Quantitative Trait Loci for Adult-Plant Resistance to *Mycosphaerella graminicola* in Two Winter Wheat Populations

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Accepted for publication 23 May 2011.

ABSTRACT

Risser, P., Ebmeyer, E., Korzun, V., Hartl, L., and Miedaner, T. 2011. Quantitative trait loci for adult-plant resistance to *Mycosphaerella graminicola* in two winter wheat populations. *Phytopathology* 101:1209-1216.

Septoria tritici blotch (STB) is one of the most important leaf spot diseases in wheat worldwide. The goal of this study was to detect chromosomal regions for adult-plant resistance in large winter wheat populations to STB. Inoculation by two isolates with virulence to *Stb6* and *Stb15*, both present in the parents, was performed and STB severity was visually scored plotwise as percent coverage of flag leaves with pycnidia-bearing lesions. 'Florett'/'Biscay' and 'Tuareg'/'Biscay', each comprising a cross of a resistant and a susceptible cultivar, with population sizes of 316 and 269 $F_{7,8}$ recombinant inbred lines, respectively, were phenotyped across four and five environments and mapped with amplified fragment length polymorphism, diversity array technology, and simple sequence repeat markers covering polymorphic regions of $\approx 1,340$

centimorgans. Phenotypic data revealed significant ($P < 0.01$) genotypic differentiation for STB, heading date, and plant height. Entry-mean heritabilities (h^2) for STB were 0.73 for 'Florett'/'Biscay' and 0.38 for 'Tuareg'/'Biscay'. All correlations between STB and heading date as well as between STB and plant height were low ($r = -0.13$ to -0.20). In quantitative trait loci (QTL) analysis, nine and six QTL were found for STB ratings explaining, together, 55 and 51% of phenotypic variation in 'Florett'/'Biscay' and 'Tuareg'/'Biscay', respectively. Genotype–environment and QTL–environment interactions had a large impact. Two major QTL were detected consistently across environments on chromosomes 3B and 6D from 'Florett' and chromosomes 4B and 6B from 'Tuareg', each explaining 12 to 17% of normalized adjusted phenotypic variance. These results indicate that adult-plant resistance to STB in both mapping populations was of a quantitative nature.

Additional key words: *Triticum aestivum*.

Septoria tritici blotch (STB), caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*), is one of the most important diseases in wheat (*Triticum aestivum* L.) worldwide, responsible for severe damage of the leaves and causing yield losses of 30 to 40% (14). Control of STB includes crop rotation, soil tillage, fungicide application, and cultivation of resistant cultivars. Some fungicides, including widely used strobilurins, are no longer effective due to mutations in the highly variable pathogen population of *M. graminicola* (34). Therefore, resistance breeding provides a promising strategy for controlling the disease. In the wheat–*M. graminicola* pathosystem, both qualitative, isolate-specific, vertical resistance depending on major genes and quantitative, isolate-nonspecific, horizontal resistance with polygenic inheritance have been reported. Until now, 13 major genes for resistance to STB (*Stb1* to *Stb12* and *Stb15*) have been mapped (10,15). *Stb6* and *Stb15* are the most common resistance genes in European germplasm (4). Isolate-specific resistance genes could be effective at seedling and adult-plant stage (3) or just at seedling and not at adult-plant stage (20), depending on the gene. Given the high diversity within local populations of *M. graminicola* (41), quantitative resistances should be favored (23). Until now,

little is known about the inheritance of adult-plant resistance to STB in the field. Three previous studies about genetic mapping for STB resistance included small populations of 65 to 106 progenies and one to three environments (7,13,32). A very recent study concentrated on seedling resistance (42). Moreover, Simón et al. (32) detected only two isolate-specific loci. In this study, two biparental recombinant inbred line (RIL) populations with a high number of progenies ($n > 250$) were developed to analyze the inheritance of adult-plant resistance to STB by inoculation in field trials across four to five environments.

MATERIALS AND METHODS

Plant material. In total, 316 and 269 $F_{7,8}$ RILs were developed from two crosses between 'Florett' and 'Biscay' and 'Tuareg' and 'Biscay', respectively. Test entries were derived from F_7 lines and tested after a seed multiplication as F_8 bulks. 'Florett' (PBIS95-82/Cortez) from RAGT and 'Tuareg' (Kris/Dekan) from Nordsaat are the resistant parents containing *Stb6* and *Stb15* or *Stb6* alone, respectively (26). 'Biscay' (CPB79/Hussar) from KWS LOCHOW is the common susceptible parent without any known race-specific resistance genes. All three cultivars are high-yielding elite parents containing the dwarfing allele *Rht-D1b*. The two progenies have been kindly provided by KWS LOCHOW.

