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ORIGINAL PAPER

Cytogenetic analysis of the susceptibility of the wheat line Hobbit sib (Dwarf A) to *Septoria tritici* blotch

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Abstract *Septoria tritici* blotch, caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*), is one of the most important foliar diseases of wheat in much of the world. Susceptibility of host plants to septoria was investigated by cytogenetic analysis. A line of Hobbit sib (Dwarf A) in which translocated chromosome 5BS–7BS was nominally substituted by chromosome arms 5BS and 7BS from Bezostaya 1 had a much lower mean level of septoria than Hobbit sib itself. By the use of microsatellite markers, it was shown that the 5BS arm of this line had in fact been substituted by the homologous arm of Chinese Spring. Further investigation of substitution and nullitetrasomic lines demonstrated that chromosome arm 5BS of Hobbit sib possesses genes, which either promote susceptibility to septoria or suppress resistance. This chromosome arm has previously been shown to carry genes for resistance to yellow (stripe) rust and powdery mildew, implying a trade-off between resistances to these two diseases and to septoria in wheat breeding. Bezostaya 1 was found to have specific resistance to *M. graminicola* isolate IPO323, probably controlled by the gene *Stb6* on chromosome arm 3AS, present in numerous wheat cultivars. It also had partial resistance to septoria distributed over several chromosomes, which may explain the value of this cultivar as a source of septoria resistance.

Introduction

Septoria tritici blotch (STB) of wheat, caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*), has a considerable economic impact on production in major wheat growing areas worldwide (Goodwin et al. 2003). In the UK, for example, it has been the most serious foliar disease of wheat since the early 1990s (Hardwick et al. 2003; Pillinger et al. 2004). Control of STB relies to a large extent on the use of fungicides, but the high cost of chemical control as well as the recent discovery of resistance to strobilurin (QoI) fungicides (Fraaije et al. 2005) has raised interest in other forms of control. As a result, there is considerable interest in breeding and growing cultivars resistant to STB as a cost-effective means of controlling the disease.

In recent years, several major genes for resistance to *M. graminicola* have been identified and mapped (Somasco et al. 1996; Arraiano et al. 2001b, 2007; Brading et al. 2002; McCartney et al. 2003; Adhikari et al. 2003, 2004a, b, c; Chartrain 2004; Chartrain et al. 2005a, b) as well as quantitative trait loci conferring partial resistance (Eriksen et al. 2003; Chartrain et al. 2004a; Simón et al. 2004). A gene-for-gene relationship has been demonstrated for *Stb6* on wheat chromosome 3A (Brading et al. 2002), which is widespread in sources of resistance to STB worldwide (Chartrain et al. 2005c).

The wheat lines studied in this paper are Bezostaya 1, Hobbit sib and Cappelle Desprez. The Russian winter wheat cultivar Bezostaya 1 (Lutescens 17/Skorospelka 2) is one of the most widely grown wheat varieties in the world (Worland et al. 1998). It has high levels of durable resistance

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to yellow and brown rusts (Worland and Law 1986, 1991; Law and Worland 1996, 1997; Johnson et al. 2000) and has been used as a source of resistance to *M. graminicola* in wheat breeding programmes worldwide (Danon et al. 1982; Ackermann et al. 1995; Annone et al. 1995; Gilchrist et al. 1999). Bezostaya 1 has been reported to have resistance to a *M. graminicola* isolate from Israel, controlled by a small number of genes (Danon et al. 1982). In field trials in the UK, Bezostaya 1 was specifically resistant to the Dutch isolate IPO323, which is avirulent to *Stb6*, but not to four other isolates (Brown et al. 2001). It was also resistant to IPO323 in tests on seedlings in glasshouse conditions and in a laboratory detached leaf assay (Arraiano et al. 2001a). Hobbit sib, also known as Dwarf A, is a semi-dwarf winter wheat line which is closely related to cultivars grown commercially in the UK during the 1970s. It is susceptible to fusarium head blight (Buerstmayr et al. 1996), powdery mildew, yellow and brown rusts (Dugall et al. 2000). Hobbit sib is also susceptible to STB while the French winter wheat, Cappelle Desprez, is moderately resistant to all isolates of *M. graminicola* tested (Brown et al. 2001).

