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The genetic architecture of seedling resistance to Septoria tritici blotch in the winter wheat doubled-haploid population Solitär × Mazurka

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Abstract Breeding for resistance to Septoria tritici blotch (STB), caused by *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), is an essential component in controlling this important foliar disease of wheat. Inheritance of seedling resistance to seven worldwide pathogen isolates has been studied in a doubled-haploid (DH) population derived from a cross between the field resistant cultivar Solitär and the susceptible cultivar Mazurka. Multiple quantitative trait locus (QTL) mapping revealed major and minor genetic effects on resistance as well as several epistatic relationships in the seedling stage. Solitär conferred resistance to isolate IPO323, governed by *Stb6* on chromosome 3A, as well as to IPO99015, IPO92034, Hu1 and Hu2 controlled by a QTL on chromosome arm 1BS, possibly corresponding to *Stb11* and minor QTL on chromosomes 1B, 3D, 6B and 7D. Resistance of Mazurka to IPO90015 and BBA22 was caused by a QTL located in a region on 4AL which harbours *Stb7* or *Stb12*. QTL specific to pycnidial coverage on 3B and specific to necrosis on 1A could be discovered for isolate IPO92034. Pairwise epistatic interactions were reliably detected with five isolates. Although their contributions to the total variance are generally low, the genotypic effect of the QTL by QTL interaction of 4AL (*Stb7* or *Stb12*) and 3AS (*Stb6*) made up almost 15% of disease expression. Altogether, the results suggest a complex inheritance of resistance to STB in the seedling stage in terms of isolate-specificity and resistance mechanisms, which have implications for marker-assisted breeding in an attempt to pyramid STB resistance genes.

Keywords *Triticum aestivum* · *Mycosphaerella graminicola* · Septoria tritici blotch · Resistance · QTL · Epistasis

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Introduction

Septoria tritici blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt. (anamorph: *Septoria tritici*), is one of the most serious foliar diseases in wheat worldwide and may result in severe yield losses through reduction of the photosynthetic area (Eyal et al. 1987). High humidity and moderate temperature conditions are conducive to the spread of asexual pycnidiospores in the field and to disease development (Palmer and Skinner 2002). Field populations of *M. graminicola* are genetically diverse due to a high level of sexual recombination (Zhan et al. 2003). Fungal resistance to strobilurins (Fraaije et al. 2005) and azoles (Zhan et al. 2006) has hampered the chemical control of the disease by fungicides, and therefore deployment of effective resistance genes in wheat varieties plays a key role in the control of STB. The inheritance of resistance to STB has been described as quantitative, incomplete and non-specific to fungal isolates, as well as qualitative, monogenic or oligogenic, and complete (Rillo and Caldwell 1966; Chartrain et al. 2004). In diallel analyses, general combining ability (GCA) effects were found to be more important for resistance expression than specific combining ability (SCA) (van Ginkel and Scharen 1987; Jlibene et al. 1994; Simón and Cordo 1998). Kema et al. (2000) found an avirulence gene in the *M. graminicola* isolate IPO323, and identification of the corresponding resistance gene *Stb6 (*Brading et al. 2002) in the wheat variety Flame provided the first evidence for a gene-for-gene relationship.

To date, fifteen isolate-specific resistance genes with major effects against STB have been mapped in hexaploid wheat. A thorough review of identification and mapping of these *Stb* genes was given by Goodwin (2007). With quantitative trait locus (QTL) analysis, Eriksen et al. (2003) identified in addition to *Stb6* a QTL for seedling resistance with minor effects on 3BL and QTL for adult plant resistance on 2B and 7B. QTL with minor and major effects in the adult plant and seedling stage were mapped to 3AS, different from *Stb6* (Eriksen et al. 2003) and to 6B (Chartrain et al. 2004). Further minor QTL for seedling resistance were found to be located on chromosomes 1D, 2D and 7DS, and for adult plant resistance on 3D and 7B (Simón et al. 2004; Arraiano et al. 2007). In a genetic and physical mapping study, Raman et al. (2009) suggested allelism to *Stb11* for a major QTL on 1BS accounting for 60–98% of the phenotypic variance. Interaction between genes or QTL has not been investigated so far.

In disease assessment, both the loss of photosynthetic activity by necrosis and the production of pycnida, the asexual fructifications which play an important epidemiological role, are relevant in characterizing STB resistance. Kema et al. (1996a, b) suggested a different genetic control for the two traits. There are only few reports on the underlying mechanisms of STB resistance. Histological observations by Kema et al. (1996a) showed a different degree of colonization in terms of both necrosis and pycnidia formation, between a resistant and a susceptible variety.

Arraiano and Brown (2006) investigated the distribution and frequency of STB resistance genes in 238 European cultivars and breeding lines using seven isolates in a detached-leaf assay, and identified resistance to IPO88004 (*Stb15*) and IPO323 (*Stb6*) as the most frequent. Resistance that follows a gene-for-gene relationship is prone to breakdown by isolates with novel virulence specificities. Collapse of field resistance was observed in cultivars Gene and Tadinia carrying *Stb4* (Cowger et al. 2000; Jackson et al. 2000). Krenz et al. (2008) demonstrated the adaptation of *M. graminicola* on a moderately resistant cultivar. However, it is still unclear why resistance conferred by some isolate-specific genes is more durable than that of others. For managing STB resistance it has been proposed to pyramid effective genes in single varieties or to assemble genes by the use of cultivar mixtures in the field. Indeed, a decrease of disease severity in cultivar mixtures could be observed (Mille et al. 2006) but appeared to be inconsistent (Cowger and Mundt 2002). Stacking of isolate-specific STB genes requires the availability of molecular markers. Validation of such markers in different genetic backgrounds and their applicability to high-throughput analysis is crucial for marker-assisted selection (MAS) strategies.

In this study we carried out a QTL analysis of STB resistance to seven isolates of *M. graminicola* at the seedling stage using a doubled-haploid (DH) population derived from a cross between the German bread wheat cultivar Solitär and the susceptible Hungarian cultivar Mazurka. Since its release in

2004, Solitär has expressed the highest level of STB resistance in the field among the registered varieties in Germany (Anomymous 2004). The aims of the study were (1) to identify isolate-specific resistance in the parental cultivars with a diverse set of *M. graminicola* isolates (2) to locate QTL with major and minor effects conferring STB resistance at the level of necrosis and pycnidial coverage using a subset of isolates, and (3) to study epistatic interactions among resistance QTL.

Materials and methods

Plant and fungal materials

The German winter wheat (*Triticum aestivum* L.) cultivar Solitär, resistant to STB in the field, was crossed with the susceptible Hungarian winter wheat cultivar Mazurka. A DH population consisting of 134 lines was generated from F1 seed by the KWS-Lochow breeding company (Bergen, Germany). All lines of this population, referred to as S × M DH population, were used for linkage map construction. Due to limited seed availability, 128 DH lines were screened for seedling resistance to *M. graminicola* at Plant Research International (PRI, Wageningen, The Netherlands), and 128–132 DH lines, varying between replications, at the Department of Plant Breeding, Martin-Luther-University (MLU, Halle, Germany).

The two parents were screened for STB resistance using a set of 30 *M. graminicola* isolates of *T. aestivum* collected from 15 countries worldwide (Table 1). All isolates were received as mycelia or spore culture except German isolates Ma3, Ma4 and Ta1 and Hungarian isolates Hu1, Hu2 and Hu3. These were collected as single pycnidia from leaf samples, either from the field nursery in Halle (varieties Mazurka and Taras) or from the breeding nursery at the Agricultural Research Institute (Martonvásár, Hungary).

