

Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache

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Abstract The ascomycete *Mycosphaerella graminicola* is the causal agent of septoria tritici blotch (STB), one of the most destructive foliar diseases of bread and durum wheat globally, particularly in temperate humid areas. A screening of the French bread wheat cultivars Apache and Balance with 30 *M. graminicola* isolates revealed a pattern of resistant responses that suggested the presence of new genes for STB resistance. Quantitative trait loci (QTL) analysis of a doubled haploid (DH) population with five

M. graminicola isolates in the seedling stage identified four QTLs on chromosomes 3AS, 1BS, 6DS and 7DS, and occasionally on 7DL. The QTL on chromosome 6DS flanked by SSR markers *Xgpw5176* and *Xgpw3087* is a novel QTL that now can be designated as *Stb18*. The QTLs on chromosomes 3AS and 1BS most likely represent *Stb6* and *Stb11*, respectively, and the QTLs on chromosome 7DS are most probably identical with *Stb4* and *Stb5*. However, the QTL identified on chromosome 7DL is expected to be a new *Stb* gene that still needs further characterization. Multiple isolates were used and show that not all isolates identify all QTLs, which clearly demonstrates the specificity in the *M. graminicola*–wheat pathosystem. QTL analyses were performed with various disease parameters. The development of asexual fructifications (pycnidia) in the characteristic necrotic blotches of STB, designated as parameter *P*, identified the maximum number of QTLs. All other parameters identified fewer but not different QTLs. The segregation of multiple QTLs in the Apache/Balance DH population enabled the identification of DH lines with single QTLs and multiple QTL combinations. Analyses of the marker data of these DH lines clearly demonstrated the positive effect of pyramiding QTLs to broaden resistance spectra as well as epistatic and additive interactions between these QTLs. Phenotyping of the Apache/Balance DH population in the field confirmed the presence of the QTLs that were identified in the seedling stage, but *Stb18* was inconsistently expressed and might be particularly effective in young plants. In contrast, an additional QTL for STB resistance was identified on chromosome 2DS that is exclusively and consistently expressed in mature plants over locations and time, but it was also strongly related with earliness, tallness as well as resistance to Fusarium head blight. Although to date no *Stb* gene has been reported on chromosome 2D, the data

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provide evidence that this QTL is only indirectly related to STB resistance. This study shows that detailed genetic analysis of contemporary commercial bread wheat cultivars can unveil novel *Stb* genes that can be readily applied in marker-assisted breeding programs.

Introduction

Septoria tritici blotch (STB) is a fungal wheat disease that is caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J.Schröt. The disease was first described in France (Desmazieres 1842; Sprague 1938), but was neglected for a long time due to overwhelming stripe rust and powdery mildew epidemics. Thus, STB was long considered as a secondary disease that mostly appeared in years with low levels of other cereal diseases. Nevertheless, it has been present in Europe for over a century, along with *Stagonospora nodorum* (Bearchell et al. 2005) and is currently considered to be one of the most important wheat diseases. Infections result in severe necrosis of the foliage that is filled with the asexual and sexual fructifications (Eyal 1999; Hunter et al. 1999; Kema et al. 1996c; McDonald et al. 1996; Shaw and Royle 1989). In Europe, STB usually establishes through airborne ascospores that are discharged from wheat debris and deposited in young wheat crops in the fall (Suffert et al. 2010). This is followed by rain splash driven spore dispersal during the growing season (Eriksen and Munk 2003; Halama 1996; Pastircak 2005; Scott et al. 1988; Shaw and Royle 1989, 1993). However, *M. graminicola* can reproduce sexually throughout the year, which provides the fungus with a mechanism to overcome adverse biotic or abiotic conditions (Kema et al. 1996c; Zhan et al. 2007; Ware et al. unpublished data).

STB management is largely effectuated by the application of fungicides and breeding for resistance. Due to its increased importance in Europe, STB is a main target as well as a serious concern of the agrochemical and breeding industry because of recent outbreaks of resistance to strobilurins (Cools and Fraaije 2008; Fraaije et al. 2005; Fraaije et al. 2007; McCartney et al. 2007; Stammler et al. 2008; Torriani et al. 2009) and steadily increasing levels of resistance to azole fungicides (Cools and Fraaije 2008; Fraaije et al. 2005; Fraaije et al. 2007; Mavroeidi and Shaw 2005; Stergiopoulos et al. 2003). These problems raised questions about the sustainability as well as the environmental impact of crop protection agents (Verweij et al. 2009). Hence, in several European countries, including France, Spain, Germany, Italy, the Netherlands and the UK, pesticide reduction programs have been developed and adopted by policy makers (Anonymous 2009). Therefore, a new focus on host resistance to increase the commercial lifetime of cultivars is required as part of a strategy to control STB.

In the UK, STB was unheard of as a major wheat disease before the late 1970s, but emerged as a major foliar blight in the early 1980s on susceptible cvs. such as cvs. Norman and Longbow. These cultivars were then replaced by others of similar susceptibility, and significant progress in breeding for resistance was not made until the mid-1990s (Paveley 2006). Demands for cultivars with better resistance levels resulted in the release of cv. Claire in 1999 that was replaced by cv. Alchemy (Angus and Fenwick 2008). Currently, other high-yield potential cultivars with moderate to high resistance to STB have been recommended, such as cv. Stigg (Anonymous 2010a; Angus et al. 2010). In France, 15 cultivars covered almost 77% of the total wheat acreage in 2003, in which cv. Apache ranked first with 23.7% and cvs. Isengrain, Tremy, Shango, Orvantis, Soissons, Caphorn and Charger together covered 37.3% (Anonymous 2005). Recent resistance screens indicated that the majority of these cultivars were highly susceptible to a substantial number of isolates in the seedling stage and hence, their resistances have a narrow efficacy (Tabib Ghaffary et al., unpublished data). Consequently, there is an urgent need for new resistance genes (Tabib Ghaffary et al., 2011 submitted to TAG).