Isolates, inoculum production, and inoculation. Both mapping populations were inoculated with a mixture of two German isolates (BAZ 6/1/04 and BAZ 8/8/04), kindly provided by Julius-Kühn-Institut, Quedlinburg, Germany. Both isolates were virulent to *Stb6* and *Stb15* as tested in a seedling test by G. J. Kema, Wageningen, The Netherlands as described by Kema et al. (19).

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*The e-Xtra logo stands for "electronic extra" and indicates that the online version contains one supplementary table and one supplementary figure.

doi:10.1094/PHYTO-08-10-0203

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Inoculum was produced at the Institute of Crop Science and Plant Breeding, Freising, Germany, from sporulating cultures of *M. graminicola* and grown on yeast malt agar (YMA) for 3 to 5 days under ultraviolet (UV) light for 16 h per day at 18°C and 8 h per night at 12°C. This starter culture was used to produce large amounts of inoculum for field trials. Inoculum was prepared by inoculating 150 ml of liquid yeast-malt-glucose medium (4 g of yeast, 4 g of malt, and 4 g of glucose in 1 liter of distilled water) in 300-ml Erlenmeyer flasks with fresh *M. graminicola* spores from agar plates. Several flasks per isolate were incubated for 3 to 5 days in reciprocal shakers (175 rpm) under natural light in the greenhouse at 18 to 20°C. The resultant spore suspensions were concentrated into 200-ml tubes using separating funnels. The concentrate was stored in freezers at -20°C until 24 h before inoculation. At each location, the inoculum was adjusted to a density of 5×10^6 spores/ml as determined by hemacytometer counts (Neubauer improved, depth 0.1 mm, 0.0025 mm; Laboroptik GmbH, Germany).

All trials were inoculated once at growth stage EC39–55 (39), after late genotypes' flag leaves had been fully unrolled. The populations were inoculated using a tractor-mounted sprayer. Inoculation was done after rainfall and during cloudy weather conditions so that moisture was retained on the leaf surface for several hours. At Freising 2009, mist irrigation was used after inoculation to enhance infection frequency.

Field trials. 'Florett'/'Biscay' and 'Tuareg'/'Biscay' were evaluated in five and four environments (location–year combinations), respectively. Trials with both mapping populations were conducted at Freising (FRE) (latitude N 48.45°, longitude E 11.72°, 448 m above sea level [a.s.l.], 7.5°C mean annual temperature, 775 mm mean annual precipitation) and Wohlde (WOH) (latitude N 52.80°, longitude E 9.98°, 80 m a.s.l., 8.8°C mean annual temperature, 753 mm mean annual precipitation) in 2008 and at FRE 2009. Additionally, 'Florett'/'Biscay' was tested at Stuttgart-Hohenheim (HOH, latitude N 48.80°, longitude E 9.20°, 400 m a.s.l., 8.5°C mean annual temperature, 685 mm mean annual precipitation) in 2008 and Oberer Lindenhof (OLI, latitude N 48.52°, longitude E 9.05°, 700 m a.s.l., 6.6°C mean annual temperature, 960 mm mean annual precipitation) in 2009. 'Tuareg'/'Biscay' was additionally tested at OLI 2008.

The populations were grown together with each of five replicated entries of the parental lines as α -design with two replications at each location adjacent to each other. Each entry was sown in sets of two 1-m rows with ≈ 40 to 60 kernels per row.

In all field trials, STB rating, heading date, and plant height were evaluated. STB severity was visually scored plotwise as percent coverage of flag leaves with lesions bearing pycnidia. Flag leaves of double rows were assessed three times at an interval of 4 to 7 days, starting from ≈ 30 days after inoculation. The arithmetic mean of two scorings, representing middle and end of disease development, was used in the following analyses. Heading date was scored as days after 1st January, when 50% of spikes of a plot had emerged. Plant height was measured from the soil surface to the middle of the spike on the main tillers.

Marker analysis and genetic mapping. In order to construct genetic maps with broad coverage enabling quantitative trait loci (QTL) mapping, we used a combination of amplified fragment length polymorphism (AFLP), diversity array technology (DART), and simple sequence repeat (SSR) markers. AFLP markers were generated using *Pst*I/*Mse*I restriction enzymes and 45 selective primer combinations by the Institute of Crop Science and Plant Breeding, Freising, Germany. DART markers were analyzed by the company Triticarte (Triticarte P/L, Yarralumla, Australia; <http://www.triticarte.com.au>). We used Triticarte service wheat *Pst*I (TaqI) v2.3 (2,500 markers) in 'Tuareg'/'Biscay' and v2.5 (5,000 markers) in 'Florett'/'Biscay'. Akbari et al. (1) described an integrated map for a cross between the wheat 'Cranbrook' and 'Halberd' using restriction fragment length polymorphism, SSR,

AFLP, sequence-tagged microsatellite, and DART markers that were used as a reference map (Supplementary Figure 1). KWS LOCHOW (Dr. Viktor Korzun) provided a total of 263 SSR anchor markers with 3 to 5 markers per chromosome to facilitate assignment of linkage groups to chromosomes. These markers are publicly available (Supplementary Figure 1) and were preselected for a maximal polymorphism in German wheat materials.

