment, replication within environment as well as genotype by environment interaction) were highly significant (P<0.0001, Table 1). Broad sense heritability for FHB resistance was estimated to be 0.91.

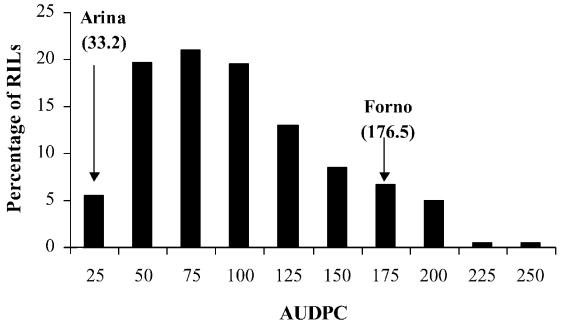

Fig. 1 Distribution of FHB AUDPC adjusted means over six environments for the 240 RILS derived from a cross between Arina and Forno. *Arrows* indicate the values of the parental lines

Table 1 Analysis of variance of genotype, environment and replication effects for Fusarium head blight (FHB) area under the disease progress curve (AUDPC) over the six environments. The degrees of freedom (*df*) and mean squares (MS) are shown for each analysis

Source	*Df*	*MS*	*F*	*P* value
Genotypes	239	25,801	34.90	<0.0001
Environments	5	84,882	114.83	<0.0001
Replications (in environment)	6	17,344	23.46	<0.0001
Genotypes × environment	1,153	1,932	2.61	<0.0001
Error	1,390	739	–	–

Within-environment heritabilities ranged from 0.84 (2000-II) to 0.92 (2000-I).

HD and PH

Adjusted means of RILs for HD and PH averaged over the six environments showed continuous and normal distributions (Fig. 2a, b). The parental lines were nearly identical for HD with 149.1 days for Forno and 150 days for Arina. One hundred and two lines were earlier than Forno and 84 lines were later than Arina. The two parents were different for PH, with an average height of 103 cm for Forno and 120.5 cm for Arina. Fifty-three lines were shorter than Forno and 22 were taller than Arina. Broad sense heritabilities were 0.76 for HD and 0.91 for PH. Significant (P<0.0001) negative correlations were found between FHB AUDPC and either HD (r=–0.29) or PH (r=–0.35).

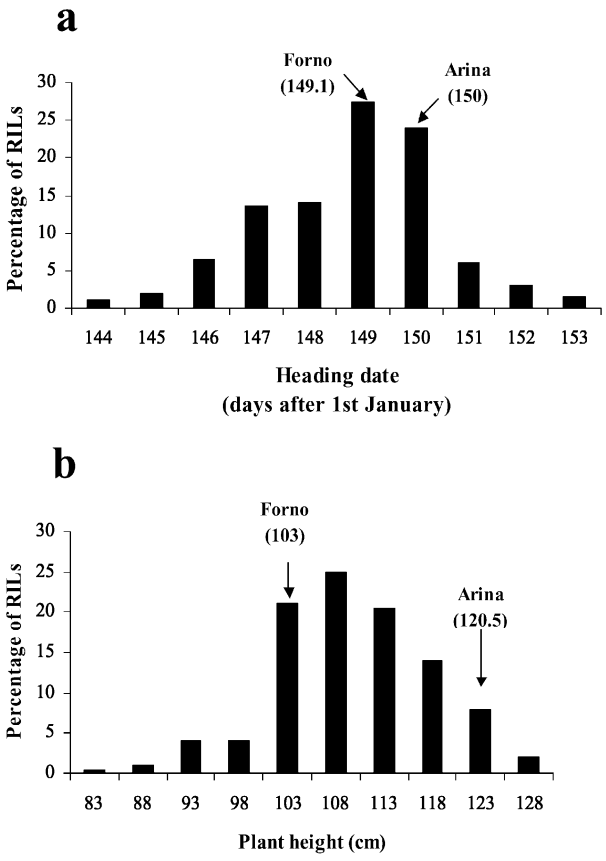

Fig. 2 Distribution of the 240 RILs derived from a cross between Arina and Forno for HD (**a**) and PH (**b**) adjusted means over six environments. *Arrows* indicate the values of the parental lines

Quantitative trait analysis

Eight QTLs were detected for the FHB AUDPC averaged over the six environments. The LOD scores ranged from 3.1 to 11.8 and the correponding R^2 ranged from 6.3 to 22.1% (Table 2). The final LOD was 40.4 and the total adjusted R^2 reached 47%. The three most consistent QTLs, detected in at least four out of six environments, were mapped on chromosomes 6DL (LOD=11.8, R^2=22.1%), 5BL (LOD=7.3, R^2=14.3%) and 4AL (LOD=5.0, R^2= 10.1%) (Table 2, Fig. 3).