The identification, characterization and processing of resistance to STB in practical wheat breeding programs, however, are not routine issues and several parameters can be used for disease scoring, such as the percentage of induced necrosis (*N*) or the percentage of pycnidia (*P*), the asexual fructifications of *M. graminicola*, in the foliage. Both parameters are strongly interwoven, as necrosis is conditional for pycnidia development, but is suggested to be under different genetic control (Kema et al. 1996d; Shetty et al. 2003, 2007, 2009). Currently, most screens involve well-characterized fungal isolates in repeated young plantlet assays and detached leaf assays (Arraiano et al. 2001a; Arraiano and Brown 2006; Kema et al. 1996a; Kema et al. 1996b; Kema and van Silfhout 1997), which have contributed to the data reliability and eventually to the mapping of resistance genes. Seedling screens offer opportunities to identify the efficacy of resistance to a wide panel of isolates, but—due to quarantine limitations—these can only be used to a limited extent under field conditions. Nevertheless, practical resistance breeding is a difficult multi-location, multi-pathogen and multi-pathotype effort responding to actual epidemiological situations—for instance for cereal rust diseases—and even legislation such as for Fusarium head blight (FHB) (Vanloqueren and Baret 2008). This resulted in 73, 89, 61 and 95 identified resistance genes for stripe rust, leaf rust, stem rust and powdery mildew, respectively, but only 17 *Stb* genes have been reported (Tabib Ghaffary et al. 2011, submitted to TAG). The majority of these *Stb* genes have a limited efficacy and hence are only sparsely deployed in breeding programs

(Arraiano et al. 2007; Chartrain et al. 2009; Goodwin 2007), whereas the resistance genes to other wheat diseases are widely applied in new commercial wheat cultivars.

The apparent need for additional resistance genes prompted us to screen a wide variety of germplasm that resulted in the identification of new *Stb* genes (Tabib Ghaffary et al. 2011, submitted to TAG; Tabib Ghaffary, unpublished data). Here, we report the characterization of STB resistance in the French winter wheat cvs. Apache and Balance with 30 *M. graminicola* isolates and the identification of new *Stb* genes and associated molecular markers that can be readily applied in marker-assisted breeding programs.

Materials and methods

Plant materials and pathogen isolates

A double-haploid (DH) population of 91 lines derived was developed from a cross between cvs. Apache and Balance. Seedling assays were performed in a greenhouse compartment. The parental cvs. Apache and Balance were planted in VQB 7 × 7 × 8 cm TEKU[®] plastic pots, 10 linearly sown seeds per pot, while the DH lines were planted in 5.5 × 5 cm round Jiffy[®] pots, five seeds per pot using a steam-sterilized peat/sand mixture. All plants were grown in a controlled greenhouse compartment with 16-h/day light supplemented with Son-T Agro 400 W lamps (Hortilux, Boca Raton, Florida, USA). Pre-inoculation temperature and relative humidity (RH) were 18/16°C (day/night rhythm) and 70% RH, whereas post-inoculation temperature and RH were 22/21°C and ≥85% RH, respectively. Adult plant experiments were carried out in 2007 and 2008 in Cappelle-en-Pévèle and Prémèsques in northern France at the breeding stations of Florimond Desprez and Serasem, respectively. Each field plot contained two 1.5-m length rows with 0.3 m spacing.

Seedling evaluations involved deep screening of the parental cultivars with 30 monopycnidial *M. graminicola* isolates in 2007 and 2008 followed by a progeny evaluation in three replications, in which eight isolates were tested in the first replication (pre-screening) and five in subsequent replications (Table 1). In all seedling experiments, an alpha lattice experimental design was adopted that considered each pot as an experimental unit with random arrangement for each isolate–replication combination on separate parallel tables in the above-mentioned greenhouse compartment. Field evaluations were performed with isolate IPO323 in a single replicated randomized block experiment in 2007 and a double replicated randomized block design at both locations in 2008.

Inoculation procedures and scoring

Pre-cultures of each isolate (Table 1) were prepared in an autoclaved 100-ml Erlenmeyer flask containing 50-ml yeast–glucose (YG) liquid medium (30 g glucose, 10 g yeast per liter of dematerialized water). The flasks were inoculated using a small piece of mycelium maintained at –80°C and incubated in a shaker (Innova 4430, New Brunswick Scientific, USA) adjusted at 125 rpm and 18°C for 5–6 days. These pre-cultures were then used to inoculate three 250-ml Erlenmeyer flasks containing 100 ml of YG media per isolate that were incubated under the aforementioned conditions to provide enough inoculum for the seedling inoculation assays at growth stage (GS) 11 (Zadoks et al. 1974). The inoculum concentration was adjusted to 10⁷ spores/ml in a total volume of 40 ml for a set of 18 plastic pots or 24 Jiffy[®] pots and was supplemented with two drops of Tween 20 (MERCK[®], Nottingham, UK).

Field inoculations were performed with a backpack air-pumped sprayer, which was calibrated at a rate of 10 L/100 m² at flag leaf appearance stage (GS 47–49), using a concentration of 10⁶ spores/ml supplemented with 36 ml of four times diluted Tween 20 (MERCK[®], Nottingham, UK) surfactant. Inoculations started when the foliage of the earliest DH lines developed and were subsequently repeated twice at 3- to 5-day intervals to compensate for earliness differences.