Genetic linkage maps were generated using JoinMap 3.0 (38) assuming Haldane's mapping function (16) as an essential prerequisite for composite interval mapping (CIM) as outlined by Zeng (40). Markers were assigned to linkage groups at logarithm of odds (LOD) ≥ 3.0 with a maximum recombination fraction of 0.4. Because of obvious clustering of markers, markers without additional information within 1 centimorgan (cM) were deleted. Clustered DART markers were deleted first, followed by AFLP. SSR markers remained in the map. In summary, we started with a larger set of polymorphic markers and ended with a subset of these as mapped markers used for QTL analysis. Abbreviations of DART markers were used as provided by Triticarte, with wPt and tPt referring to markers documented in the wheat and triticale map, respectively. AFLP loci were designated according to the standard list provided by the manufacturer (KeyGene Inc., Wageningen, The Netherlands) (21). For example, marker locus XP6451-190 refers to a fragment of ≈ 190 bp amplified with primer combination P64/M51.

Data analysis. Analyses were based on single-plot values. Estimation of variance components was done using PLABSTAT (35). Residuals of mapping populations did not follow normal distribution. Logit and arcsin transformations were applied but did not improve the normality of the data. Therefore, untransformed data were used for analysis of variance (ANOVA) and QTL analysis. Lattice-adjusted means were calculated for genotypes in each environment and used for ANOVA. Analysis across environments was performed using a general linear model with genotype and environment effects, considering genotype as random. Entry-mean heritability (h^2) (22) was calculated by PLABSTAT with

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{E} + \frac{\sigma^2}{ER}}$$

where σ_g^2 denotes the genotypic variance, σ_{ge}^2 the genotype–environment interaction variance, σ^2 the error variance, and E and R are the numbers of environments and replications per environment, respectively.

QTL analysis was performed using CIM with PLABQTL (36). For detection of QTL, the LOD threshold was set to 3.0. Critical LOD scores were determined in all populations based on 1,000 permutations as recommended by Churchill and Doerge (11) at the 0.1 probability level. Additionally, fivefold cross validation was applied to determine the magnitude of bias of phenotypic variance (R^2) explained by detected QTL. The entire data set (DS) was split into five genotypic subsamples. Means from four out of five subsamples served as estimation set (ES) for QTL detection, localization, and estimation of genetic effects. The remaining subset forms the test set (TS) in which predictions derived from the ES are tested for their validity by correlating predicted and observed data. By permutating the respective subsets used for the ES and TS, five different cross-validation runs were possible (37). In this study, fivefold cross validation with 200 replicated runs was used. Detected QTL are presented with genome position, flanking markers, distance to next marker, confidence interval (CI), and normalized adjusted R^2 (nR_{adj}^2). The 95% CI was calculated after Darvasi and Soller (12). R^2 was adjusted (R_{adj}^2) to get a more adequate estimation of explained phenotypic variance (17), and was normalized (nR_{adj}^2) so that the sum across detected QTL is equal to model R_{adj}^2 (40). In this study QTL was declared major if it explained $>10\%$ of nR_{adj}^2 . In the final simultaneous fit, the detected QTL and their positions were used to obtain esti-

mates of additive effects. These effects were calculated for each environment illustrating QTL–environment (QTL–E) interaction. QTL–E interactions were tested for significance by a sequentially rejective Bonferroni *F* test according to Bohn et al. (6).

RESULTS

Phenotypic data. Field trials revealed differentiation for STB rating, heading date, and plant height between the parents and within the two mapping populations. Both mapping populations showed a continuous distribution of mean STB rating averaged across environments (Fig. 1). Parental lines were located on either end of the distribution. ANOVA for STB revealed highly significant (*P* < 0.01) variance components of genotype (G), environment (E), and G–E interaction (Table 1). ‘Tuareg’/‘Biscay’ population had a considerably smaller genotypic variance but G–E interaction and error variances similarly high to that of ‘Florett’/‘Biscay’. Accordingly, entry-mean heritability was low

for ‘Tuareg’/‘Biscay’ compared with ‘Florett’/‘Biscay’. Correlations between STB and heading date as well as between STB and plant height were negative and low (*r* = –0.13 to –0.20) although significant (*P* < 0.01) for both populations.