Table 1 List of *M. graminicola* isolates originating from 15 countries that were used for the seedling test of the parents of the S × M DH population, Solitär and Mazurka

Isolate	Origin	Source[a]
IPO00003	USA	PRI
IPO00005	USA	PRI
IPO02159	Iran	PRI
IPO02166	Iran	PRI
IPO86013	Turkey	PRI
IPO86086	Argentina	PRI
IPO87016	Uruguay	PRI
IPO88004	Ethiopia	PRI
IPO88018	Ethiopia	PRI
IPO89011	Netherlands	PRI
IPO90006	Mexico	PRI
IPO90015	Peru	PRI
IPO92004	Portugal	PRI
IPO92034	Algeria	PRI
IPO94218	Canada	PRI
PO94269	Netherlands	PRI
IPO95036	Syria	PRI
IPO99015	Argentina	PRI
IPO323	Netherlands	PRI
IPO95054	Algeria	PRI
Ma3	Germany (Mazurka)	MLU
Ma4	Germany (Mazurka)	MLU
Ta1	Germany (Taras)	MLU
Hu1	Hungary	ARI
Hu2	Hungary	ARI
Hu3	Hungary	ARI
BBA22	Germany	JKI
BBA27	Germany	JKI
BBA39	Germany	JKI
BASF27	Germany	BASF

Isolates in bold type were selected to analyze resistance to STB in the S × M DH population

[a] *PRI* Plant Research International, Wageningen, The Netherlands; *MLU* Martin-Luther-University, Halle, Germany; *JKI* Julius-Kühn-Institute, Braunschweig, Germany; *ARI* Agricultural Research Institute, Martonvásár, Hungary; *BASF* BASF SE, Ludwigshafen, Germany

Pathogenicity assays

Seedling assays with *M. graminicola* isolates were conducted in a greenhouse cabinet (PRI) or a growth chamber (MLU). Parental screening with IPO isolates was performed in three replications at PRI and in two replications with German and Hungarian isolates at MLU. Ten plants per DH line (including the parents) and isolate were sown in pots containing a peat/sand mixture, and grown for seven to 10 days under 16 h light per day at a temperature of 18/16°C (day/night) and 70% relative humidity. Plants were inoculated

before emergence of the second leaf. Inoculum preparation and inoculation with IPO isolates were according to procedures described by Kema et al. (1996a). To produce inocula of the German and Hungarian isolates, monopycnidial spore ranks of infested leaf samples were spread on malt yeast agar (MYA) plates (1% malt, 0.4% yeast, 0.4% glucose, 2% agar w/v) and incubated at 20°C for several days. *S. tritici* spores were scraped off the agar plate and stored at −80°C. For inoculation, thawed isolates were spread on MYA plates and floated with distilled water after 7 days of growth. The inoculum was adjusted to a final concentration of 1×10^7 spores per ml. Two to three drops of Tween 20 surfactant were added and plants sprayed with approximately 2 ml of inoculum per plant and isolate until run-off occurred. Inoculated plants were kept at ≥98% relative humidity and in dark conditions for 48 h by covering with black plastic foil bags in the greenhouse or by switching off the lights in the growth chamber. After inoculation, the temperature and humidity was increased to 21°C and ≥85%, respectively. Disease development on the primary leaves was promoted by clipping the second and third leaf 10 days post-inoculation (dpi) and by the application of a compound fertilizer. Seven isolates were selected to analyse the S × M DH population (Table 1). All experiments were conducted in a randomized complete block design. As isolates IPO90015, IPO99015 and IPO92034 were tested together in a series of experiments, we applied a split-plot design with isolates as whole plot treatment and DH lines as split-plot treatment.

Disease assessment

Symptoms of STB were visually rated on the primary leaf as (1) percentage of necrotic leaf area (NEC) and (2) percentage of pycnidial coverage (PYC) on each experimental unit (10 plants). Symptoms were assessed at intervals of 2–6 days during a period of 12–29 dpi depending on disease development and isolate.

Molecular marker analysis

DNA was extracted from leaves of 10-day-old seedlings by the CTAB method (Doyle and Doyle 1990). For molecular mapping, simple sequence repeat (SSR) markers developed by IPK Gatersleben (*Xgwm*, *Xgdm*; Röder et al. 1998; Ganal and Röder 2007; Pestsova et al. 2000), Wheat Microsatellite Consortium (*Xwmc,* Gupta et al. 2002), USDS-ARS Beltsville, Agricultural Research Center (*Xbarc,* Song et al. 2005) and Genoplant (*Xgpw,* Sourdille et al. 2004a; *Xcfa*/*Xcfd*, Guyomarch et al. 2002) were used in the S × M DH population.

PCR reactions were carried out in a PT200 thermocycler (MJ Research; Bio-Rad, Munich, Germany) in a final volume of 25 µl containing 1× PCR buffer (including 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.4 µM of each primer, 1 U *Taq* polymerase (Qiagen, Hilden, Germany), and 50–100 ng template DNA. Cycling conditions were: 3 min initial denaturation at 94°C, and 45 cycles of 1 min at 94°C, 1 min annealing at 60°, 55° or 50°C depending on the primer pair, 2 min extension at 72°C. A final extension step was performed for 10 min at 72°C. One primer of each microsatellite primer pair was 5′-labelled with Cy5.5 and amplicons electrophoretically separated on an ALF Express sequencer (GE Healthcare, Freiburg, Germany).

Amplified fragment length polymorphism (AFLP) analysis followed the protocol of Vos et al. (1995) except that *Eco*RI or *Pst*I selective primers were 5′-labelled with FAM or HEX. PCR amplicon pools generated from each of a FAM- and HEX-labelled primer combination were purified using a centrifugation clean-up step with MultiScreen 96 HV well filter plates (Millipore GmbH, Schwalbach, Germany) loaded with Sephadex® G-50 (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) according to a procedure described in http://www.genome.ou.edu/protocol_book/protocol_partIV.html (validated on 20th April 2011). Amplification products were separated on a MegaBACE 1000 capillary DNA sequencer (GE Healthcare) and analyzed with MegaBACE Fragment Profiler v1.2 software (GE Healthcare). Mapped AFLP loci were named based on the nomenclature of Keygene N.V. (Wageningen, Netherlands).

Data analysis and QTL mapping

The mean disease severity in terms of NEC (in %) and PYC (in %) was calculated by averaging the AUDPC (area under the disease progress curve) values (Shaner and Finney 1977) by the period of disease assessment. Analysis of variance (ANOVA)

was conducted for IPO90015, IPO99015 and IPO92034, which were tested together in the same series of experiments. For this dataset, isolate, genotype and genotype × isolate interaction effects were estimated. Experiments (blocks) were considered as random effects, genotype and isolates as fixed effects. With isolates IPO323, Hu2, Hu1 and BBA22, only the genotype effect could be determined. Correlations between PYC and NEC were calculated with Kendall's tau rank correlation coefficient. All statistics were calculated using the statistical programming environment R v2.8 (R Development Core Team 2009).

A genetic map of the S × M DH population was generated with MAPMAKER/EXP v3.0 (Lincoln et al. 1993). For the assignment of linkage groups to chromosomes, *Xgwm* microsatellite loci were used as anchor markers according to their chromosomal location in the ITMI population (Ganal and Röder 2007). Linkage was established at a minimum LOD threshold of 3.0. Marker orders were obtained by three-point and subsequent multi-point analysis supposing an a-priori genotyping error of 1%. Only markers which could be placed in the most likely map order at a minimum LOD of 2.0 were included for the subsequent QTL analysis. Multipoint maximum-likelihood recombination fractions were converted into map distances by the Kosambi mapping function. Charts of linkage groups were drawn with Mapchart v2.1 (Voorrips 2002).