Disease severity was evaluated 21 days after inoculation in the seedling and the adult plant stage (with some variation ±2 days depending on weather conditions). In the seedling stage, the percentages of necrosis (*N*) and pycnidia (*P*; asexual fructifications) were scored separately on the first leaves, as well as *NLP* and *PLP* (days between inoculation and first *N* and *P* appearance, respectively). In the adult plant stage, the total percentage of STB symptoms on the flag leaf was recorded in 2007 and 2008, as well as earliness and tallness in 2008. Data loggers were installed at the flag leaf level to monitor the actual field conditions (RH and temperature at 10-min intervals) throughout the experiments.

FHB was established by distributing maize debris among the plants during tillering in the adult plant experiment in 2008. Disease was rated as percentage of infected spikelets per ear during STB assessments.

Mapping and QTL analysis

DNA was extracted from first leaf samples of cvs. Apache, Balance and the DH lines using the Promega Wizard[®] Magnetic DNA Purification System for Food (blc) according to the manufacturer's instructions with slight modifications. Genetic polymorphism analyses were performed with Diversity Arrays Technology (DArT) version 2.3 and 3 (Triticarte Pty Ltd, Canberra, Australia) that were

Table 1 *Mycosphaerella graminicola* isolate panels and their origin that were used for parental cultivars and Apache/Balance doubled-haploid progeny screening

	Isolate code	Origin	
		Country	Location
	IPO94218 ^a	Canada	Saskatoon
	IPO00003 ^a	USA	Colusa
	IPO00005 ^a	USA	Colusa
	IPO90006 ^a	Mexico	Toluca
	IPO90015 ^a	Peru	Unknown
	IPO87016 ^{a,d}	Uruguay	Dolores
	IPO86068 ^a	Argentina	Balcarce
	IPO99015 ^a	Argentina	Unknown
	IPO89011 ^{a,d}	Netherlands	Barendrecht
	IPO92004 ^a	Portugal	Casa Valhas
	IPO95054 ^a	Algeria	Berrahal
	IPO92034 ^{a,c}	Algeria	Guelma
	IPO88018 ^a	Ethiopia	Holetta
	IPO88004 ^a	Ethiopia	Kulumsa
	IPO95036 ^{a,c}	Syria	Minbeg
	IPO86013 ^a	Turkey	Adana
	IPO02166 ^a	Iran	Dezful,Safi Abad
	IPO02159 ^a	Iran	Gorgan, AqQaleh
	IPO95052 ^{a,e}	Algeria	Berrahal
	IPO86022 ^{a,e}	Turkey	Altinova
	IPO323 ^{b,d}	Netherlands	W.Brabant
	IPO94269 ^b	Netherlands	Kraggenburg
^a Used for parental screen in 2008	IPO98022 ^{b,d}	France	Villaines la Gonais
^b Used for parental screen in 2007	IPO98046 ^{b,d}	France	St. Pol de Leon
	IPO98047 ^b	France	Aire D'Havrincourt
	IPO98094 ^{b,c}	France	Aire D'Havrincourt
^c Used in pre-screening	IPO052461 ^{b,f}	France	Unknown (Biogemma)
^d Triplicated on DH lines	IPO052462 ^{b,f}	France	Unknown (Biogemma)
^e Durum wheat-adapted strains	IPO052463 ^{b,f}	France	Unknown (Biogemma)
^f Provided by Biogemma, Clermond-Ferrand, France	IPO052464 ^{b,f}	France	Unknown (Biogemma)

supplemented with additional SSR data. Mapping analyses were performed using JoinMap[®] 4 software with settings $\text{LOD} \geq 3$ (Log of Odds) for grouping as well as the maximum likelihood mapping option for linkage group generation (Van Ooijen 2006). The DArT markers with low quality parameters (ANOVA based P value < 80) were removed from the data set (Akbari et al. 2006) and marker positions were compared and verified using the publicly available databases at INRA (Anonymous 2010b), Triticarte (Anonymous 2010c, d) and Grain Genes (Anonymous 2010e, f).

QTL analysis was performed using MapQTL[®] 5.0 (Van Ooijen 2004) using the interval mapping (IM) option for QTL position detection, followed by MQM (Multiple QTL Model) after cofactor selection either by automatic cofactor selection (ACS) or manual investigation of the marker alignment on the linkage groups where the peaks of IM QTLs were detected. Minimum significant LOD values

were calculated by 1,000 permutation tests to determine 5% probability thresholds for seedling and adult plant stage experiments. The Excel formula option was used for Bartlett's χ^2 tests to determine the homogeneity of replication error variances enabling QTL analyses with average or individual replicate disease scores (Chu et al. 2010; Friesen et al. 2009). The QTL profiles were drawn with MapChart 2.2 software (Voorrips 2002).

The explained variance (%) of a detected QTL strongly depends on the size of a tested population. For instance, the probability of detecting a QTL that explains 10% of the total variance in a population of 200 individuals is 0,8 (Van Ooijen 2004), but it decreases almost linearly with smaller populations (Charmet 2000; Cornforth and Long 2003; Dupuis and Siegmund 1999; Knapp et al. 1990; Van Ooijen 1992). Here, the size of the Apache/Balance population was limited ($N = 91$). To increase the probability of QTL detection: (a) a wide range of isolates was used to screen

the parents, and a subset of eight highly distinctive isolates was selected for a pre-screening that was followed by tests with five of these isolates in subsequent replications; (b) the most recent release of DArT markers was used, (DArT marker V.3) that increased the genome coverage from 1,497 to 3431 cM, which strongly contributed to QTL detection; and (c) three replicated data sets were used for final QTL analysis that was preceded by Bartlett's test for homogeneity of these replicates.