Mapping data. For ‘Florett’/‘Biscay’ and ‘Tuareg’/‘Biscay’, 5,000 and 2,500 DArT markers, respectively, and 263 SSR markers per population were used for mapping. Polymorphism rate for DArT markers was 7.7 and 7.5% and that for SSR markers was 28 and 30%, respectively. In total, the number of polymorphic markers was 609 for ‘Florett’/‘Biscay’ and 384 for ‘Tuareg’/‘Biscay’. From these, 221 and 262 could be mapped for ‘Florett’/‘Biscay’ and ‘Tuareg’/‘Biscay’, respectively (Table 2). They provided maps with polymorphic regions spanning 1,341 and 1,327 cM with an average interval distance of 2.3 and 3.6 cM, respectively. Total number of linkage groups amounted to 22 in ‘Florett’/‘Biscay’ and 20 in ‘Tuareg’/‘Biscay’. Each of the linkage groups could be assigned to one of the chromosomes. In ‘Tuareg’/‘Biscay’, chromosomes 3D and 6D were missing.

Fig. 1. Histograms of *Septoria tritici* blotch rating (percent flag leaf area affected) across environments after inoculation. Mean rating of parents are indicated by an arrow. LSD_{5%} = least significant difference at *P* < 0.05.

TABLE 1. Estimates of variance components and heritabilities (± standard error) for *Septoria tritici* blotch rating (percent flag leaf area affected) of two wheat populations evaluated in four and five environments after inoculation, respectively

Population	Source of variation								Heritability
	Environment (E)		Genotype (G)		G × E		Error		
	DF ^a	Variance components	DF	Variance components	DF	Variance components	DF	Variance components	
‘Florett’/‘Biscay’	4	172.6**	315	104.3**	1,260	143.8**	1,525	49.8	0.73 ± 0.02
‘Tuareg’/‘Biscay’	3	151.7**	268	28.7**	804	140.9**	1,012	49.5	0.38 ± 0.06

^a DF = degrees of freedom; ** indicates *F* test significant at *P* < 0.01.

TABLE 2. Number of mapped amplified fragment length polymorphism (AFLP), diversity array technology (DArT), and simple sequence repeat (SSR) markers on chromosomes and genomes and total genome coverage in two wheat populations

Population, genome	Chromosome							Total	Percent	Coverage (cM) ^a
	1	2	3	4	5	6	7			
‘Florett’/‘Biscay’										
A	16	23	13	7	6	9	23	97	43.9	583
B	17	11	10	6	11	10	7	72	32.6	427
D	10	18	2	5	10	3	4	52	23.5	331
Sum	...	...	...	...	...	...	...	...	...	1,341
‘Tuareg’/‘Biscay’										
A	11	3	26	7	3	16	9	75	28.6	402
B	24	19	26	7	12	19	21	128	48.9	598
D	12	19	–	5	13	–	10	59	22.5	327
Sum	...	...	...	...	...	...	...	...	...	1,327

^a Genome coverage in centimorgans (cM).

QTL analysis. In total, nine and six QTL were detected for STB in 'Florett'/'Biscay' and 'Tuareg'/'Biscay', respectively (Table 3). Critical LOD scores based on 1,000 permutations were 3.0 to 3.2 among the two mapping populations. Two major QTL ($nR^2_{adj} \geq 10\%$) for resistance to STB were detected per population. Altogether, resistance QTL explained $\approx 50\%$ of the total phenotypic variance (total R^2_{adj}). The majority of resistance QTL showed significant ($P < 0.01$) QTL-E interactions.

Because of the high QTL-E interaction, the additive effects of resistance QTL at each location are given in Table 4. Two major

QTL of 'Florett'/'Biscay' had a small additive effect at FRE and WOH 2008 but a high impact at HOH 2008 and OLI 2009. In the combined analysis across environments, the resistant alleles of the two major QTL reduced STB rating by 5.2 and 5.8% flag leaf infection. Both susceptible and resistant parents, were donors of resistance alleles, although the susceptible 'Biscay' contributed only QTL with small effects. In 'Tuareg'/'Biscay', again, WOH 2008 contributed less to the total explained variance than the other two environments. The QTL derived from 'Biscay' had only a small effect at two of the four environments.

TABLE 3. Localization of quantitative trait loci (QTL) with logarithm of odds (LOD) > 3.0 for resistance to Septoria tritici blotch (percent flag leaf area affected) in two wheat populations (means across environments)^a