In hexaploid wheat, the development of intervarietal substitution lines has allowed the effects of individual whole chromosomes on characters of interest to be assayed. This method has been used to study the quantitative inheritance of resistance to fungal diseases (Grausgruber et al. 1997, 1999; Kerber and Aung 1999; Mentewab et al. 2000, Muranty et al. 2002; Liu et al. 2004), including STB (Arraiano et al. 2001b; Simón et al. 2001). Genes for adult plant resistance to yellow rust have been located to the translocated chromosome 5BS–7BS and to chromosome 2D (*Yr16*) of Cappelle–Desprez and to chromosomes 6B and 7D (*Yr18*) of Bezostaya 1 (Worland and Law 1986, 1991; Law and Worland 1996, 1997; Johnson et al. 2000). Genetic variation for other quantitative characters such as yield components (Miura et al 1992) and bread making quality (Turner et al. 2004) has also been determined using this technique.

Complete sets of substitution lines of chromosomes from Bezostaya 1 (Bez) into Hobbit sib (Hs) and Cappelle Desprez (CD) have been developed (Law et al. 1987; Law and Worland 1996). CD (Bez) substitution lines have been used to study the genetics of height, baking quality, disease resistance and copper toxicity tolerance (Krattiger et al. 1996; Law and Worland 1997; Ganeva et al. 2003) while Hs (Bez) substitution lines have been used to study adult plant resistance to yellow rust (Worland and Law 1991).

This paper reports experiments conducted on lines with chromosomes of Bez substituted into two different backgrounds, Hs and CD, to identify the chromosomal locations of genes promoting resistance and susceptibility to STB. Both isolate-specific and partial, isolate non-specific-resistance were investigated.

Materials and methods

Disease trials

Plant material

Hs, Bez, CD, 21 substitution lines of chromosomes from Bez into Hs, 21 substitution lines of chromosomes from Bez into CD (Law and Worland 1996), Chinese Spring (CS) and the CS nullitetrasomic lines CS-N5BT5A and CS-N5BT5D were grown in polytunnel trials of adult plants. Duplicate lines have been developed for each chromosome substitution. CD and Hs have a reciprocal translocation with respect to the CS and Bez karyotypes, with 5BL–7BL and 5BS–7BS chromosomes. The CD (Bez5BS–7BS) substitution line, for example, has a chromosome 5B which should consist of CD 5BL and Bez 5BS and a chromosome 7B with CD 7BL and Bez 7BS (Law and Worland 1996).

A group of lines, CD (Bez2D), Hs (Bez3A), CD (Bez3A), CD (Bez4A), CD (Bez5BS–7BS) and Hs (Bez5BS–7BS), as well as Bez, CD and Hs, were tested in glasshouse seedling trials to examine further some of the results of the adult plant trial.

The CD (Bez) substitution series has been checked using microsatellite markers to detect the accuracy of the chromosomes substituted (Korzun et al. 1997). Markers on chromosomes 4D and 6D were not polymorphic between Bez and CD so the presence of the Bez chromosomes in these substitution lines could not be confirmed. Errors were found in some other lines, but where Korzun et al. (1997) reported one of the duplicate substitution lines as erroneous, the correct line was tested in the polytunnel trials. Where both duplicate lines were correct, the one with the greatest number of backcrosses (Korzun et al. 1997) was tested.

Disease tests

Adult plants were grown in pots in a polytunnel and tested for responses to STB by the method of Arraiano et al. (2001b), in which plants are sprayed with a suspension of *M. graminicola* spores using a knapsack sprayer calibrated to apply a known volume per unit area, resulting in an application rate of spores of 3×10^8 m^{-2}. They were also tested as whole seedlings by the method of Arraiano et al. (2001a), in which 12- to 15-day-old plants are sprayed with a spore suspension (10^7 ml^{-1}) to run-off. Two *M. graminicola* isolates were used, IPO323 and IPO94269. These isolates were selected because they have been used in genetic analysis of the fungus (Kema et al. 2002) and because, in previous tests, Bez was resistant to IPO323 and susceptible to other isolates, including IPO94269 (Arraiano et al. 2001a; Brown et al. 2001). *M. graminicola* isolates were cultured and sprayed onto plants as described by Arraiano et al. (2001b).

Experimental design

All experiments were conducted in randomised complete block designs in layouts generated with the Experimental Design Generator and Randomiser (EDGAR, Brown 2000). Adult plants were tested in two trials in 1998 and 1999 for the substitution lines of chromosomes from Bez into Hs and two trials in 1999 and 2003 for the substitutions from Bez into CD; the tests on the two populations in 1999 were combined in a single trial, providing a point of comparison between them. In each trial, there were two blocks per isolate and five randomised plots per block. Each plant line was represented once in each plot; in 1999, each plot contained plants of both series of substitution lines. Seedlings were tested once with five replicate plants of each line per isolate.