All QTL analyses were carried out with the R/qtl package 1.11–12 (Broman et al. 2003) in the R environment using whole-genome interval mapping (Lander and Botstein 1989). Initially, all QTL analyses were performed for each experiment and isolate separately. First, in a single-QTL model a search for individual QTL was performed using maximum-likelihood estimation. If the phenotypic distribution exhibited a marked spike, a two-part model, composed of a binary and a normal model, was applied as described by Broman (2003), and DH lines with mean disease severities $\leq$2.5% of PYC and NEC, respectively, were considered resistant. Evidence for pairwise epistatic QTL interactions was tested by a two-dimensional genome scan with a two-QTL model using Haley–Knott regression (Haley and Knott 1992). LOD significance thresholds of $P = 0.05$ for the single- and two-QTL models were determined by running 10,000 permutations on the phenotypic data. Finally, all significant single QTL and QTL involved in interactions were considered and their map positions refined in the context of a multiple-QTL model (MQM). From these refined QTL positions the QTL confidence ranges, defined by a 1.5 LOD drop from the maximum LOD position, were estimated. The overall fit of the full model against the null model was tested by ANOVA. In a second step, each QTL term was dropped from the model one at a time and a comparison was made between the full model relative to the model with the term omitted (reduced model). If the omitted QTL also occurred in the interaction with another QTL, the interaction was dropped as well. From the drop-one ANOVA table the heritability of a QTL term, defined as the proportion of the phenotypic variance explained by the term, was calculated, and the effect of a QTL was estimated as the difference in the mean between the two homozygous QTL genotypes. Interaction effects were estimated as the deviation of the combined effect of alleles at two QTL from the sum of their individual effects (Fisher 1918).

A joint MQM analysis using DH line means of phenotypic data from all experiments included only those QTL and QTL interaction terms which were significant in at least two single experiments (Table 3).

Results

Parental screening for STB resistance with *M. graminicola* isolates

A total of 30 *M. graminicola* isolates originating from 15 countries throughout the world (Table 1) were used for the seedling assay with the two parents of the S × M DH population: Solitär, a German variety with outstanding field resistance to several fungal diseases, and Mazurka, a Hungarian variety with tolerance to drought and frost. Both wheat genotypes clearly differentiated in their response to STB for the majority of isolates (Fig. 1). On average, Solitär showed a lower percentage of PYC in comparison to Mazurka. With isolates IPO323, IPO86068, IPO99015 and Hu2, complete resistance was observed in Solitär. Amongst other isolates, Solitär exhibited the highest disease symptoms after infection with the German isolates BASF27, BBA22, BBA27, BBA39, Ma3 and Ma4. In contrast, Mazurka appeared to be moderately resistant to these isolates,

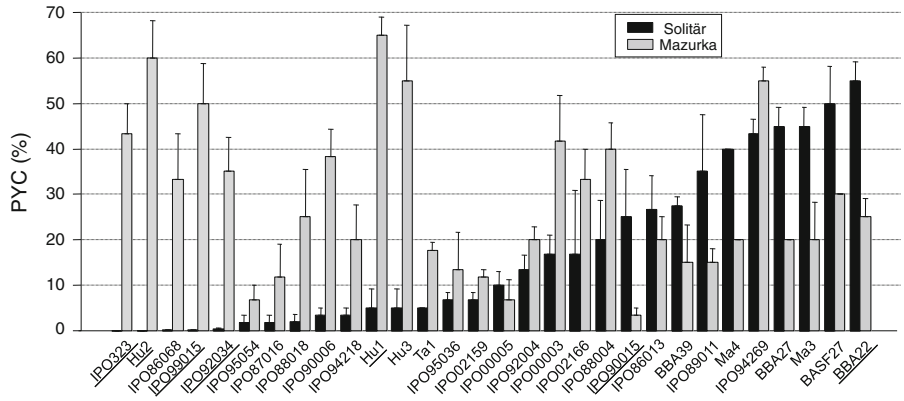

Fig. 1 Means and standard errors of pycnidial coverage (*PYC*, in %) in the parental screening of Solitär and Mazurka with 30 worldwide *M. graminicola* isolates. *Underlined* isolates were chosen for analysing the DH lines of the S × M population. IPO isolates: mean of three replicates determined at 21 dpi; BASF27, BBA isolates, Hu1, Hu2, Hu3, Ma3, Ma4, Ta1: means of two replicates determined at 29 dpi

and highly resistant to isolate IPO90015. Due to the distinct response observed in the parental genotypes, IPO323, IPO90015, IPO92034, IPO99015, BBA22, Hu1 and Hu2 were chosen for analysing STB resistance in the S × M DH population.

Phenotypic distribution of STB resistance in the S × M DH population

Between 128 and 132 DH lines were tested with the subset of isolates for STB resistance. Scatter plots and associated histograms of mean disease severity shown in Fig. 2 for IPO92034 and IPO90015 indicate a broad phenotypic variation in the S × M DH population for both NEC and PYC. Generally, two different patterns of distributions could be observed, which were more pronounced for NEC (Fig. 2). One pattern, as illustrated by isolate IPO92034, describes a symmetric continuous distribution when STB was measured by NEC and became right-skewed on the basis of PYC. The relationship between the two disease parameters appeared to be linear and only moderately correlated (Kendall's rank correlation coefficient $\tau = 0.50$). Segregation patterns of response to BBA22, Hu1 and Hu2 also suggested a right-skewed (PYC) or normal distribution (NEC) of the DH population (data not shown). A different distribution pattern is exemplified by isolate IPO90015 (Fig. 2). DH lines bearing no pycnidia and no or low necrotic area on the first true leaf stood out as a distinct spike. This distribution points to the action of a major gene superimposed on quantitative inheritance of STB resistance. If DH lines corresponding to the spike were excluded from correlation analysis, the obvious relationship between PYC and NEC became visible ($\tau = 0.57$). Such mixture distributions were also revealed with isolates IPO99015 and IPO323 (data not shown). DH lines with low PYC ($\leq$2.5%) but high NEC were also found, particularly with isolate IPO92034.

A significant genotype × isolate interaction in response to IPO90015, IPO99015 and IPO92034 pointed to isolate-specific reactions to STB in the S × M DH population (Table 2). These results imply QTL mapping needs to be carried out on single isolates. Although IPO323, Hu1, Hu2 and BBA22 were tested in separate experiments, indirect evidence from correlation analyses also suggested genotype × isolate interactions (see Electronic Supplementary Material ESM Table 1).

QTL mapping of seedling resistance to STB

A genetic framework map constructed with 145 SSRs was augmented with 120 AFLP loci. The entire map comprised 31 linkage groups which could be assigned to all 21 wheat chromosomes. Finally, 120 SSR loci, 58 AFLP and one phenotypic marker (*B1*) arranged in statistically reliable orders were chosen for QTL interval mapping. The linkage map covers 2272.8 cM with an average marker density of 12.7 cM.