Population, QTL number	Chromosome	Pos. (cM) ^b	Flanking marker ^c		LOD	d (cM) ^d	CI (cM) ^e	nR ² _{adj} (%) ^f	QTL–E ^g
			Left	Right					
'Florett'/'Biscay' (<i>n</i> = 301)									
1	1A	44	Xwmc0312	XwPt-7030	4.4	0	27–56	3.0	**
2	1B	78	XwPt-0260	Xwmc0419	4.3	0	68–88	2.9	**
3	2B	4	XP2553-222	Xwmc0344	7.1	4	0–10	4.8	**
4	3B	60	XP2553-237	Xstb10	20.7	1	57–61	12.6	**
5	4B	6	XP1754-147	XwPt-8092	14.7	1	2–10	9.3	**
6	5B	2	Xgwm0408	XwPt-4577	3.4	0	0–19	2.3	**
7	6D	16	Xgwm0469	Xcfd0013	19.4	0	13–16	11.9	**
8	7A	36	Xbarc0108	Xwmc0009	7.6	0	30–42	5.1	**
9	7D	2	XwPt-7842	XwPt-7368	4.3	2	0–13	3.0	...
Total R ² _{adj} (%) ^h	...	...	...	...	...	...	...	54.9	...
'Tuareg'/'Biscay' (<i>n</i> = 263)									
1	1A ns ⁱ	44	Xwmc0024	XwPt-3904	3.1	0	29–59	3.1	**
2	4A	6	XwPt-5434	Xwmc0219	5.8	0	0–13	5.8	...
3	4B	22	Xwmc0471	Xwmc0238	19.3	0	7–29	17.3	**
4	4D	2	Xcfd0071	Xgwm0129	5.2	0	0–12	5.2	...
5	6B	24	XwPt-6286	XP1459-119	12.5	0	18–30	11.8	**
6	7B	4	Xwmc0517	XP2255-118	8.2	0	0–14	8.0	**
Total R ² _{adj} (%)	...	...	...	...	...	...	...	51.3	...

^a Major QTL explaining $\geq 10\%$ of phenotypic variance are shaded.

^b Position in centimorgans (cM).

^c Closest marker in bold.

^d Distance in centimorgans to the next flanking marker.

^e The 95% confidence interval (CI) after Darvasi and Soller (12).

^f Normalized partial phenotypic variance explained by detected QTL.

^g QTL-environment (E) interaction tested for significance (sequentially rejective Bonferroni F test); ** indicates F test significant at $P < 0.01$.

^h Adjusted phenotypic variance explained by detected QTL (final simultaneous fit) across environments.

ⁱ LOD > 3.0 but not significant (ns) according to critical LOD score after 1,000 permutations ($\alpha = 10\%$).

TABLE 4. Additive effects of resistance alleles separately for the environments and combined for Septoria tritici blotch (percent flag leaf area affected)^a

QTL number	QTL designation	Donor of resistance	Additive effect of resistance allele (%) ^b							
			2008			2009			Combined	
			FRE	HOH	OLI	WOH	FRE	OLI		
'Florett'/'Biscay' (<i>n</i> = 301)										
1	<i>QStb.lsa_fb-1A</i>	'Florett'	-4.1	-3.3	...	-0.1	-1.0	-3.2	-2.3	
2	<i>QStb.lsa_fb-1B</i>	'Biscay'	-3.2	-3.9	...	-0.5	-0.9	-4.6	-2.4	
3	<i>QStb.lsa_fb-2B</i>	'Biscay'	-1.1	-4.4	...	-1.0	-3.6	-4.4	-2.9	
4	<i>QStb.lsa_fb-3B</i>	'Florett'	-2.0	-12.9	...	-0.8	-6.2	-7.4	-5.8	
5	<i>QStb.lsa_fb-4B</i>	'Florett'	-1.1	-7.6	...	-0.7	-3.5	-9.2	-4.5	
6	<i>QStb.lsa_fb-5B</i>	'Biscay'	-2.5	-4.9	...	-0.6	-0.6	-3.5	-2.1	
7	<i>QStb.lsa_fb-6D</i>	'Florett'	-3.4	-10.2	...	-0.7	-2.5	-9.6	-5.2	
8	<i>QStb.lsa_fb-7A</i>	'Florett'	-6.2	-3.0	...	-1.1	-2.2	-2.5	-3.1	
9	<i>QStb.lsa_fb-7D</i>	'Biscay'	-2.7	-3.8	...	-0.7	-1.6	-2.8	-2.2	
Total <i>R</i> ² _{adj} (%) ^c			...	15.7	49.8	...	16.4	40.0	42.7	54.9
'Tuareg'/'Biscay' (<i>n</i> = 263)										
1	<i>QStb.lsa_tb-1A</i>	'Biscay'	-0.6	...	-3.6	-0.3	-1.4	...	-1.5	
2	<i>QStb.lsa_tb-4A</i>	'Tuareg'	-1.6	...	-4.5	-1.3	-1.4	...	-2.2	
3	<i>QStb.lsa_tb-4B</i>	'Tuareg'	-3.5	...	-11.5	-1.1	-2.1	...	-4.0	
4	<i>QStb.lsa_tb-4D</i>	'Tuareg'	-2.1	...	-2.5	-1.4	-1.7	...	-1.9	
5	<i>QStb.lsa_tb-6B</i>	'Tuareg'	-1.1	...	-6.4	-1.1	-4.5	...	-3.3	
6	<i>QStb.lsa_tb-7B</i>	'Tuareg'	-0.7	...	-7.7	-0.9	-1.1	...	-2.6	
Total <i>R</i> ² _{adj} (%) ^c			...	12.5	...	42.1	3.4	22.4	...	51.3

^a Major QTL explaining $\geq 10\%$ of phenotypic variance are shaded. FRE = Freising, HOH = Hohenheim, OLI = Oberer Lindenhof, and WOH = Wohlde.