Data analysis

In polytunnel trials of adult plants, disease was scored on flag leaves as the percentage leaf area covered by lesions bearing pycnidia (Arraiano et al. 2001b). In seedling tests, the percentage leaf area covered by lesions bearing pycnidia was scored four times between 21 and 29 days after inoculation (Arraiano et al. 2001a), allowing the area under the disease progress curve (AUDPC, Shaner and Finney 1977) to be calculated from the combined scores. Data were analysed by generalised linear mixed modelling of binomial proportions (GLMM, Welham 2002). The significance of fixed effects was tested by F tests of Wald statistics (Elston 1998). For adult plants the variate analysed was the percentage leaf area covered by pycnidia. The variate analysed for seedling trials was the AUDPC as a proportion of the maximum AUDPC (maxAUDPC, calculated assuming a score of 100% on every day the test was scored) (Arraiano et al. 2001b). The ratio of AUDPC to maxAUDPC was analysed with a binomial distribution and a logit link function. The analyses were done using the statistical package GenStat® for Windows Release 8.2 (Genstat 5 Committee 2005) (VSN International, Oxford, UK).

Analysis of the Xgwm369 microsatellite marker locus

A wheat microsatellite marker specific to chromosome 3A, *Xgwm369* (Röder et al 1998), was used to test the authenticity of the duplicates of inter-varietal chromosomal substitution lines CD (Bez2D), CD (Bez3A), Hs (Bez2D) and Hs (Bez3A), together with lines Bez, CS, CD, Hs and Flame, the cultivar in which *Stb6* was identified (Brading et al. 2002). DNA was extracted from leaves of 16-day-old seedlings using the CTAB method (Saghai-Maroof et al. 1984) and its concentration measured with a spectrophotometer (Ultrospec 2000, Pharmacia Biotech). The PCR reaction was done as described by Röder et al (1998) and products visualised on a silver-stained acrylamide gel as described by Bryan et al. (1997).

Molecular mapping of Hobbit sib (Bezostaya 5BS–7BS)

Plant material

Substitution lines Hs (Bez 5BS–7BS), CD (Bez 5BS–7BS), the parents Hs, CD and Bez, CS and the nullitetrasomics CS-N5BT5A and CS-N5BT5D were tested with a set of 26 SSR markers mapping to chromosome 5B and 13 SSR markers mapping to chromosome 7B (Table 1), to test if Bez chromosome 5BS–7BS was indeed present in the substitution lines.

Microsatellite analysis

DNA from the eight lines was extracted from 8-day-old seedlings using The DNeasy® 96 Plant Kit (Qiagen). The PCR was done using HotStarTaq Master Mix (Qiagen) (2005) conditions. Fluorescent primers were labelled with 6-FAM, NED, PET, VIC or HEX dye labels. Fluorescent PCR products were separated on an ABI377 DNA sequencer and their sizes measured using Applied Biosystem's GeneScan and Genotyper fragment analysis software (Applied Biosystems, Foster City, CA, USA).

Results

There were significant differences in the levels of STB on adult plants of lines with different substituted Bez chromosomes (S). However, the effects of Bez chromosomes depended significantly on interactions between the chromosome substitution and the isolate (I; IPO323 or IPO94269) or the recipient cultivar (C; CD or Hs) (S, S.I and S.C terms in Table 2). The main contribution to the S.C interaction came from the line carrying Bez chromosome 5BS–7BS (Fig. 1). The substitution line carrying this translocation was resistant to both isolates in the Hs (Bez) series but heavily diseased in the CD (Bez) series (Fig. 1).

The contrast between the effects of substituting CD and Hs chromosome arms 5BS and 7BS for the homologous chromosome arms of Bez prompted an investigation to verify the reliability of the substitution lines containing these translocated chromosome arms. Study of SSR alleles in these lines showed that in Hs (Bez 5BS-7BS), the Hs chromosome arm 5BS was not substituted by the homologous Bez arm in the 5BS segment. Instead, the 5BS–7BS arm of

Table 1 SSR markers mapping to 5B and 7B, with position on the Komugi composite wheat map (in cM; http://shigen.lab.nig.ac.jp/wheat/komugi/maps/markerMap.jsp) and allele sizes (bp) for lines Hobbit sib, Cappelle Desprez, Bezostaya, Chinese Spring, Hs (Bez5BS–7BS) and CD (Bez5BS–7BS)