The single-QTL analysis of resistance to isolates IPO90015, IPO99015, IPO323 employed a two-part QTL model (Broman 2003), whereas for isolates

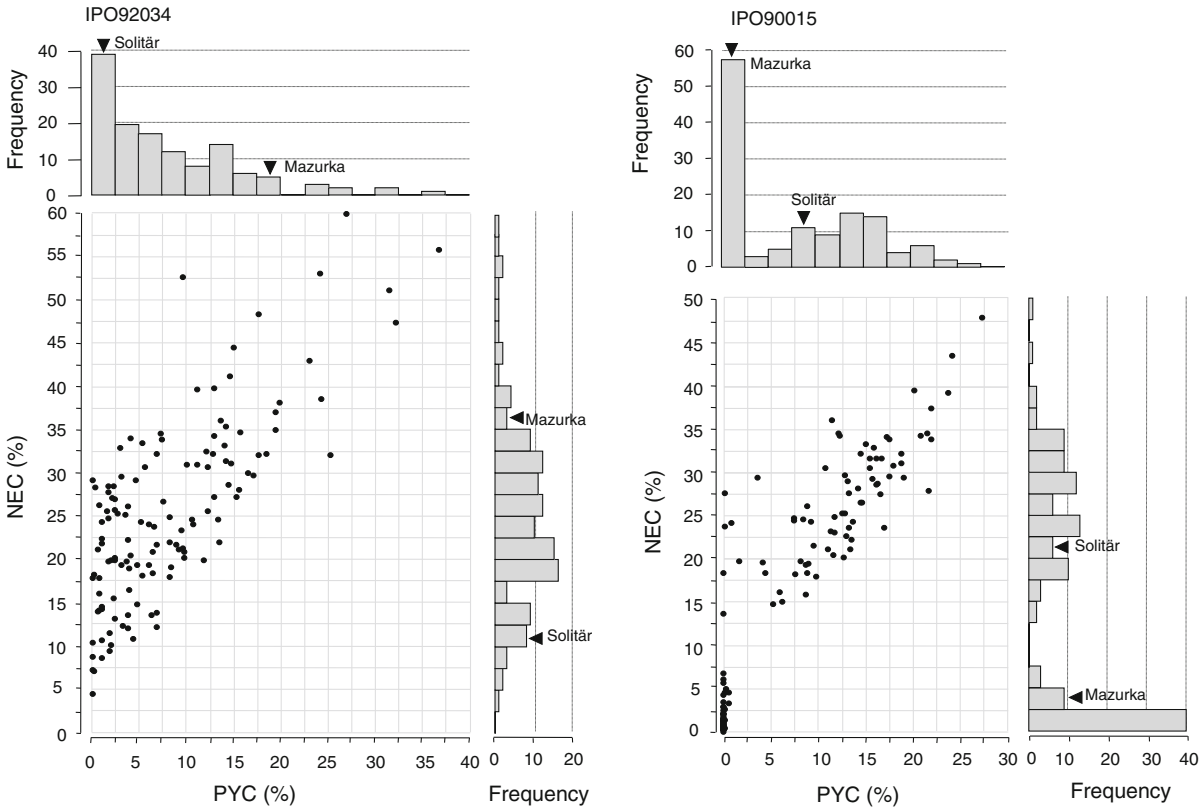

Fig. 2 Scatterplots with marginal histograms for necrotic leaf area (*NEC*, in %) and pycnidial coverage (*PYC*, in %) in the seedling stage (mean of three replicates) of Solitär, Mazurka and their DH offspring ($n = 128$). Data are given for *M. graminicola* isolates IPO92034 (*left*) and IPO90015 (*right*)

Table 2 Analysis of variance (ANOVA) of isolate (IPO90015, IPO99015 and IPO92034) and line effects conducted in a split-plot experiment

Source of variation	Degrees of freedom	PYC		NEC	
		Mean square	*F*-value	Mean square	*F*-value
Isolate	2	32.0	0.2	7576.7	26.3**
Error (block * isolate)	4	213.9		288.5	
DH line	127	276.6	18.4***	851.4	24.5***
Error (block * DH line)	254	15.1			
DH line * isolate	254	121.1	10.1***	280.3	13.1***
Error (block * isolate * DH line)	508	12.0		21.4	

Computations were done separately for pycnidial coverage (PYC, in %) and necrotic leaf area (NEC, in %)

** $P = 0.01$; *** $P = 0.001$

IPO92034, Hu1, Hu2, BBA22 a normal model was applied. Significant pairwise QTL interactions, i.e. deviations from purely additive effects, could be established for five isolates in a two-QTL analysis (data not shown). In Table 3, the given QTL parameters from the MQM analyses are based on the means of the three experiments. Two QTL interaction pairs, one detected with isolate IPO92034, the other with isolate Hu2, closely missed the significance level of $P = 0.05$ ($P = 0.06$ and

Table 3 QTL and QTL by QTL interactions for STB resistance (means of three experiments) to seven *M. graminicola* isolates in the seedling stage identified in the S × M DH population by multiple QTL mapping

Isolate	Disease trait[a]	No. of experiments[b]	QTL/QTL pair[c]	Resistance donor[d]	Position(s) (cM)[e]	Nearest marker/ marker pair	QTL heritability[f] (%)	Genotypic effect[g] (%)	*F*-value[h]	Putative gene
IPO90015	PYC	3	*QStb.4AL*	M	12	*Xwmc313*	47.9	12.8	165.8***	*Stb7/Stb12*
		3	*QStb.1B.c:QStb.2AL*	S:M; M:S	108:8	*Xgwm806:Xgpw2046*	3.2	6.6	11.2**	
	NEC	3	*QStb.4AL*	M	18	*Xwmc313*	75.8	24.8	393.9***	*Stb7/Stb12*
IPO323	PYC	3	*QStb.3AS*	S	0	*Xgwm369*	68.8	19.1[i]	152.0***	*Stb6*
		3	*QStb.4AL*	M	18	*Xwmc313*	14.7	6.2[i]	32.5***	*Stb7/Stb12*
		3	*QStb.3AS:QStb.4AL*	S:M	0:18	*Xgwm369:Xwmc313*	5.4	13.3[i]	24.0***	
	NEC	3	*QStb.3AS*	S	0	*Xgwm369*	84.1	42.7[i]	329.2***	*Stb6*
		3	*QStb.4AL*	M	18	*Xwmc313*	5.0	6.8[i]	19.7***	*Stb7Stb12*
		3	*QStb.3AS:QStb.4AL*	S:M	0:18	*Xgwm369:Xwmc313*	1.9	14.7[i]	14.6***	
IPO99015	PYC	3	*QStb.1B.a*	S	66	*Xgwm752.1B*	42.8	11.7	120.6***	*Stb11*
		3	*QStb.3DS*	S	12	*Xgwm1243*	4.6	3.7	12.9***	
		3	*QStb.7DS*	S	2	*E34M58_134*	1.4	1.9	4.0*	*Stb4/Stb5*
	NEC	3	*QStb.1B.a*	S	64	*Xgwm752.1B*	30.0	17.8	103.4***	*Stb11*
		3	*QStb.3DS*	S	10	*Xgwm1243*	8.0	8.5	27.7***	
		3	*QStb.7DS*	S	33	*Xgwm885*	1.8	3.9	6.2*	*Stb4/Stb5*
		3	*QStb.2AL:QStb.7DL*	S:M; M:S	0:28	*Xgwm1151:Xgwm1242*	3.3	10.4	11.2**	
IPO92034	PYC	3	*QStb.3B*	S	15	*E35M52_129*	38.4	9.0[i]	32.0***	*Stb2/Stb14*
		3	*QStb.1B.a*	S	76	*Xgwm752.1B*	11.5	4.7[i]	14.4***	*Stb11*
		3	*QStb.6B*	S	72	*Xgwm1076*	10.2	4.0[i]	12.8***	
		3	*QStb.3B:QStb.1B.a*	S:S	15:76	*E35M52_129:Xgwm752.1B*	4.2	7.3[i]	10.4**	
		2	*QStb.3B:QStb.6B*	S:S	15:72	*E35M52_129:Xgwm1076*	4.7	7.2[i]	11.8***	
	NEC	3	*QStb.1B.a*	S	66	*Xgwm752.1B*	19.7	11.1	37.4***	*Stb11*
		3	*QStb.6B*	S	88	*Xgwm219*	7.4	6.5	14.0***	
		3	*QStb.1A*	M	14	*E32M56_95*	10.1	7.1	19.2***	
Hu1	PYC	3	*QStb.1B.a*	S	46	*Xgwm752.1B*	12.2	3.7	18.1***	*Stb11*
	NEC	3	*QStb.1B.a*	S	48	*Xgwm752.1B*	16.1	4.8	24.9***	*Stb11*