Results

Mapping

A total of 962 polymorphic markers between cvs. Balance and Apache, including 169 SSR and 793 DArT markers (231 and 562 DArT markers of polymorphic chip versions 2.3 and 3, respectively), were used for mapping. A genetic map with 36 linkages group was constructed, containing 786 DArT and SSR markers (428 and 205 DArT markers of V3 and V2.3, respectively; as well as 153 SSR markers) covering 3,431 cM of the total wheat genome. A total of 176 markers (134 and 26 DArT markers of V3 and V2.3, respectively; plus 16 SSR markers) were excluded from mapping due to marker similarity (109 loci), significant segregation distortions or unreliable DArT scores (67 loci).

Isolate selection and QTL analyses for seedling resistance to septoria tritici blotch

Disease development in all seedling assays was excellent with maxima of 100% *N* and 83% *P* on the susceptible checks. The field evaluations were prone to strong environmental fluctuations, but resulted in adequate STB levels in 2007 and 2008 at both locations. The initial screening of parental cvs. Apache and Balance with 30 *M. graminicola* isolates showed a clear contrast ($P = 0.05$) with 15 isolates (Tables 1, 2). Nine isolates differentiated the parents for *N*, and 12 showed significant differences for *P*. Finally, isolates, IPO87016, IPO92034, IPO323, IPO98022, IPO89011 and IPO98094, as well as IPO95036 and IPO98046, were selected for a single replicated pre-screening of the DH lines. QTL analysis with *P* phenotypic data resulted in five significant QTLs on chromosomes 3AS, 1BS, 6DS and 7D (7DS/7DL switch) with higher LOD values than the threshold (LOD = 3.5) that was determined by permutation test at $P = 0.05$ (Fig. 1; Table 3). The highest LOD values per QTL were obtained with isolates IPO323, IPO98022, IPO98046 and IPO87016 (Table 4); hence, these isolates were selected, along with IPO89011 that also detected a major QTL on chromosome 6DS, to complete the data set with two additional replications.

Not all isolates detected all QTLs, which underscores the specificity in the *M. graminicola*–wheat pathosystem. The results clearly show that *P* is the most efficient parameter for QTL detection as nine QTLs were detected using this parameter compared to three for *N* (Table 3). Isolates IPO323 and IPO87016 specifically detected the 3AS and 1BS QTLs, respectively. With the exception of IPO87016, all isolates detected the 6DS QTL. The 7D QTLs were detected by isolates IPO98022, IPO89011 and IPO98046, but the genomic position of the associated marker as not consistent. Moreover, despite the fact that some isolates did not show a significant difference between both parents, DH analyses detected QTLs for *N* and/or *P*. For instance, IPO98046 induced a non-significantly different *P* level in both parents (Table 2), but in the DH analysis it detected the QTLs on 6DS and 7D. The 7D QTL, however, was not consistent in all replications (7DS or 7DL). Isolate IPO323 did not differentiate the parents for *N*, but still detected the 3AS QTL in the DH analysis. *NLP* data enabled the detection of more QTLs than *N*, but *PLP* reduced their number compared to *P* (not shown). *NLP* and *PLP* also detected two additional minor QTLs with LODs of 4.8 and 3.9 on chromosomes 5A and 2B, respectively (not shown).

The details of the mapped QTLs in seedling experiments are shown in Table 4. The LOD values and explained variances vary substantially with the applied isolates and also with the presence of additional QTLs. For instance the 6DS QTL explains only approximately 10% of the observed variation in the presence of the 3AS QTL in tests with IPO323, but close to 68% in the presence of the 7DL QTL in tests with IPO89011. In tests with isolate IPO98046, both the 6DS and 7D (7DS/7DL switch) QTLs explain about 20% of the observed variation. Remarkably, in the case of the resistance to isolate IPO98046, the 6DS QTL is expressed in all replications, whereas the QTL on 7DS is identified in two of the replications (Table 4).

Since multiple QTLs in the Apache/Balance DH population (Tables 3, 4) were detected, additional analyses of the interaction between these QTLs were performed (Figs. 2, 3). Isolate IPO323 detected the 3AS and 6DS QTLs. Four groups that significantly differed in *P* were generated by averaging the *P* levels for all DH lines with and without the resistant and susceptible alleles of DArT marker *wPt-0836* and flanking SSR markers, *Xgpw5176-Xgpw3087*. Marker *wPt-0836* is present in cv. Apache and diagnostic for its susceptibility. The absence of the resistance alleles from both parents resulted in 39% of *P*. The presence of the resistance alleles of the flanking markers linked to the 6DS QTL reduced it to 14%, but without the DArT marker *wPt-0836* (the QTL on 3AS), the average of *P* dropped to just 1%. Accumulation of both resistance alleles associated with the 6DS and 3AS QTLs did not significantly lower *P*. Hence, the 3AS QTL is epistatic over the 6DS QTL in

Table 2 Screening with 30 *Mycosphaerella graminicola* isolates from diverse origin resulted in significant differences (Δ) between the parental cvs. Apache and Balance (* $P = 0.05$, labeled)