Marker	Chromosome	Position (cM)	Line					
			Hs	CD	Bez	CS	Hs (Bez 5BS-7BS)	CD (Bez 5BS-7BS)
Xgwm443	5BS	11.58	129	121	133	140	129[a]	121[b]
Xwmc149	5BS	19.39	179	179	178	178	179[a]	178[c]
Xgwm234	5BS	20.57	235	226	237	239	235[a]	237[c]
Xwmc376	5BS	29.09	234	234	234	208	234[a,c]	234[b,c]
Xwmc386	5BS	29.09	Null	Null	178	174	174[d]	178[c]
Xwmc363	5BS	29.57	120	120	120	116	116[d]	120[b,c]
Xbarc216	5BS	36.81	115	115	103	119	119[d]	103[c]
Xbarc340	5BS	38.84	239	239	237	237	237[d]	237[c]
Xgwm544	5BS	41.41	Null	Null	176	168	168[d]	176[c]
Xgwm133	5BS	43.99	138	133	129	129	129[d]	129[c]
Xgwm66b	5BS	44.08	Null	Null	158	154	154[d]	158[c]
Xgwm540	5BS	44.63	121	121	128	126	126[d]	128[c]
Xbarc4	5BS	44.99	203	203	173	164	164[d]	173[c]
Xbarc109	5BS	46.65	243	243	240	237	237[d]	240[c]
Xwmc616	5BS	–[e]	145	145	149	162	162[d]	149[c]
Xgwm67	5BS	48.61	92	92	83	83	83[d]	83[c]
Xgwm371	5B	50.00	196	196	186	176	196[a]	196[b]
Xgwm213	5B	50.00	172	172	166	189	189[d]	172[b]
Xgwm335	5B	51.00	217	217	211	235	235[d]	217[b]
Xbarc74	5B	52.00	187	187	179	184	184[d]	187[b]
Xwmc537	5BL	52.00	Null	Null	202	193	Null[a]	Null[b]
Xwmc75	5BL	88.75	201	201	222	222	201[a]	201[b]
Xgwm408	5BL	108.06	153	153	185	181	153[a]	153[b]
Xwmx235	5BL	113.12	251	251	251	249	251[a]	251[b]
Xwmc28	5BL	125.49	210	212	212	206	210[a]	212[b]
Xbarc59	5BL	131.23	200	204	200	200	200[a]	204[b]
Xwmc338	7BS	10.00	Null	Null	144	Null	Null	144[c]
Xgwm537	7BS	20.00	216	216	225	215	225[c]	225[c]
Xgwm400	7BS	23.21	154	154	140	142	140[c]	140[c]
Xwmc476	7BS	28.36	217	217	209	209	209[c]	209[c]
Xgwm46	7BS	41.00	170	170	174	186	174[c]	174[c]
Xbarc72	7BS	42.00	187	187	185	185	185[c]	185[c]
Xbarc267	7BS	43.00	152	152	164	160	164[c]	164[c]
Xgwm297	7BS	44.00	161	*[f]	159	157	159[c]	159[c]
Xgwm43	7BS	48.00	185	183	179	177	179[c]	179[c]
Xbarc90	7BL	49.00	159	Null	162	162	159[a]	Null[b]
Xpsp3033	7BL	75.63	187	187	187	190	187[a,c]	187[b]
Xgwm577	7BL	105.10	155	169	134	169	155[a]	169[b]
Xbarc50	7BL	106.92	140	132	132	128	140[a]	132[b]

[a] Hobbit sib allele, [b]Cappelle Desprez allele, [c]Bezostaya allele, [d]Chinese Spring allele, [e]–marker not present in Komugi composite map, [f]missing data

this line includes a large segment of CS material with a smaller segment of Hs in the telomeric region (Table 1). The 7BS arm was correctly substituted by the Bez chromosome arm. This chromosome arrangement could have arisen because the Hs monosomic set used to develop the Hs (Bez) substitution series was developed from the original CS monosomic series (Law and Worland 1972). It appears that the 5BS–7BS translocation, present in Hs but

Table 2 Analysis of variance from generalised linear mixed modelling of septoria tritici blotch (*Mycosphaerella graminicola*) on wheat plants of substitution series Hobbit sib (Bezostaya 1) and Cappelle Desprez (Bezostaya 1)

Factor	*df*	Variance	Variance ratio	P^a
Year (Y)	2	167.1	77.7	***[b]
Substitution (S)	22	32.2	15.0	***
Isolate (I)	1	318.6	148.2	***
Cultivar (C)	1	0.7	0.3	NS
S.I	22	26.1	12.1	***
S.C	22	19.2	8.9	***
S.I.C	22	4.4	2.1	**
Y.I	2	270.7	125.9	***
I.C	1	1.8	0.8	NS
Y.I.Block	58	1.9	0.9	NS
Residual	1,811	2.2		

Isolates IPO323 and IPO94269 were inoculated onto adult plants in polytunnel trials. The variate analysed was percentage of leaf area with lesions bearing pycnidia, as a proportion of total leaf area

df numerator degrees of freedom

[a] Significance of variance ratio (*F* test): NS $P > 0.05$; **$0.01 > P > 0.001$; ***$P \leq 0.001$

[b] Comparison with year, isolate, block effect; other comparisons with Residual (between plants)

not CS, was not correctly replaced and a large CS segment remained in the proximal part of the 5BS arm of the Hs 5BS–7BS monosomic line, which was then passed on during the development of the Hs (Bez 5BS–7BS) line.