Table 3 continued

Isolate	Disease trait[a]	No. of experiments[b]	QTL/QTL pair[c]	Resistance donor[d]	Position(s) (cM)[e]	Nearest marker/ marker pair	QTL heritability[f] (%)	Genotypic effect[g] (%)	*F*-value[h]	Putative gene
Hu2	PYC	3	*QStb.1B.a*	S	68	*Xgwm752.1B*	32.6	7.0	75.1***	*Stb11*
		2	*QStb.2B:QStb.7DL*	S:M; M:S	20:34	*Xgwm374:E39M56_184*	6.7	5.9	15.3***	
	NEC	3	*QStb.1B.a*	S	68	*Xgwm752.1B*	26.0	7.4	54.5***	*Stb11*
		3	*QStb.2B:QStb.7DL*	S:M; M:S	20:34	*Xgwm374:E39M56_184*	9.6	8.4	20.1***	
BBA22	PYC	3	*QStb.4AL*	M	6	*Xgwm160*	18.3	4.1	32.1***	*Stb7/Stb12*
		3	*QStb.1B.b*	S	4	*Xgwm1078*	7.0	2.4	12.3***	*Stb11*
	NEC	3	*QStb.4AL*	M	18	*Xwmc313*	11.2	2.9	16.2***	*Stb7/Stb12*

[a] *PYC* pycnidial coverage, *NEC* necrotic leaf area

[b] Number of experiments in which a single QTL and QTL by QTL effect was identified, respectively

[c] QTL name described by chromosome or chromosome arm; a lower-case character indicates a different QTL on the same chromosome

[d] Single QTL allele, QTL by QTL interaction allele combination(s) conferring resistance; *S* cv Solitär, *M* cv Mazurka

[e] QTL position(s) determined by refined MQM analysis

[f] QTL heritability defined as phenotypic variance explained by the QTL or QTL by QTL interaction

[g] QTL effect was estimated as the difference in the mean between the two homozygous QTL genotypes

[h] * $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$

[i] Estimated single QTL effect and QTL by QTL interaction effect not unambiguously distinguishable

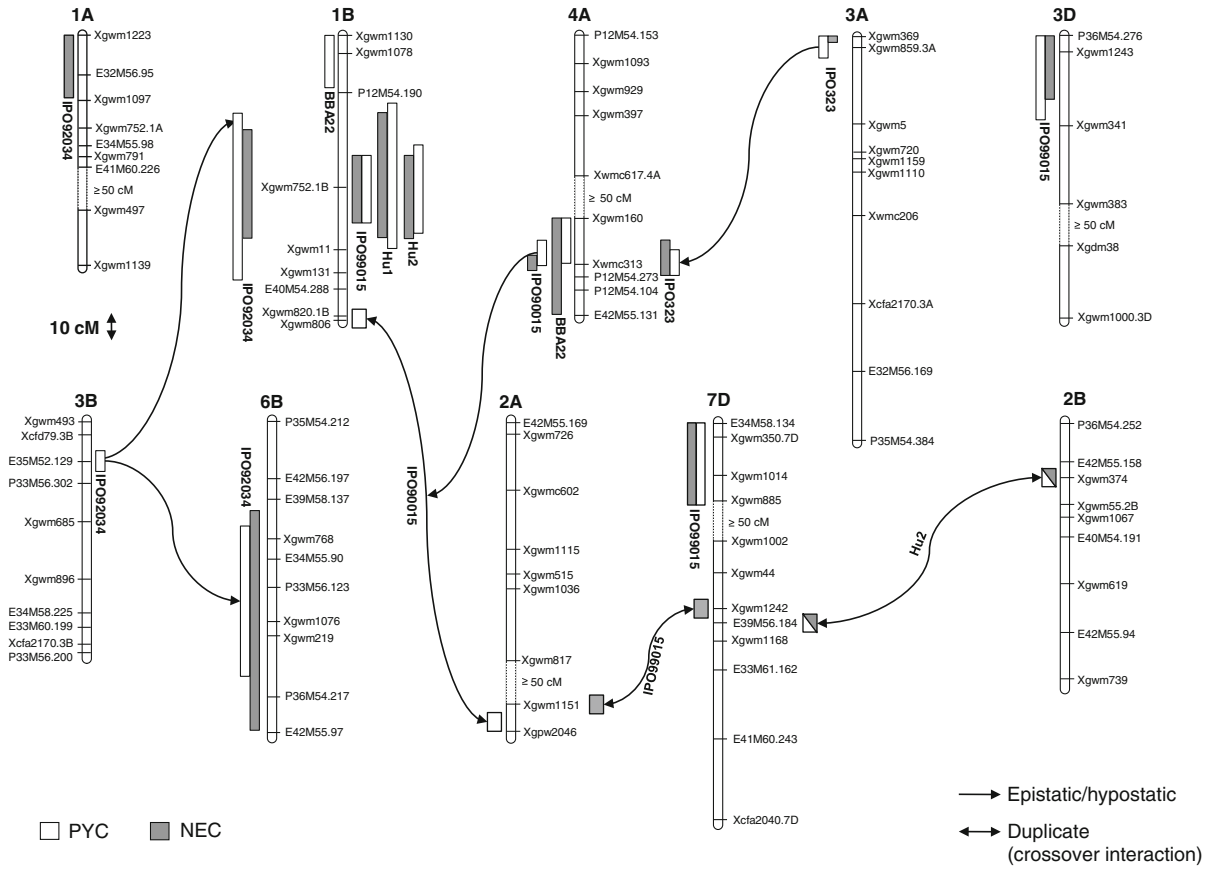

Fig. 3 Location of main resistance QTL effects for STB on the genetic map of the S × M DH population detected with seven isolates in the seedling stage for pycnidial coverage (*PYC*) and necrotic leaf area (*NEC*). The *bar size* indicates the maximum LOD: 1.5 LOD. An epistatic/hypostatic QTL by QTL interaction is indicated by a *single arrow*, duplicate (crossover) interaction by a *double arrow*. For the latter, confidence ranges could not be determined and *boxes* next to the closest linked marker were used instead

$P = 0.07$, respectively) in one experiment each. The confidence ranges of QTL for different isolates and disease parameters are shown in Fig. 3. If a map region affected resistance to more than one isolate, a single QTL name was assigned to overlapping ranges.

Seedling resistance to IPO323, conferred by Solitär, was predominantly controlled by a QTL located distally on chromosome arm 3AS. This locus explained 68.8% (PYC) or 84.1% (NEC) of the phenotypic variance, respectively. On average, *QStb.3AS* caused a difference in PYC of 19.1%. In the two-part model, a QTL with small effects on PYC (6.2%) and NEC (6.8%) was identified on 4AL, closely linked to *Xwmc313*. Conditional on observations above 2.5% disease severity, *QStb.4AL* accounted for 5.0% (NEC) to 14.7% (PYC) of the phenotypic variance. QTL *QStb.4AL* was also detected with IPO90015 but with a QTL heritability ranging from 47.9% (PYC) to 75.8% (NEC) and a decrease in disease severity of 24.8% (NEC) and 12.8% (PYC). This QTL is responsible for the spike of resistant DH lines in the distribution of PYC and NEC (Fig. 2). *QStb.4AL* was also identified in response to the German isolate BBA22, although the maximum LOD positions differed slightly among the two disease traits (Table 3). There it accounted for 11% (NEC) or 18% (PYC), respectively, of the phenotypic variance.

Solitär imparted the main component of resistance to IPO99015. This locus, *QStb.1B*.a, linked to *Xgwm11*, could be assigned to 1BS based on a deletion bin (Sourdille et al. 2004b) and explained between 30% (NEC) and 42.8% (PYC) of the

phenotypic variance. Two further QTL with minor effects on PYC and NEC could be detected on chromosomes 3D and 7D (Table 3). QTL overlapping with the *QStb.1B.a* interval were also found upon infection with Hu1, Hu2 and IPO92034, and resistance was mediated by Solitär likewise. QTL heritabilities and effects of *QStb.1B.a* were found to be higher for Hu2 than Hu1, suggesting less favourable infection conditions for the latter isolate.