^b Estimated additive effects (less percent flag leaf infection) in final simultaneous fit of the resistance allele.

^c Adjusted phenotypic variance explained by detected QTL in final simultaneous fit in each environment and in the series.

QTL mapping of ‘Florett’/‘Biscay’ and ‘Tuareg’/‘Biscay’ revealed four and eight QTL with LOD > 3.0 for plant height and nine and six QTL for heading date, respectively. A few of these QTL had a CI similar to that for STB rating (Table 5). The effects of these co-localizing QTL were rather small, with nR^2_{adj} of 3.8 to 5.3%.

Fivefold cross validation verified six out of nine QTL in the ES compared with the DS in ‘Florett’/‘Biscay’ and all QTL in ‘Tuareg’/‘Biscay’ (Supplementary Table 1).

Individual effects of the two largest QTL per population are shown in Figures 2 and 3. Total data set was divided into four subpopulations, each carrying different alleles of the two major QTL. In ‘Florett’/‘Biscay’, the first subpopulation carrying the susceptible allele (S) at both resistance loci had a mean STB rating of 46% (Fig. 2). The second and third subpopulation carried the resistant allele (R) at 3B locus and the S allele at 6D locus or vice versa. Mean STB rating was reduced by 10% in both subpopulations. The fourth subpopulation carrying both resistant alleles reduced STB rating by 20% compared with the susceptible allele situation. In ‘Tuareg’/‘Biscay’, similar effects were obtained by analyzing the two major QTL for their phenotypic effects (Fig. 3). The maximal difference between the subpopulation having both resistance alleles and that having both susceptibility alleles was smaller, with 13.2% mean STB rating. The variation within the resistant subpopulations gives the possibility for further phenotypic selection in both wheat populations.

DISCUSSION

This study was based on multi-environmental field trials of large mapping populations inoculated with *M. graminicola* isolates. By spray inoculation of the flag leaves, as done in this study, a similar STB differentiation was achieved than by natural infections of 24 cultivars across nine environments (28), indicating that our inoculation method reflects the natural situation. ‘Florett’ and ‘Tuareg’ were among the most resistant entries within their progeny. In a companion article including both parents, only one cultivar (‘Solitär’) was significantly better than ‘Florett’ and ‘Tuareg’ (28). The inoculated isolates BAZ 6/1/04 and BAZ 8/8/04 were virulent to all parents in the seedling stage to exclude effects of the isolate-specific *Stb6* and *Stb15* genes known to segregate in the populations. Results reported here revealed no QTL for STB resistance near the locations of the mentioned genes on chromosomes 3A and 6A (4).

Genotypic variation for STB was significant in both populations. For ‘Tuareg’/‘Biscay’, however, genotypic variation was much smaller than the effect of environment and G–E interaction, leading to a low heritability. One cause for this might be that the resistance of ‘Tuareg’ proved to be environmentally rather unstable (28). Because of the high G–E interaction of ‘Tuareg’/‘Biscay’, the number of QTL and the effects of the detected QTL might be underestimated. Moreover, the susceptible parent ‘Biscay’ was less susceptible in this population compared with ‘Florett’/‘Biscay’, a fact that was caused by differing environments, but also reduced genotypic difference of the progeny.

Plant height and heading date may affect STB resistance considerably as reported in literature (2,18,27,32). In our mapping populations, phenotypic correlations between the three traits were significantly low, indicating that only a small number of QTL might interfere between these traits (Table 5). Indeed, only in ‘Florett’/‘Biscay’ was the CI of a QTL on chromosome 2B mapped for plant height directly overlapping with a minor STB-resistance QTL. The QTL for heading date on chromosome 2B and the QTL for heading date and plant height on chromosome 3B were mapped in positions neighboring the STB resistance QTL; however, without an overlap of CIs. This is especially important for the QTL on chromosome 3B with its high impact on STB resistance. Accordingly, there is a chance for identifying recombinants that display the QTL for STB resistance but not for unwanted morphological traits. An explanation for the low importance of morphological traits could be that inoculation was done once after latest genotypes’ flag leaves had been fully unrolled. Also, we exactly rated the leaf insertion that has been inoculated, not regarding any epidemiological development of the pathogen from leaf to leaf. Additionally, parents were very similar in their plant height and heading date; for example, both populations were fixed for the dwarfing allele *Rht-D1b*. In conclusion, achieving precise phenotyping data in field experiments is a challenge in this pathosystem.