S.I interactions involved specific resistance to isolate IPO323. Bez was resistant to IPO323 but susceptible to IPO94269 while cultivars Hs and CD were susceptible to both isolates (Fig. 1). The S.I interaction arose from the Bez 3A substitution lines, which showed high levels of resistance to IPO323 but not to IPO94269, similar to those of Bez in both substitution series (Fig. 1).

Adult plants of lines CD (Bez2D) and CD (Bez4A) also had significantly less disease than CD with isolate IPO323 (Fig. 1). In the case of line CD (Bez2D), this was because the B duplicate line of CD (Bez2D) used in the adult and seedling trials contains the distal part of the Bez 3A chromosome arm, as indicated by the presence of the Bez allele of *Xgwm369* (Fig. 2), which is linked to *Stb6* that confers resistance to IPO323. The A duplicate line of CD (Bez2D) was susceptible to IPO323 in seedling trials (Table 3) and has the CD allele of *Xgwm369* (Fig. 2). By contrast, Line CD (Bez4A) was susceptible to IPO323 in seedling trials (Table 3), indicating that it does not express Stb6 resistance.

The effect of the recipient cultivar on the S.I interaction was small but significant (S.I.C term in Table 2). This was mainly due to the different responses to isolate IPO323 of lines with chromosomes 5BS–7BS or 2D of Bez in the two backgrounds (Fig. 1). When the data for lines CD (Bez 3A), Hs (Bez 3A), CD (Bez 2D) and Bez, with specific resistance to IPO323, were removed from the analysis, the S.I.C interaction effect was not significant. Although the remaining S.I effect was significant at $P = 0.03$, no individual chromosome contributed strongly to this interaction. The substitution main effect was significant ($P < 0.001$), mostly because of the contrasting effects of chromosomes 2D (61% IPO94269 infection) and chromosomes 5BS–7BS (25%) and 7A (28%). This suggests that, in general, replacement of the Hs and CD 2D chromosomes by Bez increases susceptibility to STB. Replacement of chromosomes 5BS–7BS and 7A of CD by Bez decreases disease levels. In the Hs series, replacement of 7A and 7BS by Bez decreases STB, as does replacement of 5BS by CS

Adult plants of CS and the two nulli-5B lines of CS had similar levels of STB with isolate IPO94269. This indicates that removal of chromosome 5B of CS and its replacement by a homoeologous group 5 chromosome does not affect susceptibility to a virulent isolate of *M. graminicola* like IPO94269. As CS has *Stb6* on chromosome 3A (Chartrain et al. 2005c), all three lines are resistant to IPO323.

Discussion

A gene promoting susceptibility to STB

One substitution line, Hs (Bez 5BS–7BS), had significantly less STB than the euploid line with both isolates in adult plant trials (Fig. 1) but not in seedling tests (Table 3). By contrast, CD (Bez 5BS–7BS) had similar disease scores to those on euploid CD with both isolates in both sets of experiments (Fig. 1; Table 3). This indicates that the 5BS-7BS substitution reduces STB in adult plants in the Hs series but not in the CD series.

In the CD series, 5BS-7BS has been almost completely correctly substituted by Bez, except for a small part of the telomeric end of 5BS, which has not been substituted (Table 1). As the Bez 7BS chromosome arm does not confer resistance to STB in CD (Bez 5BS–7BS), the fact that this arm has been substituted correctly into Hs (Bez 5BS–7BS) (Table 1) does not explain the resistance of the Hs substitution line.

An alternative hypothesis is that CS arm 5BS carries resistance to STB. However, euploid CS is susceptible to isolate IPO94269 (Fig. 1), although it is resistant to IPO323 because of *Stb6* on chromosome 3A (Chartrain et al. 2005c). This indicates that genes from CS chromosome arm 5BS do not confer resistance in the Hs (Bez 5BS–7BS) line. A critical test is provided by the nullitetrasomic lines, CS-N5BT5A and CS-N5BT5D. If CS arm 5BS carried resistance genes, the nulli-5B lines would be more susceptible