QFhs.fal-6DL was mapped between marker loci *Xpsr915* and *Xcfd19a*, *QFhs.fal-5BL.1* between *Xgwm639a* and *Xpsr120a* and *QFhs.fal-4AL* between *Xcdo545* and *Xgwm160*. *QFhs.fal-6DL* and *QFhs.fal-4AL* originated from the resistant parent Arina, whereas *QFhs.fal-5BL.1* originated from the susceptible parent Forno. Out of the five other QTLs detected for the FHB AUDPC averaged over six environments, four were detected only in one or two environments (*QFhs.fal-3AL, QFhs.fal-3BL, QFhs.fal-3DS* and *QFhs.fal-5AL.1*) and one was only detected for the mean (*QFhs.fal-2AL*). Six other QTLs were detected in one environment but not across environments: *QFhs.fal-1BL, QFhs.fal-2DL, QFhs.fal-5AL.2, QFhs.fal-5BL.2, QFhs.fal-5BL.3* and *QFhs.fal-6AL.* Among a total of 14 QTLs detected in single environments, six originated from the susceptible parent Forno: *QFhs.fal-3AL, QFhs.fal-3DS, QFhs.fal-5BL1, QFhs.fal-5BL.2, QFhs.fal-5BL.3* and *QFhs.fal-6AL.* Four QTL by QTL interactions were detected, with low effects (1.7%<R^2<4%), and each interaction was detected only once. In three cases *QFhs.fal-5BL.1* was involved in these interactions (Table 2). The five-fold cross-validation performed on FHB AUDPC averaged over six environments confirmed that the QTLs detected on chromosomes 4AL, 5BL and 6DL were consistent (Table 3); they were detected in each of the cross-validation splits. For the 100-fold cross-validation, *QFhs.fal-6DL* occurred in 99 splits, *QFhs.fal-5BL.1* in 95 splits and *QFhs.fal-4AL* in 81 splits. Single marker analysis performed using SAS GLM procedure also identified these three chromosomic regions as significantly (P<0.0001) associated with FHB resistance (data not shown).

Six QTLs were detected for the average HD across six environments (Table 4). The three most important QTLs were located on chromosomes 6DL (LOD=9.1, R^2= 16.0%), 3DL (LOD=6.7, R^2=13.7%) and 7BL (LOD= 7.4, R^2=13.2%) and originated from Arina, whereas the three others, located on chromosomes 2AL, 5BL and 6AL

Table 2 QTLs for FHB AUDPC detected by CIM in the Arina/Forno RILs population for each environment and the mean over the six environments. The marker intervals cited are those flanking the peak of the LOD scan. The location of each QTL peak (in cM) is given referring to the map of Paillard et al. 2003. The chromosomal location of each QTL is also given. R^2 represents the percentage of phenotypic variance explained for each QTL