Each of the above QTL was evident in either disease trait. In contrast, resistance specific to PYC and NEC was observed for IPO92034 as the formation of pycnidia was markedly affected by a QTL located on chromosome 3B and the size of necrotic lesions by a QTL on 1A (Table 3). *QStb.3B* accounted for 38.4% of the phenotypic variance. Whereas Solitär conferred resistance at *QStb.3B*, Mazurka carried the resistant allele at *QStb.1A* which contributed only 11.5% of the variance associated with NEC. A further QTL, denoted as *QStb.1B.b*, controlling PYC-specific resistance to BBA22 was detected on 1B, but in a different position from *QStb.1B*.a (Fig. 3). Only a small proportion of the phenotypic variance (7.0%) could be attributed to *QStb.1B.b*. Resistance of this PYC-specific locus is mediated by Solitär.

Epistatic QTL effects on STB resistance

Epistatic interactions were detected for both resistance traits with IPO323 and Hu2. However, three interactions showed specificity to PYC (IPO90015, IPO92034) and one interaction specificity to NEC (IPO99015) (Table 3; Fig. 3). Two types of epistasis that could be distinguished by presence or absence of a single locus effect were observed in the S × M DH population, and these are illustrated for IPO323 and IPO90015 (Fig. 4). The two QTL involved in the epistatic interaction in response to IPO323, *QStb.3AS* (marker *Xgwm369*) and *QStb.4AL* (marker *Xwmc313*) were also identified in the single-QTL model via a two-part analysis. However, inclusion of the interaction effect yielded a significantly better model fit. The presence of the Solitär allele at *Xgwm369* (=epistatic)

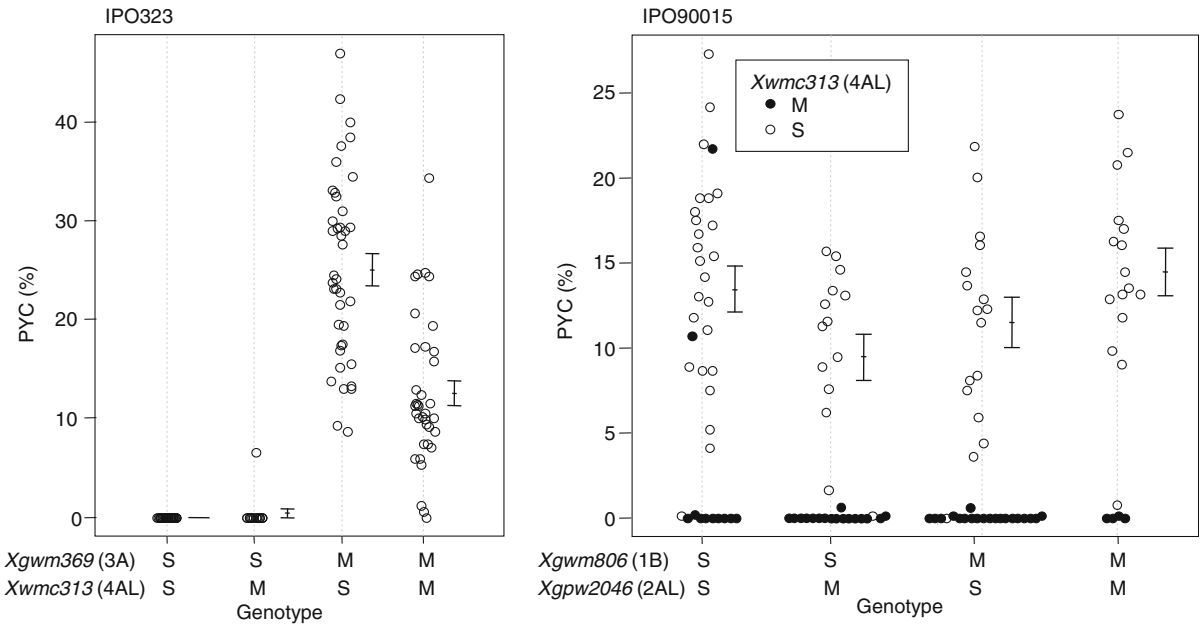

Fig. 4 Epistatic effects revealed in the S × M DH population. Means and standard errors of pycnidial coverage (*PYC*, in %) for the Solitär (*S*) and Mazurka (*M*) allele pairs at loci *Xgwm369* (3A) and *Xwmc313* (4L) determined by testing with IPO323 (*left*) and allele pairs at *Xgwm806* (1B) and *Xgpw2046* (2AL) after infection with IPO90015 (*right*). In the *right panel, filled circles* represent the Mazurka allele, *open circles* the Solitär allele at locus *Xwmc313*. For isolate 90015, standard errors were calculated conditional on the Solitär allele at this locus

ensures seedling resistance independent of the allelic state at *QStb.4AL* (=hypostatic). Lines carrying the Mazurka alleles at loci *Xgwm369* and *Xwmc313* were less susceptible. On average, this interaction explained 5.4% (PYC) and 1.9% (NEC) of the phenotypic variance (Table 3). The PYC-specific QTL *QStb.3B* detected with IPO92034 interacted with each of two single QTL (*QStb.6B* and *QStb.1B.a*) with smaller effects on pycnidia formation (see Electronic Supplementary Material ESM Fig. 1). The Solitär allele at *QStb.3B* (=epistatic) conferred a high level of resistance to PYC, but when absent the Solitär allele at *QStb.6B* or *QStb.1B.a* (=hypostatic) still reduced pycnidia formation. Thus, apart from an additive mode of action, epistatic effects of *QStb.3B* and *QStb.1B.a* (explained phenotypic variance = 4.2%) and between *QStb.3B* and *QStb.6B* (explained phenotypic variance = 4.7%) were involved in resistance to pycnidia formation.

QTL by QTL interactions without marginal single-QTL effects were uncovered with isolates IPO90015, IPO99015 and Hu2. This crossover interaction, sometimes termed duplicate epistasis, only gave rise to resistance (or susceptibility) in genotypes with opposite allelic configurations in a pair of QTL. As an example, the interaction between *QStb.1B.c* (tightly linked to *Xgwm806*) and *QStb.2AL* (closely linked to *Xgpw2046*) conditional on the allelic state of *QStb.4AL* (locus *Xwmc313*) in response to IPO90015 is presented in Fig. 4. Genotypes carrying the Mazurka allele at *Xwmc313* respond with disease severities of PYC $\leq$ 2.5% regardless of the alleles at *QStb.1B.c* and *QStb.2AL*, with only a few exceptions. The interaction between the latter resulted in a higher resistance to PYC whenever one QTL carried the Solitär allele and the other QTL the Mazurka allele, and explained 3.2% of the phenotypic variance independent of the major effect of 4AL. A crossover interaction could also be observed between *QStb.2B* (linked to *Xgwm374*) and *QStb.7DL* (linked to *E39M56_184*) in response to Hu2, and explained 6.7% (PYC) or 9.6% (NEC) of the phenotypic variance, respectively. A NEC-specific crossover interaction has been detected with IPO99015 between *QStb.2AL* (linked to *Xgwm1151*) and *QStb.7DL* (linked to *Xgwm1242*), and explained 3.3% of the phenotypic variance (see ESM Fig. 1). Chromosome arm 2AL, covered by only two markers, was already involved in the interaction with *QStb.1B.c*. *Xgpw2046* and *Xgwm1151* are separated by only 8 cM. Therefore it cannot be ruled out that this part of 2AL is involved in multiple interactions. This might also be true for the QTL region on 7DL since *Xgwm1242* is only 6 cM apart from *E39M56_184*, linked to *QStb.7DL*, which has already been shown to interact with *QStb.2B* in response to Hu2.