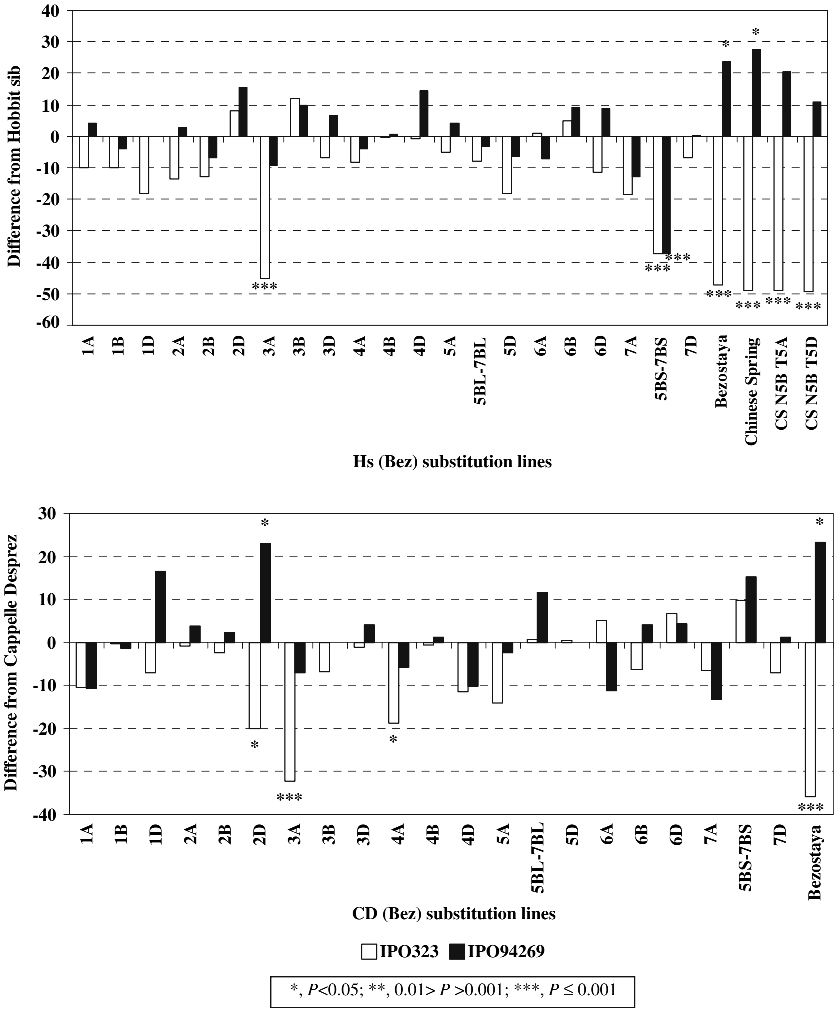

Fig. 1 Percentage area of flag (top) leaves of adult wheat plants covered by lesions bearing pycnidia of *Mycosphaerella graminicola*, the septoria tritici blotch pathogen of wheat. Deviations from euploid in **a** Hobbit sib (Bezostaya1) substitution series (Hobbit sib with isolate IPO323 = 50%; IPO94269 = 48%, **b** Cappelle Desprez (Bezostaya1) substitution series (Cappelle Desprez with isolate IPO323 = 38%; IPO94269 = 49%)

to STB than euploid CS. In fact, they have similar disease scores (Fig. 1), so it is concluded that genes from CS chromosome arm 5BS do not contribute resistance to the Hs (Bez 5BS–7BS) line.

As genes from CS and Bez in the substituted chromosome 5BS–7BS do not confer resistance, it is concluded that Hs carries a gene or genes promoting susceptibility to STB on chromosome arm 5BS, the removal of which increases the resistance of the Hs (Bez 5BS–7BS) substitution line. Such a gene might enhance susceptibility to STB, in which case removing it in the Hs (Bez 5BS–7BS) line would reduce levels of STB. Alternatively, it might be a gene, which suppresses resistance in Hs, so that substitution of chromosome arm 5BS of Hs by CS would nullify the effect of the suppressor, allowing expression of a previously inhibited resistance gene.

The former hypothesis is perhaps more likely as resistance is only observed in adult plants, not seedlings, and is effective against two *M. graminicola* isolates. Moreover, Hs has no known gene for resistance to STB (Arraiano et al. 2001a, Fig. 1; Table 3).

Genes promoting susceptibility

In a study of CS aneuploid lines, the short arms of all homoeologous group 5 chromosomes promoted adult plant resistance (APR) to both yellow rust and powdery mildew while the long arms carried genes either inhibiting resistance or promoting susceptibility to both diseases (Pink et al. 1983). Subsequent research on the two substitution series studied here, Hs (Bez) and CD (Bez), detected genes promoting adult plant susceptibility to yellow rust on chromosomes 4B, 5BL–7BL and 5D of Hobbit sib; in contrast, substitution of the 5BS–7BS translocated chromosome of both Hs and CD increased susceptibility to yellow rust (Worland and Law 1991; Law and Worland 1996). This indicates that chromosome 5BS–7BS has genes for APR to yellow rust in both Hs and CD. In CD, study of lines ditelosomic for 5BS and 7BS confirmed location of genes promoting susceptibility on the 5BS arm of CD (Law and Worland 1997). Recently, Boyd et al. (2006) reported mutations in Hs, which enhanced field resistance to rusts and powdery mildew at different growth stages.