Isolate	Necrosis %			Pycnidia %		
	Apache	Balance	Δ	Apache	Balance	Δ
IPO00003	90	65	ns	7	5	ns
IPO00005	3	8	ns	0	1	ns
IPO02159	84	89	ns	0	20	*
IPO02166	62	33	ns	2	11	ns
IPO86013	91	77	ns	23	25	ns
IPO86022	6	27	ns	0	0	ns
IPO86068	4	57	*	1	2	ns
IPO87016	10	90	*	0	51	*
IPO88004	98	71	*	18	14	ns
IPO88018	13	97	*	1	14	*
IPO89011	54	15	ns	23	1	*
IPO90006	16	15	ns	0	2	ns
IPO90015	26	58	ns	4	7	ns
IPO92004	18	85	*	0	16	*
IPO92034	30	84	ns	0	27	*
IPO94218	5	22	ns	0	4	*
IPO95036	52	79	ns	8	31	ns
IPO95052	16	2	ns	0	0	ns
IPO95054	16	80	*	0	4	*
IPO99015	3	98	*	0	23	*
IPO323	100	96	ns	25	0	*
IPO94269	100	100	ns	13	19	ns
IPO98022	<i>100</i>	86	*	32	8	*
IPO98046	100	100	ns	24	44	ns
IPO98047	100	100	ns	16	10	ns
IPO98094	96	100	ns	32	<i>10</i>	*
IPO052461	100	98	ns	0	0	ns
IPO052462	100	100	ns	0	0	ns
IPO052463	100	58	*	0	0	ns
IPO052464	100	96	ns	0	0	ns

(Bold) Resistance source cv. Apache, (Italic) Resistance source cv. Balance

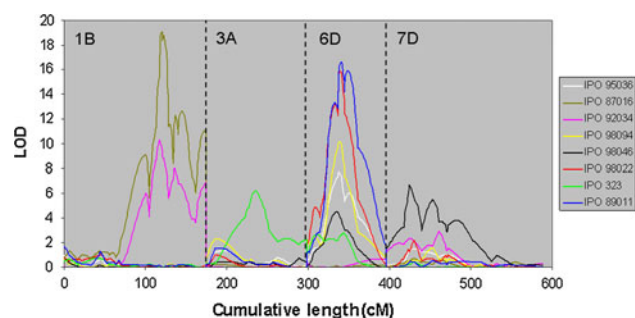


Fig. 1 Interval mapping LOD profile of the Apache/Balance DH mapping population using eight *Mycosphaerella graminicola* isolates in a pre-screening test (P)

the analysis with isolate IPO323. In tests with isolates IPO98022 and IPO89011, the 6DS QTL had a larger effect than the 7D QTL (7DS/7DL switch), but the presence of both

QTLs lowered P to 7%. This shows that 6DS and 7D had an additive effect, but the additive effect of the former QTL is much stronger as it has a higher LOD value. This was also shown for tests with isolate IPO98046 where the individual QTLs contributed equally to disease reduction, but the combination of both QTLs minimized the disease level. Eventually, the accumulation of four QTLs in the Apache/Balance DH population for average STB levels over all used isolates was tested, which clearly demonstrated that the pyramiding of the associated markers gradually and significantly reduced disease levels (Fig. 3).

Detection of QTLs associated with resistance to STB in the adult plant stage

Due to field size limitations, the Apache/Balance DH population was only tested with isolate IPO323 in both

Table 3 Summary of detected quantitative trait loci for necrosis (*N*) and pycnidia (*P*) in the Apache/Balance mapping population with five *Mycosphaerella graminicola* isolates

Chromosomal position	IPO 323	IPO 98022	IPO89011	IPO98046	IPO 87016
3AS	<i>N P</i>				
6DS	<i>P</i>	<i>N P</i>	<i>P</i>	<i>P</i>	
7D ^a		<i>P</i>	<i>P</i>	<i>P</i>	
1BS					<i>N P</i>

^a The QTLs detected on 7D vary over isolates. Isolate IPO98022 detected a QTL on 7DS, while IPO89011 detected a QTL on 7DL and isolate IPO98046 detected QTLs on both 7DS and 7DL

Table 4 Quantitative trait loci (QTLs) associated with necrosis (*N*) and pycnidia development (*P*) in the Apache/Balance DH population after inoculation with five *Mycosphaerella graminicola* isolates in the seedling stage

Isolate	Closest marker	Chromosome position	Phenotypic data set ^a	Resistance source	<i>N</i>			<i>P</i>		
					PD ^b (cM)	LOD	Exp. (%)	PD (cM)	LOD	Exp. (%)
IPO323	<i>wPt-0836</i>	3AS	R1	Balance	0	12.2	46.1	1	7.3	27.7
			R2		1	25.5	73.7	1	11.1	39.7
			R3		1	25.6	73.1	1	10.8	38.7
	<i>Xgpw5176-Xgpw3087^c</i>	6DS	R1	Balance				3.2–5	3.6	12.7
			R2					0.3–8	3.1	8.9
			R3					4.3–4	3.5	11
IPO98022	<i>Xgpw5176-Xgpw3087^c</i>	6DS	R1	Balance	6.3–2	6.4	30.4	5.3–3	16.3	47
			R2		8.3–0	5.4	21.6	5.3–3	13.1	47.4
			R3		0.3–8	4.4	18.8	5.3–3	12.3	48
	<i>Xgwm111</i>	7DS	R1	Apache				1.1	6.2	11.8
			R2					0	5.2	11.2
			R3					0	2.2 ^d	5.9
IPO89011	<i>Xgpw5176-Xgpw3087^c</i>	6DS	Ave.	Balance				5.3–3	23.16	67.5
		7DL	Ave.	Apache				0	4.5	8
IPO98046	<i>Xgwm111</i>	7DS	R1	Apache				0	9.5	27.5
			R2					– ^e	–	–
			R3					0	6.2	20.8
	<i>Xgpw313</i>	7DL	R1	Apache				–	–	–
			R2					5	6.8	20.5
			R3					–	–	–
	<i>Xgpw5176-Xgpw3087^c</i>	6DS	R1	Balance				8.3–0	7	19
			R2					8.3–0	7.9	24.2
			R3					6.3–2	7.4	27.1
IPO87016	<i>wPt-2019</i>	1BS	R1	Apache				2	19.1	63.3
			R2					1	21.1	68.3
			R3					0	17.8	59.3
			Ave.		1	21.11	67.3			