The polymorphism rate in both elite populations was low when summarized across DArT, SSR, and AFLP markers. There were, however, considerable differences among marker types. Only 5 and 2% of analyzed DArT markers could be mapped in ‘Florett’/‘Biscay’ and ‘Tuareg’/‘Biscay’, respectively. Polymorphism rate was $\approx 7\%$ in both populations but many polymorphic DArT markers did not remain in the final map due to clustering. The polymorphism rate of the SSR markers was much higher, with $\approx 30\%$ due to preselection of those markers highly polymorphic in

TABLE 5. Localization of quantitative trait loci (QTL) with logarithm of odds (LOD) > 3.0 for plant height and heading date coinciding with QTL for resistance to Septoria tritici blotch in two wheat populations (means across environments)^a

Trait, population, QTL number ^b	Chromosome	Pos. (cM) ^c	Flanking marker ^d		LOD	d (cM) ^e	CI (cM) ^f	nR ² _{adj} (%) ^g	Effect ^h	
			Left	Right					Height (cm)	Days
Plant height										
‘Florett’/‘Biscay’										
3	2B	4	XP2553-222	Xwmc0344	3.5	4	0–15	3.9	–1.0	...
4	3B	44	XP2553-237	Xstb10	3.5	6	33–55	5.3	–1.4	...
Heading date										
‘Florett’/‘Biscay’										
3	2B	18	XwPt-1140	XwPt-3132	6.7	1	10–26	4.6	...	–0.4
4	3B	40	XP2553-237	Xstb10	4.5	2	26–54	3.8	...	–0.3
‘Tuareg’/‘Biscay’										
4	4D ns ⁱ	4	Xgwm0129	XP2255-265	2.7	1	0–14	4.0	...	–0.3

^a Number of QTL refers to Table 3.

^b Wheat populations: ‘Florett’/‘Biscay’, $n = 301$; and ‘Tuareg’/‘Biscay’, $n = 263$.

^c Position in centimorgans (cM).

^d Closest marker in bold.

^e Distance in centimorgans to the next flanking marker.

^f The 95% confidence interval (CI) after Darvasi and Soller (12).

^g Normalized partial phenotypic variance explained by detected QTL.

^h Estimated additive effects in final simultaneous fit of the QTL allele combined across all field trials; numbers reflect reduced height and earlier heading, respectively.

ⁱ LOD > 3.0 but not significant (ns) according to critical LOD score after 1,000 permutations ($\alpha = 10\%$).

German wheat material (V. Korzun, *personal communication*). It should be noted that DArT markers for wheat were developed primarily with Australian wheat material. In AFLPs in both populations, only 3 to 4 bands per primer combination were polymorphic compared with 7 to 8 in ‘History’/‘Rubens’ (Holzapfel, *personal communication*) and, on average, 6.4 in ‘Arina/NK93604’ (30). Obviously, the resistant, high-yielding elite parents are genetically rather related to the susceptible ‘Biscay’, although this is not visible from their pedigrees. Genetic similarities (*GS*) between ‘Florett’, ‘Tuareg’, and ‘Biscay’ were quite high (*GS* = 0.70 to 0.73), illustrating their relatedness as analyzed by 221 SSR markers scattered evenly across the genome (V. Korzun, *personal communication*). Pairwise comparisons of other German wheats resulted in a *GS* of 0.58 in the same analysis. However, the small average interval distances (2.3 to 3.6 cM) indicate high-density maps, although genome coverage is limited to $\approx 1,350$ cM according to Haldane’s mapping function. We had to use this mapping function to fulfill a necessary assumption for CIM (40) although it provides longer maps than the Kosambi function. Somers’ high-density consensus map covered 2,569 cM, with an average interval distance of 2.2 cM (33). Taken together, these results indicate that, although a high number of markers have been tested, short maps were achieved because the parents

‘Florett’, ‘Tuareg’, and ‘Biscay’ possess highly conserved genomic regions, most probably due to similarity by descent. In adult plants, resistance to STB can be isolate-specific or quantitative (15). No known *Stb* gene is located on chromosomes 1A, 2B, 4B, 4D, and 6D where seven QTL for STB resistance have been found in our populations. Thus, additional sources for STB resistance have been detected here. Three QTL (*QStb.lsa_fb-1B*, *QStb.lsa_fb-4A*, and *QStb.lsa_fb-7A*) were mapped on chromosomes with common markers next to the genes *Stb11*, *Stb12*, and *Stb3*, respectively (8,9,15). Only marker *Xwmc0219* next to *Stb12* on chromosome 4A was mapped within the CI of *QStb.lsa_fb-4A*. Phenotypic data of both mapping populations suggested a typical quantitative inheritance of STB resistance in the field. This was confirmed by QTL analyses, revealing nine and six QTL, respectively, with small individual effects. Altogether, detected QTL in each population explained >50% of adjusted phenotypic variance (R^2_{adj}). Both parents contributed resistant alleles. Major QTL, however, were all from the respective resistant parent. Two QTL per population were declared major, explaining >10% of nR^2_{adj} . This is quite strict in comparison with other QTL studies (31). Major QTL in this study were found consistently across environments on chromosomes 3B and 6D from ‘Florett’ and 4B and 6B from ‘Tuareg’.