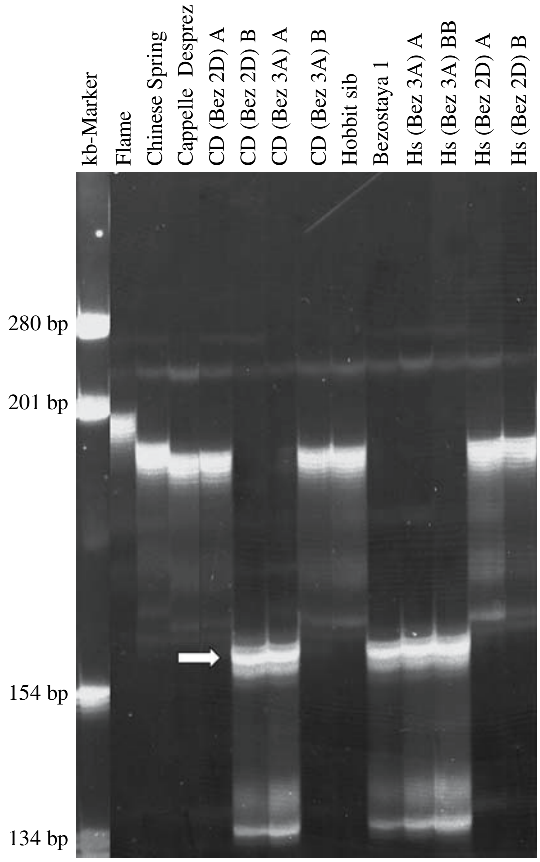

Fig. 2 Microsatellite gel showing alleles of the *Xgwm369* marker, closely linked to *Stb6* on chromosome 3A. The gel includes duplicate substitution lines of Cappelle Desprez (Bezostaya 1) and Hobbit sib (Bezostaya 1) for chromosomes 3A and 2D, recipient and donor varieties, Flame and Chinese Spring. It shows that CD (Bez2D) duplicate B, CD (Bez3A) duplicate A and both duplicates of Hs (Bez3A) have the segment of Bezostaya 1 chromosome 3A linked to *Stb6* (indicated by *arrow*)

Table 3 Mean percent leaf area of wheat seedling leaves covered by lesions bearing pycnidia of *Mycosphaerella graminicola* isolates IPO323 and IPO94269 in lines of substitution series Hobbit sib (Bezostaya 1) and Cappelle-Desprez (Bezostaya 1)

Lines	Isolates		
	Duplicate	IPO323	IPO94269
CD (Bez2D)	A	35[a]	NT
	B[b]	0***[c]	31
Hs (Bez3A)	A[b]	0***	56
	BB	4***	47
CD (Bez3A)	A[b]	0***	55
	B	52	43
CD (Bez4A)	A	68	33
	B[b]	62	45
Hs (Bez5BS–7BS)	A[b]	56	58
	CA	59	59
CD (Bez5BS–7BS)	A[b]	39	31
Bezostaya 1		0***	40
Cappelle-Desprez		56	41
Hobbit sib		54	68
Isolate mean		37	54

NT not tested

[a] Means over replicate plants and dates estimated by generalised linear mixed modelling

[b] Duplicate used in polytunnel trial of adult plants

[c] Lines with a significantly lower level of disease than CD or Hs. ***$P \leq 0.001$, Student´s *t* test

Disease susceptibility genes in plants are poorly understood compared to resistance genes. The *MLO* gene in barley is required for susceptibility to powdery mildew (*Blumeria graminis*) and its deletion or inactivation confers almost complete resistance to the fungus (Buschges et al. 1997). Several genes required for susceptibility to powdery mildew in *Arabidopsis thaliana* have also been identified (Vogel and Sommerville 2000; Vogel et al. 2002). In wheat, the *Tsn1* gene located on chromosome 5BL confers sensitivity to a toxin produced by tan spot (*Pyrenophora tritici-repentis*) and its absence results in insensitivity to this disease (Haen et al. 2004).