	Name	Marker interval	Peak	Chr	2000				2001				2002				Mean over six environments	
					2000-I		2000-II		2001-I		2001-II		2002-I		2002-II			
					LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
1	*QFhs.fal-1BL*	*Xgwm268-Xwmc44*	146	1BL	–	–	–	–	–	–	–	–	–	–	3.2	12.4	–	–
2	*QFhs.fal-2AL*	*Xcfa2086-Xgwm311*	198	2AL	–	–	–	–	–	–	–	–	–	–	–	–	3.2	6.8
3	*QFhs.fal-2DL*	*Xglk302b-Xgwm539*	118	2DL	–	–	–	–	–	–	3.2	6.7	–	–	–	–	–	–
4	*QFhs.fal-3AL*[a]	*Xwmc264-Xgwm155*	62	3AL	–	–	–	–	–	–	–	–	3.9	7.3	7.9	14	5.0	10.0
5	*QFhs.fal-3BL*	*Xcfa2134b-Xgwm131b*	84	3BL	–	–	–	–	–	–	–	–	3.2	6.0	4.9	9	3.1	6.3
6	*QFhs.fal-3DS*[a]	*Xbcd907c-Xgwm161*	10	3DS	–	–	–	–	–	–	–	–	–	–	4.7	8.9	3.9	8.1
7	*QFhs.fal-4AL*	*Xcdo545-Xgwm160*	78	4AL	–	–	3.3	6.3	–	–	–	–	3.8	7.1	10	17.5	5.0	10.1
		Xgbx3480b-Xbcd907g	74	–	–	–	–	–	–	–	3.3	6.6	–	–	–	–	–	–
8	*QFhs.fal-5AL.1*	*Xgwm291-Xglk348c*	198	5AL	3.5	7.1	–	–	–	–	–	–	–	–	–	–	3.2	7.0
9	*QFhs.fal-5AL.2*	*Xfbb166a-Xpsr426*	112	–	–	–	–	–	–	–	–	–	–	–	4.7	8.7	–	–
10	*QFhs.fal-5BL.1*[a]	*Xgwm371-Xgwm639a*	60	5BL	4.9	9.1	–	–	–	–	–	–	7.5	13.6	–	–	–	–
		Xgwm639a-Xpsr120a	62	–	–	–	9.0	16.1	3.8	7.5	–	–	–	–	5.9	10.8	7.3	14.3
11	*QFhs.fal-5BL.2*[a]	*Xpsr1201-Xgwm371*	38	–	–	–	–	–	–	–	5.2	10.1	–	–	–	–	–	–
12	*QFhs.fal-5BL.3*[a]	*Xgwm1246-Xpsr145a*	4	–	–	–	–	–	–	–	–	–	–	–	4.1	8.0	–	–
13	*QFhs.fal-6AL*[a]	*Xgwm169—Xpsr966b*	162	6AL	–	–	–	–	–	–	–	–	–	–	5.0	9.7	–	–
14	*QFhs.fal-6DL*	*Xcfd19a-Xcfd47*	118	6DL	11.5	20.0	–	–	10.5	19.4	–	–	–	–	–	–	–	–
		Xpsr915-Xcfd19a	116	–	–	–	7.5	13.7	–	–	–	–	10.4	18.3	–	–	11.8	22.1
		Xcfd19b-Xgdm14b	108	–	–	–	–	–	–	–	–	–	–	–	17.2	28.2	–	–
Digenic effects			–	–	–	–	7×10	1.8*	–	–	–	–	4×10	4.0*	8×10	2.1*	–	–
Q×Q interaction (%)			–	–	–	–	–	–	–	–	–	–	5×13	2.1*	–	–	–	–
Final simultaneous fit (adj. R^2 and final LOD)			–	–	17.3	25.7	21.1	31.2	15.1	25.1	14.7	23.1	31.9	41.2	41.9	47.7	40.4	46.6