^a R1, R2 and R3 represent first, second and third replicate data. QTL analysis was performed on averaged data (Ave) when Bartlett's χ^2 test indicated nonsignificant phenotypic variation over replicates, otherwise replicates were processed individually

^b PD, QTL peak distance in cM

^c Flanking markers

^d Not significant, but consistent QTL position

^e No QTL detected

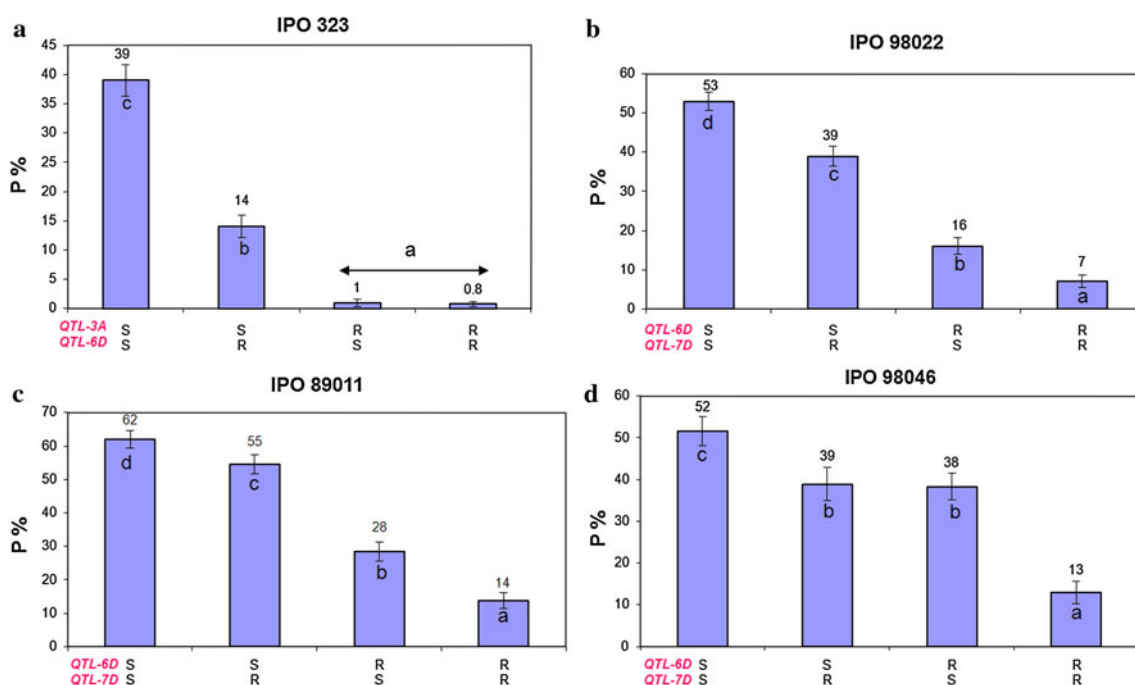


Fig. 2 Various interactions between QTLs in the Apache/Balance DH mapping population detected by single isolates controlling *P*. SS, individual DH lines merely carrying susceptibility alleles of two markers associated with QTLs; RS and SR, individual DH lines carrying a resistance allele of a marker linked to one QTL and a susceptibility allele of a marker of another QTL; RR, individual DH

lines with both resistance alleles. Same letters in the columns indicate not significantly different *P* values ($P = 0.05$). **a** Epistatic effect of the major QTL-3AS over the minor QTL-6DS detected by IPO323; **b** and **c** QTLs detected by IPO98022 and IPO89011, respectively, show a mutual additive effect; **d** Additive interaction between two QTLs with almost equal LOD scores

years. The weather conditions for STB development were conducive in both years, resulting in differentiating STB levels (quantified by the average severity of DH lines with/without 3AS associated DArT markers and with/without

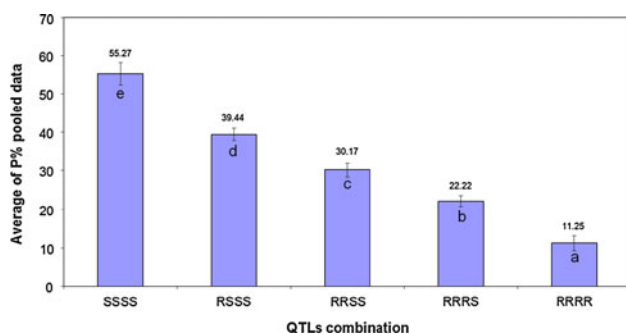


Fig. 3 Stacking effect of individual QTLs that were detected in the Apache/Balance DH mapping population. Overall resistance to the five employed *M. graminicola* isolates (*P*) significantly ($P = 0.01$) increased with the number of QTLs in DH lines. SSSS and RRRR, representative of individual DH lines merely carrying markers of susceptibility or resistance alleles associated with QTLs on chromosomes 3AS, 6DS, 7DS (only the most common marker *Xgwm111*) and 1BS, respectively. RSSS and RRRS, individual DH lines with combinations of one resistance allele linked to a QTL and three susceptibility alleles or vice versa. RRSS, individual DH lines carrying two QTLs associated with resistance and two QTLs associated with susceptibility

6DS flanking SSR markers that were identified in the seedling stage with isolate IPO323). QTL analyses revealed three QTLs on chromosomes 3AS, 2DS and 6DS that were associated with STB resistance (Table 5; Fig. 4). The 3AS and 6DS QTLs were also detected at the seedling stage. The former QTL was consistently expressed at both locations in both years, but the latter QTL was only detected in 2008 at the Serasem location and, similar to the seedling analyses, explained a lower percentage of the observed variation. Interestingly, the 2DS QTL was exclusively and consistently detected throughout all adult plant tests, but was also significantly correlated with earliness (-0.48 and -0.25 , $P = 0.05$ at Florimond Desprez and Serasem, respectively), tallness (-0.36 , $P = 0.05$ at Serasem) and resistance to FHB (Fig. 4e). Subsequent regression analyses that fitted means of logit-transformed STB values on earliness and tallness left no residual STB resistance effect for the 2D locus ($P = 0.359$).