Suppressor genes

There are several reports of resistance to fungal diseases such as rusts and powdery mildew being inhibited by suppressor genes (Kerber and Green 1980; Kema et al. 1995; Ma et al. 1995; Zeller and Hsam 1996; Hanušová et al. 1997; Assefa and Fehrmann 2004). In most cases, the suppressors have specific interactions with particular resistance genes (Kema et al. 1995; Ma et al. 1995; Jørgensen 1996; Nelson et al. 1997; Ren et al. 1997). In the wheat cultivar Canthatch, removal of a suppressor gene on chromosome 7DL allowed expression of resistance to several races of stem rust but not to all those tested (Kerber and Green 1980). Here, the effect of the 5BS–7BS substitution appeared not to be resistance gene-specific, as it reduced susceptibility to both isolates tested, IPO323 (avirulent to *Stb6*: Brading et al. 2002) and IPO94269 (avirulent to *Stb5*: Arraiano et al. 2001b and *Stb10*: Chartrain et al. 2005a).

The resistance of Hs (Bez 5BS–7BS) was only effective in adult plants (Fig. 1; cf Table 3). In a study of wheat yellow (stripe) rust, some postulated suppressor genes were effective in seedlings but not in adult plants (Kema et al. 1995), an example of suppression activity, which is dependent on plant growth stage, though in the opposite direction to that reported here. In Canthatch, the suppressor gene reduced the resistance of seedlings to all rust races tested and that of adult plants to most races (Kerber and Green 1980). Hence the Hs (Bez 5BS–7BS) line might carry a suppressor of resistance but against this hypothesis, Hs itself is very susceptible (Fig. 1; Brown et al. 2001) and has no known gene for STB resistance.

Septoria-promoting genes in wheat breeding

Comparison of the data presented in this paper with published reports of susceptibility and suppressor genes indicate that the disease-promoting gene on chromosome arm 5BS of Hs could be of either type. However, a suppressor gene would operate by inhibiting the activity of a resistance gene. Not only is Hs susceptible to all *M. graminicola* isolates tested, both in seedling (Arraiano et al. 2001a) and adult plant trials (Brown et al. 2001), but no resistance gene has been identified in this line. It is therefore concluded that it is more likely that the 5BS arm of Hs carries a gene or genes promoting susceptibility, but the possibility that it has a gene suppressing resistance cannot be excluded on the basis of current data.

The 5BS arm of several important cultivars and lines used in wheat breeding, including Hs and CD, carries genes promoting durable APR to yellow rust and powdery mildew (Pink et al. 1983; Law and Worland 1997). Resistance to these two diseases has been a major priority for wheat breeders, not least in the UK. If this chromosome arm has been selected in breeding programmes for this reason, it would have promoted susceptibility to STB. This might explain why many of the most popular cultivars grown in the UK since the 1970s have been susceptible to STB (Arraiano and Brown 2006), although recombination would eventually break any linkage between genes promoting septoria and those reducing biotrophic fungal diseases.

Specific resistance gene

In field (Brown et al. 2001), polytunnel (Fig. 2) and whole seedling (Table 3) trials, Bez was specifically resistant to IPO323 but susceptible to IPO94269 while Hs and CD were susceptible to both isolates. Analysis of the chromosome substitution series of Bez in two different backgrounds, CD and Hs, indicated that the specific resistance of Bez to IPO323 is carried by chromosome 3A (Fig. 1). This strongly suggests that Bez has the resistance gene *Stb6*, located on the short arm of chromosome 3A, which confers specific resistance to IPO323 but not IPO94269. Among the other varieties now known to have *Stb6* is Kavkaz, a daughter of Bez (Chartrain et al. 2005c).

It is important to identify the types of resistance carried by known sources of resistance, such as that of Bez to *M. graminicola*, so that they may be used appropriately in breeding programmes. Here, we report that Bez has isolate-specific resistance to *M. graminicola* controlled by a gene (or genes) on chromosome 3A, which is probably *Stb6*. The resistance of Bez to a wide spectrum of isolates from Israel (Danon et al. 1982) had previously suggested this. Although the specific resistance of Bez is unlikely to be durable, appropriate marker assisted selection for the isolate-non-specific component of resistance could allow it to be used as a source of resistance in a breeding programme which aims to combine different sources of resistance to obtain more durable resistance to septoria tritici blotch.

Conclusion

This study is the first to report the existence of genes in wheat that suppress resistance or increase susceptibility to septoria tritici blotch. Hs is closely related to important current European wheat cultivars and the presence of a gene or genes suppressing resistance to STB or promoting susceptibility on chromosome 5BS–7BS, which carries genes for resistance to other fungal diseases, might partly explain why susceptibility to septoria tritici blotch increased so rapidly from the mid 1970s and why it is now widespread in modern European cultivars.

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