Discussion

Differential parental responses to *M. graminicola* isolates

In several studies, specificity in the *T. aestivum–M. graminicola* pathosystem has been identified as a significant isolate × genotype interaction in experiments using differential sets (Kema et al. 1996a, b). Solitär and Mazurka, the parents of the S × M DH population, were included in a larger differential set of thirteen *T. aestivum* genotypes representing all fifteen mapped *Stb* resistance genes (Tabib Ghaffary et al. 2008). Among the twenty IPO isolates tested at PRI, four isolates were avirulent to cultivars carrying *Stb6* (IPO323), *Stb5* (IPO94269), *Stb9* (IPO89011) or *Stb15* (IPO88004), respectively (Brading et al. 2002; Arraiano et al. 2001; Chartrain et al. 2009; Arraiano et al. 2007). From the screening results we hypothesize that Mazurka probably possesses none of the four *Stb* genes whereas Solitär carries *Stb6* and other resistance genes not covered by the set of isolates. Quite often, differentiation for STB between the two cultivars is not clear-cut enough to distinguish between qualitative and quantitative resistance to single isolates. In addition to the IPO isolates, we also tested locally adapted German and Hungarian fungal isolates. It is remarkable that the German fungal isolates BBA22, BASF27, BBA39, Ma3 and Ma4, the latter two collected from Mazurka, caused lower PYC on Mazurka but were aggressive on Solitär. Conversely, while Solitär was resistant to the three Hungarian isolates Hu1, Hu2 and Hu3, Mazurka was highly susceptible. These findings apparently indicate adaptation of *M. graminicola* isolates to German and Hungarian cultivars, respectively. Adaptations of *M. graminicola* to resistant and moderately resistant wheat cultivars are known and well documented (Jackson et al. 2000; Krenz et al. 2008), as the high sexual recombination in *M. graminicola* populations

increases the chance of generating novel virulence combinations. The low acreage of Solitär in Germany in combination with isolate non-specific resistance might explain the high field resistance of this variety.

Isolate-specific major and minor QTL identified in the S × M DH population

In many studies major resistance genes, designated *Stb* genes, to specific *M. graminicola* isolates have been identified because in a single-isolate assay almost complete resistance was conditioned by a corresponding gene pair (Goodwin 2007). However, owing to the concerted action of several genes and environmental effects, resistance to single isolates appeared also as a quantitative character (Eriksen et al. 2003, Simón et al. 2004). QTL mapping exploits the total observed variation to dissect the genetics of STB resistance including minor genetic effects and, as with classical genetics, to disclose epistatic relationships. In the S × M DH population we detected QTL explaining the bulk of the phenotypic variance, depending on the isolate, on chromosomes 3A, 4A and 1B for both resistance traits and on 3B with specificity to PYC. Besides these major genes, QTL which contributed moderately or little to the phenotypic variance were localized on chromosomes 1A, 1B, 3D, 6B and 7D. For a more reliable detection of those minor QTL with low heritabilities, the population size is the limiting factor. *Stb6* was characterized by conferring resistance to IPO323 but susceptibility to IPO94269 and by its co-segregation with SSR locus *Xgwm369*. Our pathogenicity assays and QTL analyses demonstrate that Solitär possesses *Stb6* and that *QStb.3AS* corresponds to *Stb6*. Varieties carrying *Stb6* still show genetic variation in disease severity (Arraiano and Brown 2006), and Chartrain et al. (2005c) assumed allelic variation in the *Stb6* gene itself or gene modifiers. Kema et al. (2000) provided evidence that IPO323 carries more *Avr* genes besides the *Stb6* matching avirulence gene. Chartrain et al. (2005a) showed that the spring wheat line Kavkaz-K4500 L.6.A.4 (KK) has an additional gene for resistance to IPO323 besides *Stb6*. In our study *QStb.4AL* also contributed to resistance against IPO323 but was not as effective as *Stb6*, and the underlying gene acts downstream of the epistatic *Stb6* gene. The fact that the Mazurka allele at *QStb.4AL* not only enhanced resistance to IPO323 but also to IPO90015 and BBA22 points to a single gene or a complex of linked genes. It is likely that among the published *Stb* genes, *Stb7* and *Stb12*, both located distally on chromosome arm 4AL, are candidates for *QStb.4AL*. *Stb7* has been mapped in proximity to *Xwmc313* in crosses with the spring wheat variety Estanzuel Federal (McCartney et al. 2003) and independently in a population derived from a cross between KK and cv. Shafir (Chartrain et al. 2005a). *Stb12*, first mentioned in the latter study, has been distinguished from *Stb7* by the differential response of the parents to two Israeli isolates and was found to be more closely linked to *Xwmc219* than to *Xwmc313*. According to pedigree data (L. Láng, personal communication) it is unlikely that Mazurka could have received *Stb12*. Based on this evidence and the strong linkage to *Xwmc313,* we assume that Q*Stb.4AL* identified in the S × M DH population is likely to correspond to *Stb7*.

A large LOD confidence interval on chromosome 1B defined as *QStb.1B.a* conferred resistance to four isolates in the S × M DH population, with the positive allele being contributed by Solitär. The phenotypic effects that vary with the isolate could reflect the action of a major gene modulated by interacting genes, or, as suspected for Hu1, less favourable conditions for disease development. Until now, the only gene mapped to 1B is *Stb11* identified in the Portuguese breeding line TE9111 (Chartrain et al. 2005b). By physical mapping Raman et al. (2009) could refine the location of *Stb11* to the flanking markers *Xwmc230* and *Xbarc119b*. In our study, *QStb.1B*.a is closely linked to *Xgwm752.1B* and by comparison with the consensus map (Sourdille et al. 2004b) its confidence range includes *Stb11*.

Two minor QTL were localized with IPO99015 on the short arms of chromosomes 3D and 7D. *QStb.3DS* should be different from a QTL for adult plant resistance that has been mapped to the long arm of chromosome 3D by Simón et al. (2004). Hence, *QStb.3DS* constitutes a newly identified QTL. Two published genes, *Stb4* and *Stb5*, are clustered on the short arm of 7D. *Stb5* can be excluded as a candidate because of the susceptibility of Solitär to IPO94269 being indicative for the absence of *Stb5* (Arraiano et al. 2001). *Stb4*, first described by Somasco et al. (1996), exhibited good resistance in field and greenhouse experiments and mapped near the centromere closely linked to *Xgwm111* (Adhikari et al. 2004b).

As yet, no *Stb* gene has been mapped to the distal end of 7DS (Goodwin 2007). However, a QTL on 7DS with minor effects was identified by Arraiano et al. (2007) in the Swiss wheat cv. Arina and its location is distal to *Stb4*. *QStb.7DS* could be unambiguously mapped to a 31-cM interval between the AFLP marker *E34M58_134* and *Xgwm885,* demonstrating that *QStb.7DS* is not identical with *Stb4* but possibly located in the same region on 7DS as the QTL identified by Arraiano et al. (2007). QTL with low heritabilities like *QStb.7DS* probably have no impact on field seedling resistance and would not withstand a cross validation.

A QTL with minor effect on the long arm of chromosome 6B was identified in all replicates in response to IPO92034, and the most likely position is between *Xgwm219* and *Xgwm1078*. While none of the known *Stb* genes mapped to this chromosome, some studies reported several QTL on 6B. Eriksen et al. (2003) located two different minor QTL on 6BS in the seedling stage after inoculation with IPO323 and a Danish isolate, respectively. With IPO323, a QTL on 6B could not be detected in the S × M DH population, indicating that *QStb.6B* is another QTL. In the ITMI mapping population, Simón et al. (2004) found a minor QTL on 6BS in the seedling stage for two independent isolates. In adult plant tests with three isolates, Chartrain et al. (2004a) revealed a QTL with minor effects linked to *Xgwm133* and *Xgwm219*. Possibly this QTL coincides with *QStb.6B* because they cover roughly the same region. Unfortunately, a conclusive comparative QTL analysis is often complicated by the lack of common polymorphic markers between different mapping populations.