* Significant at the 5% level

[a] QTLs originating from Forno

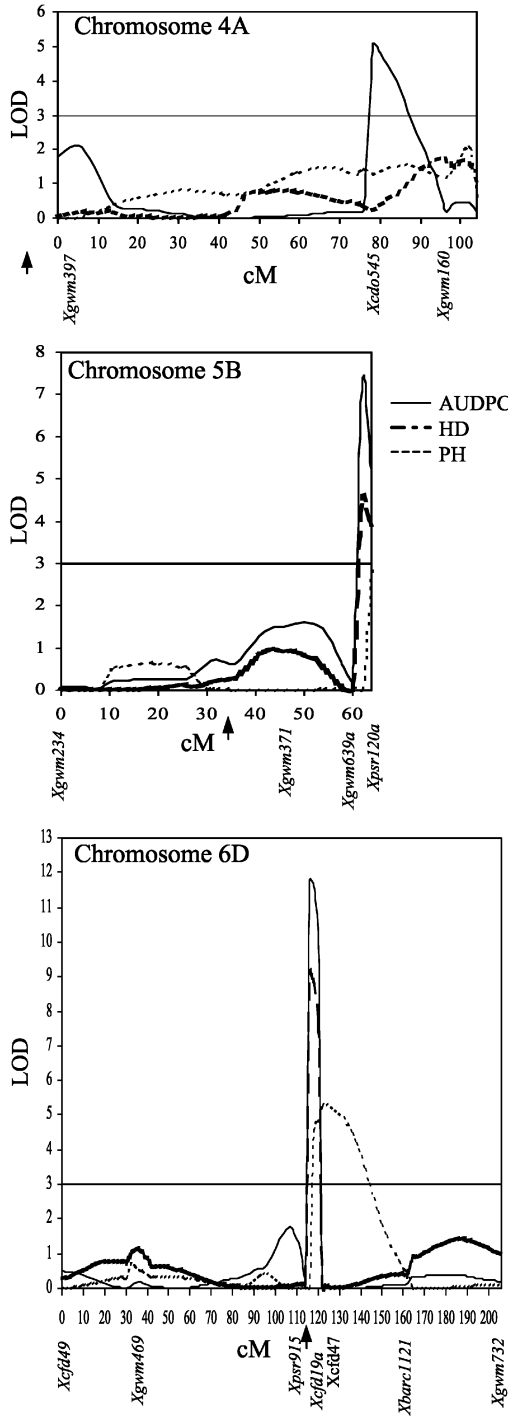

Fig. 3 LOD score scan on chromosomes 4A, 5B and 6D for QTLs for FHB AUDPC, HD and PH adjusted means over environments. LOD curves were calculated with CIM with an F-to-enter of 10. The estimated centromere position is indicated by an *arrow*. Molecular markers are in the order according to Paillard et al. (2003)

originated from Forno (R^2 ranging from 8.3% to 8.9%). *QEet.fal-6DL* and *QEet.fal-5BL* coincided with QTLs for FHB resistance consistent across the environments (Fig. 3).

The analysis of the average PH across the six environments revealed five QTLs (Table 5). These QTLs were located on chromosome 1AS, 1BL, 2AL, 5AL and 6DL with LOD scores and R^2 ranging from 5.1 to 13.3 and from 9.2% to 22.7%, respectively. Only one QTL, *QHt.fal-1BL*, originated from the short parent Forno. *QHt.fal-6DL* (LOD=5.3, R^2=9.8%) partly overlapped with QTLs for FHB resistance and heading time (Fig. 3). The two strongest QTLs, *QHt.fal-5AL* (LOD=13.3, R^2=22.7%) and *QHt.fal-2AL* (LOD=11.9, R^2=20.9%) did not overlap with QTLs for FHB resistance (Tables 2, 5).

To confirm that the two main QTLs detected for FHB AUDPC on chromosomes 5BL and 6DL both coinciding with HD QTLs were not an artifact due to the inoculation and/or scoring methods, we performed a QTL analysis on a sub-sample of the lines heading within 3 days. Lines heading (average data over six experiments) between the median value (149.3) and 1.5 days before and after this date were selected. In this subset of 123 lines the correlations between FHB AUDPC and either HD or FT were no more significant (r=–0.16, P=0.073 and r=–0.15, P=0.088 respectively), whereas the correlation between FHB AUDPC and PH was still significant (r=–0.41, P<0.0001). The ANOVA on FHB AUDPC revealed that all effects (genotype, environment, replication within environment as well as genotype by environment interaction) were still highly significant (P<0.0001, data not shown) and broad sense heritability remained very high (0.92). No QTL was detected for HD (neither for FT) in this sub-sample of lines, whereas QTLs for FHB AUDPC were still detected on chromosomes 5BL and 6DL (Table 6). For PH, CIM revealed the same QTLs as the ones mapped in the total set of lines, except two additional QTLs on chromosome 1BS and 4AL (Table 7).

Discussion

The distribution of the RILs for FHB AUDPC revealed that FHB resistance is a quantitatively inherited trait in the Arina/Forno population. Similar findings were described in several studies in spring wheat (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002), and recent studies in winter wheat (Buerstmayr et al. 2000; Gervais et al. 2003; Shen et al. 2003). In contrast to the observations reported by Bai et al. (1999), Waldron et al. (1999) and Buerstmayr et al. (2002) the distribution in our study was not bimodal. This is an indication that FHB resistance in the Arina/Forno population is polygenic and not controlled by a major QTL. Heritabilities were high (0.91 for broad-sense heritability and ranging from 0.84 to 0.92 for within-environment heritabilities). This reflects the accuracy and reproducibility of the experimental conditions and of the scoring method used for FHB evaluation under field conditions. Gervais et al. (2003) also found high heritabilities for FHB field resistance in the Renan/Récital population. Within-year heritabilities ranged from 0.75 to 0.84. Similar findings were also reported in studies