Fig. 2. Notched boxplots for subpopulations of ‘Florett’/‘Biscay’ illustrating four different allele situations at two major quantitative trait loci (QTL) (*QStb.lsa_fb-3B* and *-6D*) for resistance to Septoria tritici blotch (STB) (S = susceptible allele present and R = resistant allele present); if notches do not overlap, medians are different according to McGill et al. (24); horizontal line within boxes = median, + = mean, o = outliers, dashed lines indicate mean STB rating of susceptible and resistant parent, respectively, across environments.

In two other studies, detection of QTL for STB resistance was reported. Eriksen et al. (13) mapped QTL for STB resistance, with some of the alleles providing resistance at seedling stage, others at adult-plant stage, and some being effective at both stages. Four resistance QTL for adult-plant resistance from the resistant parent ‘Senat’ were mapped on chromosomes 2B, 3A, 6B, and 7B (*QStb.risø-2B*, *QStb.risø-3A.2*, *QStb.risø-6B.2*, and *QStb.risø-7B*), altogether explaining 62 to 77% of phenotypic variance. There was an overlap of resistance QTL and QTL for plant height at chromosome 3A. In this study, we mapped QTL on the same positions on chromosome 2B in ‘Florett’/‘Biscay’ and on chromosomes 6B and 7B in ‘Tuareg’/‘Biscay’. SSR markers *Xwmc0344* on chromosome 2B close to *QStb.lsa_fb-2B* and *Xwmc517* on chromosome 7B close to *QStb.lsa_tb-7B* were the only markers in accordance to the map of ‘Savannah’/Senat. Support intervals of resistance QTL in Savannah/Senat on chromosomes 2B and 7B did not include the common markers indicating independent QTL. Chartrain et al. (7) detected one QTL from the susceptible parent ‘Riband’ (*QStb.psr-6B-1*), explaining 24% of phenotypic variance (R^2) in one polytunnel experiment. The position of their QTL was close to SSR marker *Xgwm0219*, which is also present in the ‘Tuareg’/‘Biscay’ population but ≈60 cM distal to our QTL on this chromosome. Both previous mapping

studies had small population sizes of 65 to 106 progenies affecting the power of QTL detection (25,29).

In summary, adult-plant resistance to *M. graminicola* analyzed in large populations was inherited quantitatively depending on several loci, each explaining only a smaller part of the phenotypic variance. Major QTL described in this study for the first time were found consistently across environments on chromosomes 3B and 6D from ‘Florett’ and 4B and 6B from ‘Tuareg’. For providing maximal selection gain in marker-assisted selection (MAS), pyramiding of additively inherited major QTL from different parents in elite breeding populations seems promising (Figs. 2 and 3). Another strategy is to use molecular markers next to effective *Stb* genes to combine isolate-specific and quantitative resistances. Fine mapping of these QTL with markers common across populations will locate QTL positions more precisely (5), facilitate meta-analysis, and, thus, promote MAS in wheat breeding.

ACKNOWLEDGMENTS

This project was financially supported by the Bundesministerium für Bildung und Forschung (BMBF, Bonn) via PTJ (Jülich) and the KWS LOCHOW GmbH within the German-French-Spanish ERA-Net PG-CEREHEALTH Consortium (project number 0313992A). We thank the

Fig. 3. Notched boxplots for subpopulations of ‘Tuareg’/‘Biscay’ illustrating four different allele situations at two major quantitative trait loci (QTL) (*QStb.lsa_tb-4B* and *-6B*) for resistance to Septoria tritici blotch (STB) (S = susceptible allele present and R = resistant allele present); if notches do not overlap, medians are different according to McGill et al. (24); horizontal line within boxes = median, + = mean, o = outliers, dashed lines indicate mean STB rating of susceptible and resistant parent, respectively, across environments.

teams at all locations for their excellent technical assistance in data collection, particularly B. Lieberherr and M. Buck (Universität Hohenheim), L. Giehl and T. Wirth (Institute of Crop Science and Plant Breeding, Freising), and M. Scholz (KWS LOCHOW, Wohldle); and J. Groh (Institute of Crop Science and Plant Breeding, Freising) for providing the AFLP data.

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