QTL with specificity to necrosis and pycnidia formation

Separate analyses were carried out for the parameters NEC and PYC in order to disclose resistance QTL involved in different stages of disease development. The positive relationship between NEC and PYC detected in the S × M DH population was expected since pycnidia formation usually relies on the presence of necrotic lesions (Simón et al. 2005). In the *T. aestivum–M. graminicola* pathosystem, pycnidia formation is conditioned by collapsed but not necessarily necrotic plant tissue (Kema and van Silfhout 1997). At 10–14 dpi, the fungus switches from a symptomless to a necrotrophic stage by the induction of cell collapse, release of nutrients and formation of pycnidia. Assessment of the disease using necrotic leaf area is not always reliable as other biotic and abiotic stress-related factors may mimic chlorotic or necrotic symptoms, thereby overestimating the actual infestation.

In this study we worked with whole seedlings under optimal growing conditions in the greenhouse and senescence was only visible on mock plants 21 dpi after scoring was already finished on inoculated DH lines. The loose relationship between PYC and NEC found in isolates IPO92034 (Fig. 2) and BBA22 already indicated the occurrence of development-specific resistance mechanisms. Likewise, Chartrain et al. (2005b) determined a moderate correlation between necrosis and pycnidia formation in a mapping population screened with IPO323 and suspected partial resistance of one parent, TE9111, to be the cause. In contrast, strong necrosis was always accompanied with high pycnidial coverage in the S × M DH population; i.e. PYC-specific resistance factors are absent in Mazurka. Two PYC-specific QTL, both contributed by Solitär, were mapped to chromosome arm 3BS with isolate IPO92034 and to chromosome arm 1BS with isolate BBA22 (Table 3). It is evident that *QStb.1B.b* is different from *QStb.1B.a* as it resides at a more distal region (Fig. 3). *QStb.3B* had a major effect on pycnidia formation. As a possible candidate gene for *QStb.3B,* we considered *Stb2,* first identified by Wilson (1985) under natural conditions and mapped by Adhikari et al. (2004a) to the short arm of chromosome 3B, tightly linked to *Xgwm389* and proximal to *Xgwm493*. Since *QStb.3B* is located distal to *Xgwm493* in the S × M DH population it is evident that a different gene is involved. Eriksen et al. (2003) mentioned a QTL with minor effects on 3BL in the seedling stage. Unfortunately the authors did not consider measures of pycnidia formation. Another *Stb* gene, *Stb14,* also mapped to 3BS. However, no further information on this gene is available in the catalogue of gene symbols (McIntosh et al. 2007). It appears that *QStb.3B* affects initial pycnidia formation whereas *QStb.1B.a* and *QStb.6B* are more generally involved in the suppression of the infection process. Besides PYC-specific QTL, one NEC-specific QTL with minor effects, also obtained with isolate IPO92034, could be identified. Its position on

1A does not coincide with any known *Stb* gene or QTL and hence this is the first report of a QTL on this chromosome.

Epistatic relationships in STB seedling resistance

Complete epistasis could be shown for *QStb.3AS* and *QStb.4AL* with isolate IPO323. Epistatic effects up to 13.3% for PYC were observed which do not differ greatly from the single main effect at 3AS of 19.1%. This means that epistasis can make an important contribution to the genetic variance of STB resistance. Setting up an appropriate statistical model in such a situation is challenging because effects are confounded. Firstly, the epistatic locus should rather be considered as a binomial variate, and the residual genetic variation accounted for by the hypostatic locus should be approximated as a normal distribution. This can be handled roughly by composite interval mapping (Zeng 1994) or exactly as a two-part model as suggested by Broman et al. (2003). We have found 2.5% disease severity to be a reasonable cut-off point to separate the phenotypic spike from the residual distribution (Fig. 2), and slightly different values did not affect the outcome. Secondly, QTL main effects are not easily interpretable in the presence of interaction and are prone to bias. The effect of the epistatic locus (*QStb.3AS*) is less affected than the effects of the hypostatic locus (*QStb.4AL*) and the interaction. Meaningful estimates for *QStb.4AL* are obtained conditional on the *QStb.3AS* genotype. The situation is even more intricate for crossover interactions when resistance alleles at a locus pair originate from different parents and therefore single locus effects cancel out each other. We detected only a few such effects and due to their marginal contribution they can be neglected in breeding programs.

The efficacy of a resistance gene, i.e. whether it is considered a major or minor gene, strongly depends upon the presence of specific alleles at other resistance loci. In the same way efficacy is affected by the frequency of corresponding allele combinations at avirulence-determining loci in the pathogen population. For instance, *QStb.4AL* had a major effect on resistance to IPO90015 and probably matches *Stb7* or *Stb12, w*hereas its effect is masked in individuals carrying the resistant allele at *QStb.3AS* (*Stb6)* when exposed to IPO323. When challenged with IPO90015, *QStb.4AL* is a major QTL which is epistatic to the PYC-specific crossover interaction between *QStb.1B.c* and *QStb.2AL*. These interrelationships constitute a three-way interaction. Combining the results of the IPO323 and IPO90015 assays, we hypothesize a resistance control pathway in which *Stb6* is hierarchical over *Stb7* (or *Stb12*) which again acts on top of the *QStb.1B.c–QStb.2AL* interaction.

Evidence of epistatic and disease development-specific gene action possibly reflects differences at the histological, biochemical and molecular levels found between susceptible and resistant genotypes in early and late events of the infection process (Shetty et al. 2003; Adhikari et al. 2007; Keon et al. 2007). Isolate IPO323 has been shown to hijack plant resistance signalling of a susceptible host by accelerating programmed cell death (PCD) (Keon et al. 2007). Possibly, *Stb6* is active during the penetration stage and shortly after, thereby preventing PCD and as a consequence necrosis and pycnidia formation is suppressed. *Stb7*, when challenged to IPO90015, may act like *Stb6*, or be attenuated after infection with IPO323 and BBA22, by a reduction of fungal growth, accompanied with less necrotization and pycnidia formation. The PYC-specific *QStb.1B.c–QStb.2AL* interaction might interfere at a later stage of pycnidia formation and influence pycnidia maturation by inhibiting fungal synthesis of reactive oxygen species. Likewise, such responses can be assumed for interactions detected with Hu2 and IPO99015.

Concluding remarks and outlook

By adoption of multiple QTL models on a set of isolates, we unravelled seedling resistance to STB as an intricate pathway involving genes at different stages of the infection process. How *Stb* genes, which usually have large effects, relate to QTL with small effects is still a matter of discussion. It is evident that QTL with major effects found in this study coincide with previously described *Stb* genes. One hypothesis introduced the notion of QTL with minor effects being weak alleles of 'major' resistance genes as a result of gene erosion due to pathogen co-evolution (Poland et al. 2008).

QTL analysis revealed that Solitär carries at least two *Stb* genes and few minor QTL. Whether any of these resistance factors, single or in combination, is

involved in the remarkable field resistance of Solitär remains to be demonstrated. Field testing of the S × M DH population is currently under way. Breeding of resistance to STB relies on efficacy and durability of employed resistance genes in the field, and a strong effect by pyramiding *Stb* genes has not been reported to this day. From the *Stb* genes identified here, only *Stb6* has been shown to be effective in the field (Arraiano et al. 2009). Knowledge of additive and epistatic action of *Stb* genes (or QTL) might allow MAS to be more efficient and targeted. Taking into account the dynamic virulence structure of *M. graminicola*, breeding for field resistance to STB still remains a challenging task.

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