Table 3 Five-fold cross-validation of QTLs for FHB AUDPC. Data were split in five subsets of 192 lines used for QTL detection (calibration) and the remaining 48 lines were used for QTL validation

Cross-validation split (CV)		Calibration		Validation
Subset	QTLs detected	LOD	Adj. R^2 (%)	Adj. R^2 (%)
1	3A 3B 4A* 5B* 6D*	24.26	41.2	29.6
2	2A 3A 3B 4A* 5B* 6D*	29.87	46.8	14.5
3	3A 3D 4A* 5A 5B* 6D*	30.94	48.1	19.7
4	4A* 5B* 6D*	19.81	36.4	25.9
5	3B 4A* 5B* 6D*	20.66	36.4	32.6
5-fold CV	Mean phenotypic R^2		41.8	24.4
100-fold CV	Mean phenotypic R^2		40.1	23.4

* QTLs detected in each of the five cross-validation sets

Table 4 QTLs for HD detected by CIM in the Arina/Forno RILs population for the mean over six environments. The marker intervals cited are those flanking the peak of the LOD scan. The location of each QTL peak (in cM) is given referring to the map of Paillard et al. 2003. The chromosomal location of each QTL is also given. R^2 represents the percentage of phenotypic variance explained for each QTL

	Name	Marker interval	Peak	Chr.	LOD	R^2 (%)
1	*QEet.fal-2AL*[a]	*Xcfa2086-Xgwm382*	196	2AL	4.7	8.9
2	*QEet.fal-3DL*	*Xgwm645-Xgwm383a*	10	3DL	6.7	13.7
3	*QEet.fal-5BL*[a]	*Xgwm639a-Xpsr120a*	62	5BL	4.5	8.3
4	*QEet.fal-6AL*[a]	*OA97-Xsfr.AtB5.1a*	126	6AL	4.7	8.7
5	*QEet.fal-6DL*	*Xpsr915-Xcfd19a*	116	6DL	9.1	16.0
6	*QEet.fal-7BL*	*Xpsr958-Xpsr927*	50	7BL	7.4	13.2
Digenic effects QTL×QTL interaction (%)		–	–	1×5	–	2.6*
Final simultaneous fit (adj. R^2 and final LOD)		–	–	–	28.7	35.0

* Significant at the 5% level
[a] QTLs originating from Forno

Table 5 QTLs for PH detected by CIM in the Arina/Forno RILs population for the mean over six environments. The marker intervals cited are those flanking the peak of the LOD scan. The location of each QTL peak (in cM) is given referring to the map of Paillard et al. 2003. The chromosomal location of each QTL is also given. R^2 represents the percentage of phenotypic variance explained for each QTL

	Name	Marker interval	Peak	Chr.	LOD	R^2 (%)
1	*QHt.fal-1AS*	*Xksu18a-Xsfr.AtB5.1b*	30	1AS	5.1	9.2
2	*QHt.fal-1Bc*[a]	*Xgwm11-OA93*	48	1B	8.1	14.4
3	*QHt.fal-2AL*	*Xpsr934a-Xcfa2086*	188	2AL	11.9	20.9
4	*QHt.fal-5AL*	*Xglk317a-Xpsr386b*	68	5AL	13.3	22.7
5	*QHt.fal-6DL*	*Xcfd47-Xbarc1121*	122	6DL	5.3	9.8
Digenic effects		–	–	3×5	–	1.7*
QTL×QTL interaction (%)		–	–	–	–	—
Final simultaneous fit (adj. R^2 and final LOD)		–	–	–	33.4	42.5

* Significant at the 5% level
[a] QTLs originating from Forno

Table 6 QTLs for FHB AUDPC detected by CIM in the subsample of lines heading within 3 days for the mean over six environments. The marker intervals cited are those flanking the peak of the LOD scan. The location of each QTL peak (in cM) is given referring to the map of Paillard et al. 2003. The chromosomal location of each QTL is also given. R^2 represents the percentage of phenotypic variance explained for each QTL