Discussion

The present data show that both cvs. Apache and Balance contributed specific resistance to the DH population. The resistance in both parents could be easily differentiated using the 30 *M. graminicola* isolates panel and enabled the

Table 5 Quantitative trait loci (QTL) associated with resistance to STB evoked by inoculations with *Mycosphaerella graminicola* IPO323, earliness and tallness in the adult plant stage under field conditions

Location	Plant tallness																						
	Flag leaf lesion				Earliness				Plant tallness														
	Seasem 2007	Serasem 2008	Florimond Desprez 2008	Serasem 2008	Florimond Desprez 2008	Serasem 2008	Florimond Desprez 2008	Serasem 2008	Florimond Desprez 2008	Serasem 2008	Florimond Desprez 2008												
Closest marker	Chromosome	PD ^a	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %	PD	LOD	Exp %										
<i>Xgpw332b</i>	2DS	2	8.9	30.9	6	3.5	7.8	11.9	6.7	30.9	5	24	77.5	3	27.3	79.3	6	5.6	24.8	7	6.56	23.3	
<i>wPt-0797</i>	3AS	0	6.2	19.1																			
<i>wPt-0836</i>	3AS				1	14.2	35.6	1	8.6	28.6													
<i>Xgpw5176-Xgpw3087^b</i>	6DS				8.3-0	5.9	12.5																

^a PD, QTL peak distance in cM^b Flanking markers

selection of multiple isolates with significant differences that increased the detection of QTLs and helped to understand both the specificity of and interactions between these QTLs. So far, only 17 resistance genes and QTLs have been reported to STB (Arraiano et al. 2007; Chartrain et al. 2009; Goodwin 2007; Tabib Ghaffary et al. 2011, submitted to TAG) and there is a clear need for an extended arsenal of resistance genes to support resistance breeding. The QTL on chromosome 6DS is a new resistance gene, as no other *Stb* gene has been mapped to this chromosome, except for the erroneous location of *Stb3* (Adhikari et al. 2004) that was later correctly assigned to chromosome 7AS (Goodwin 2007). Hence, the 6DS QTL that was detected in the French winter wheat cv. Balance with four *M. graminicola* isolates and flanked by the SSR markers *Xgpw3087* and *Xgpw5176* is associated with a new resistance gene to STB that is designated as *Stb18*.

This is an isolate-specific resistance gene that was detected with the French *M. graminicola* isolates, IPO98 022 and IPO98046, and with the Dutch isolates, IPO89011 and IPO323. Isolate IPO89011 detected *Stb18* at the seedling stage, whereas IPO323 identified it in both the seedling and adult plant stage. IPO89011 is also avirulent on *Stb9* (Chartrain et al. 2009) and *Stb5* (Arraiano et al. 2001b), confirming the presence of multiple avirulence factors in *M. graminicola* isolates. Isolate IPO87016 from Uruguay is specifically virulent to *Stb18* as no QTL other than the IBS QTL was detected with this isolate, which was also confirmed by additional phenotyping assays. In the adult plant stage, *Stb18* was detected only in 2007, but this is most likely due to the epistatic effect of the QTL on chromosome 3AS. All other QTLs also demonstrated gene-for-gene interactions that were operational in the *M. graminicola*-wheat pathosystem (Brading et al. 2002). Earlier findings that *P* rather than *N* is a reliable disease parameter (Kema et al. 1996a) are supported by the current data, as *Stb18* was only detected once for *N* but multiple times for *P*. Previously, Kema et al. (1996a) concluded that *N* and *P* are under different genetic control, which is in accordance with the current MapQTL analyses.

The publicly available map databases show that the flanking markers of *Stb18* on 6DS, *Xgpw3087* and *Xgpw5176*, have also been mapped on chromosomes 6A and 2D, respectively. However, in the Apache/Balance population, these markers were linked with *Xgpw4440*, *Xgwm325*, *Xgpw4350*, *Xgpw43* and *Xgm469*, which are positioned on chromosome 6DS in the aforementioned linkage map databases. In the mapping process, the marker alignment of chromosome 6DS was sorted by an LOD score of 4, indicating a 10,000-fold higher likelihood of linkage. It is therefore concluded that *Stb18* and its closest flanking markers, *Xgpw3087* and *Xgpw5176*, are mapped on chromosomes 6DS.

confirmed that IPO87016 is avirulent on TE9111 (Tabib Ghaffary et al. unpublished data) that is reported to carry *Stb11*, *Stb6* and *Stb7*, which are mapped on chromosomes 1BS, 3AS and 4AL, respectively (Chartrain et al. 2005c). The isolate IPO87016 is virulent on *Stb6*, but avirulent on *Stb7* (Tabib Ghaffary et al. unpublished data). Therefore, the observed resistance in cv. Apache can be due to *Stb7* or *Stb11*. As the only detected QTL was positioned on chromosome 1BS and not on chromosome 4AL, we conclude that the QTL in cv. Apache represents *Stb11*, which was also confirmed by map comparison and additional phenotypic data. The QTL associated with *Stb11* in the Apache/Balance population is linked to DArT marker *wPt-2019* that can be used in addition to *Xbarc008* as an alternative for marker-assisted selection.