	Name	Marker interval	Peak	Chr.	LOD	R^2 (%)
1	*QFhs.fal-5BL.1*[a]	*Xgwm371-Xgwm639a*	50	5BL	5.5	18.5
2	*QFhs.fal-6DL*	*Xpsr915-Xcfd19a*	118	6DL	4.7	16.2
Digenic effects		–	–	none	–	–
QTL×QTL interaction (%)						
Final simultaneous fit (adj. R^2 and final LOD)		–	–	–	11.2	31.5

[a] QTLs originating from Forno

focusing on type II resistance: Shen et al. (2003) found a broad sense heritability of 0.76 in the population derived from the cross between two winter wheats, Patterson and Funduela 201R. Studies on spring wheat crosses, including Sumai 3 or one of its derivatives as a parent of the population, also reported high broad sense heritabilities for FHB type II resistance (Bai et al. 1999; Waldron et al. 1999).

In total, we detected 14 QTLs for FHB resistance. Three out of these were found consistently across at least four environments and were confirmed by cross-validation: *QFhs.fal-6DL*, *QFhs.fal-5BL* and *Qfhs.fal-4AL*. These QTLs are different from previously described

Table 7 QTLs for PH detected by CIM in the sub-sample of lines heading within 3 days for the mean over six environments. The marker intervals cited are those flanking the peak of the LOD scan. The location of each QTL peak (in cM) is given referring to the map of Paillard et al. 2003. The chromosomal location of each QTL is also given. R^2 represents the percentage of phenotypic variance explained for each QTL

	Name	Marker interval	Peak	Chr.	LOD	R^2 (%)
1	*QHt.fal-1AS*	*Xksu18a-Xsfr.AtB5.1b*	28	1AS	3.2	10.5
2	*QHt.fal-1BS*[a]	*Xgwm550-lrk10b*	4	1B	3.1	18.1
3	*QHt.fal-1Bc*[a]	*OA93-Xgwm131a*	54	1Bc	3.6	11.7
4	*QHt.fal-2AL*	*Xpsr934a-Xcfa2086*	190	2AL	8.0	24.8
5	*QHt.fal-4AL*[a]	*Xgbx3480b-Xbcd907g*	66	4AL	3.2	10.5
6	*QHt.fal-5AL*	*Xglk317a-Xpsr386b*	68	5AL	6.2	19.6
7	*QHt.fal-6DL*	*Xpsr915-Xcfd19a*	120	6DL	3.3	10.9
Digenic effects		–	–	2×7	–	3.9*
QTL×QTL interaction (%)		–	–	4×5	–	4.0*
Final simultaneous fit (adj. R^2 and final LOD)		–	–	–	28.61	49.5

* Significant at the 5% level
[a] QTLs originating from Forno

QTLs for FHB resistance. One QTL with minor effects (R^2=6.3%) was detected across environments on chromosome 3B. However, this QTL mapped to the long arm of the chromosome and thus it is different from the major QTL identified on chromosome 3BS in Sumai 3 (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002). Our results, together with the results described by Gervais et al. (2003) and Shen et al. (2003), indicate that different genomic regions are involved in FHB resistance in winter wheat compared to the spring wheat cultivar Sumai 3 and its derivatives. In Sumai 3, two major QTLs on chromosomes 3B and 5A contribute to the resistance, explaining from 35% to 60% of the phenotypic variance, depending on the cross and the study (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Guo et al. 2003). In contrast, the resistance in the three winter wheat populations seems to be based on an accumulation of moderate (10%<R^2<20%) and minor undetected QTLs. Interestingly, the QTL detected on 3BL in the Renan/Récital population mapped to the same region as the one detected in the Arina/Forno population (near locus *Xgwm131b*). Possibly, these QTLs are identical. One minor QTL detected on 2AL for the mean over six environments in our population (R^2=6.8%) mapped at the same position (near locus *Xgwm311*) as the one originating from Stoa, a moderately susceptible spring wheat cultivar (Waldron et al. 1999; Anderson et al. 2001). Gervais et al. (2003) also found a QTL consistent over environments at the same position. In addition, a minor QTL detected on chromosome 5AL in our population mapped at the same position (top of the chromosome, 20 cM from *Xgwm595*) as one of the QTLs detected on 5AL by Gervais et al. (2003). Buerstmayr et al. (2002) and Shen et al. (2003) also detected QTLs on chromosome 5A, but they mapped to the short arm of the chromosome. The minor QTL that we detected on 3DS is also different from the one detected by Shen et al. (2003); their QTL was located near the centromere, whereas our QTL mapped at approximately 50 cM from the centromere.

Despite the high heritability for FHB resistance, the QTLs detected in this study explain only 47% of the phenotypic variation. One possible explanation is the incomplete coverage of our genetic map (gaps on 1AL, 4AS, 4BS, 4DS, 6BL) which may hamper the detection of one or several QTLs with strong effects. A second hypothesis is that the resistance of Arina is controlled by many QTLs with minor effects, which remained undetected in this study.

HD and PH were used as co-variates for the calculation of adjusted means or for the ANOVA for single marker analyses. Despite this, we detected FHB resistance QTLs coinciding with HD and/or PH QTLs. The major QTLs detected on 6D and 5B overlapped completely with QTLs for HD and partially with a QTL for PH in the case of the 6D QTL. The QTL analysis carried out on the sub-sample of lines heading within 3 days showed that the co-localization of QTLs for FHB resistance and HD is not due to an escape of the late genotypes, or an advantage of these genotypes resulting from the experiment itself. No QTL for HD was detected in the sub-sample, whereas both QTLs for FHB resistance on 5BL an 6DL remained with strong effects (R^2=18.5% and 16.2% respectively). In the same sub-sample the significant negative correlation between FHB AUDPC and PH and the partially overlapping QTLs for the two characters were detected again. In the Renan/Récital population, stable QTLs on chromosomes 2B and 5A also overlapped with QTLs for PH and/or FT (Gervais et al. 2003). Studies on barley (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Dahleen et al. 2003, Mesfin et al. 2003) revealed co-localizations between QTLs for FHB resistance and QTLs for either PH, earliness or traits of spike architecture.

Negative correlations between FHB symptoms and developmental traits were described in wheat (Mesterhazy 1995; Miedaner 1997; Hilton et al. 1999; Buerstmayr et al. 2000, Somers et al. 2003) and in barley. The relationship between PH and FHB symptoms was studied by Buerstmayr et al. (2000). In two different populations negative correlation coefficients of –0.37 and –0.38 were found. Despite this, recombination made it possible to select shorter genotypes with improved FHB resistance. A high correlation between FHB field resistance and PH (r=–0.65) as well as coinciding QTLs for both characters were also described by Somers et al. (2003).

In addition to chromosome 6D, a QTL for PH with a LOD slightly inferior to the LOD threshold of 3 over-

lapped with the FHB AUDPC QTL on chromosome 5B. Interestingly, no QTL for FHB resistance overlapped with the two major QTLs for PH detected on chromosomes 5AL and 2AL. Our data do not resolve if there is a true pleiotropic effect of PH or if there are linked genes in this interval controlling the traits independently. Arina is a tall variety with a long and loose spike. Taller lines with loose spikes may escape infection by having the heads under different humidity levels. Zhu et al. (1999) found a positive correlation between inflorescence density and FHB severity in barley. Additional studies should be carried out to investigate if the spike morphology explains a part of the resistance in our population.

In our study, the QTLs for FHB resistance exhibited mainly additive effects. We rarely found epistatic effects, and no epistasis was detected for the analysis carried out on the mean over six environments. Most of the studies on FHB resistance show that genetic variation is mainly based on additive effects and that epistasis occurs only in few crosses (Bai et al. 2000; Buerstmayr et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002; Gervais et al. 2003). This suggests that the accumulation of resistance components from different origins could be an efficient strategy to enhance FHB resistance. The detection of new QTLs from different sources of resistance opens up the possibility to combine such QTLs through marker assisted selection for the further improvement of resistance. A number of molecular markers linked to FHB resistance QTLs have been reported (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Kolb et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; del Blanco et al. 2003; Gervais et al. 2003; Guo et al. 2003; Shen et al. 2003). Zhou et al. (2003) have already demonstrated that marker-assisted selection for the major QTL on chromosome 3BS improved the efficiency of selection for FHB resistance. The map-based cloning of this QTL is a long-term goal (Liu and Anderson 2003) and will substantially contribute to a better understanding of FHB resistance mechanisms.

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