The QTL on chromosome 3AS is associated with DArT marker *wPt-0836* (v2.3). This marker is clustered with *wPt-2478* that is also mapped in the Avalon/Cadenza reference map (Anonymous 2010d) close (2.1 cM) to marker *Xgwm369* that was determined as a closely linked marker of *Stb6* (Brading et al. 2002). This gene confers resistance to isolate IPO323 and is prevalent among a worldwide set of cultivars and breeding lines (Arraiano and Brown 2006; Chartrain et al. 2005b; Eriksen et al. 2003). As no other gene has been mapped on chromosome 3AS in the Apache/Balance population, the 3AS QTL must represent *Stb6* in cv. Balance. Additional evidence is provided by screening with the other isolates that did not detect the 3AS QTL and are all virulent on cv. Shafir that carries *Stb6* (Tabib Ghaffary et al. unpublished data). Unfortunately, the DArT marker *wPt-0836* cannot be used for detecting *Stb6*, as it is associated with susceptibility to isolate IPO323 in cv. Apache.

Adhikari et al. (2004) and Arraiano et al. (2001b) have reported *Stb4* and *Stb5* on chromosome 7DS, respectively, and linkage with SSR marker *Xgwm111*, which is recognized as a specific marker for *Stb4* (0.7 cM). This marker is also present on the Apache/Balance 7D linkage group and is associated with QTLs that were detected with isolates, IPO98046 and IPO98022. IPO98046 is avirulent on cv. Tadinia (Tabib Ghaffary et al. unpublished data) that carries *Stb4* and *Stb6* (Arraiano and Brown 2006; Chartrain et al. 2005b), but virulent on cv. Shafir that carries *Stb6* (Brading et al. 2002, Tabib Ghaffary et al. unpublished data). The present data confirm this observation, as IPO98046 did not, but IPO323 did detect *Stb6* on chromosome 3AS. Hence, the 7DS QTL that was detected with IPO98046 in cv. Apache seems identical to *Stb4*. *Stb5*, present in CS/synthetic 6x, is also reported on chromosome 7DS (Arraiano et al. 2001b). Despite that isolate IPO98022 is avirulent on CS/synthetic 6x and detected a QTL on chromosome 7DS that is linked to SSR marker *Xgwm111*, it is dissimilar with *Stb5* as this isolate is virulent on cv.

Tadinia that carries both *Stb4* and *Stb6* (Tabib Ghaffary et al. unpublished data). The QTL detected with IPO89011 is associated with DArT marker *wPt-1859* that is positioned amidst SSR markers on chromosome 7DL (Fig. 4d). No *Stb* genes have been mapped to this chromosome arm and, hence, cv. Apache carries one or more unknown *Stb* genes on chromosome 7DL that require further characterization.

Finally, the data show that accumulation of QTL-associated markers incrementally contributes to higher and broader levels of STB resistance. Chartrain et al. (2004, 2005a, c) thoroughly analyzed STB resistance in cvs. KK4500 and TE9111. They described several *Stb* genes in these cultivars and suggested that gene pyramiding might be an effective method of resistance breeding, but neither interactions between these genes nor phenotype/genotype associations were addressed. Still, KK4500 and TE9111 have relatively broad efficacy (Kema et al. 1996a, b). This was in accordance with our findings that *Stb* resistance gene accumulation is a valid strategy to breed for wide efficacy resistance in wheat to STB, as was also shown in many other breeding programs dealing with other crops and various single or multiple biotic stresses (Barloy et al. 2007; Song et al. 2009). Therefore, a detailed characterization of known and new *Stb* genes is indispensable and contributes greatly to their deployment in marker-assisted stacking strategies in commercial breeding programs.

QTL analysis in adult plants

The field experiments confirmed the presence of the 3AS and 6DS QTLs that were identified as *Stb6* and *Stb18*. The latter is inconsistently expressed in the presence of *Stb6*, which also provides mature plant resistance to specific *M. graminicola* isolates, as reported earlier (Arraiano and Brown 2006; Brading et al. 2002; Chartrain et al. 2005b). The new QTL on chromosome 2D was consistently and exclusively expressed in adult plants in both years at both locations. However, this QTL is strongly associated with earliness and tallness, and regression analyses did not show a significant residual effect on STB resistance. We are therefore reluctant to assign STB resistance to the 2D QTL and rather suggest that it indirectly influences STB resistance by regulating earliness and tallness that are known to affect STB severity (Arama et al. 1999; Arraiano et al. 2009; Simon et al. 2005). The associated SSR marker *Xgpw332* is also associated with *Rht8* and *Pp1* that are involved in the regulation of wheat tallness and earliness (Korzun et al. 1998; Worland et al. 1988; Anonymous 2010a). These physiological parameters also influence FHB resistance (Somers et al. 2003; Steiner et al. 2004). Interestingly, a QTL for FHB resistance was mapped on the same position in the Apache/Balance population.

Previously, Handa et al. (2008) identified a possible multidrug resistance associated protein (MRP) at this 2D chromosomal location that is involved in the wheat–Fusarium interaction. We tentatively conclude that the 2D QTL confers earliness/tallness in wheat and therefore indirectly contributes to multiple pathogen resistance.

This project showed that new *Stb* loci can still be identified in contemporary commercial wheat cultivars by using panels of carefully characterized *M. graminicola* isolates. Such screens also demonstrate the efficacy of *Stb* genes in various production environments and therefore contribute to STB resistance management.

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