Theor Appl Genet (2001) 103:758–764

L.S. Arraiano · A.J. Worland · C. Ellerbrook
J.K.M. Brown

Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6*x*'

Received: 1 February 2001 / Accepted: 17 April 2001

Abstract Septoria tritici blotch, caused by the fungus *Mycosphaerella graminicola*, is currently the major foliar disease of wheat world-wide, and new sources of resistance and knowledge about the genetics of resistance are needed to improve breeding for resistance to this disease. Sears's 'Synthetic 6*x*' hexaploid wheat, derived from a hybrid of *Triticum dicoccoides* and *Triticum tauschii*, was resistant to 12 of 13 isolates of *M. graminicola* tested. Chromosome 7D of 'Synthetic 6*x*' was identified as carrying resistance to all 12 isolates in tests of seedlings of inter-varietal chromosome substitution lines of 'Synthetic 6*x*' into 'Chinese Spring' and to two isolates in tests of adult plants. A septoria tritici blotch resistance gene, named *Stb5*, was identified using the *M. graminicola* isolate IPO94269 and mapped on the short arm of chromosome 7D, near the centromere, in a population of single homozygous chromosome-recombinant lines for the 7D chromosome.

Keywords Septoria tritici blotch · *Mycosphaerella graminicola* · Synthetic hexaploid wheat · Disease resistance · Genetic mapping · *Stb5*

Introduction

Septoria tritici blotch, caused by the ascomycete fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*), is an important disease in all major wheat-growing areas. The disease has the potential to cause considerable reductions in total yield throughout the world (Scharen 1999) and is an important target for fungicide applications (Cook 1999). Owing to the rising cost of fungicides and the difficulty of controlling the disease where chemical control is expensive, farmers are increasingly interested in varieties which combine resistance to septoria tritici blotch with high agronomic value. New sources of resistance are required in order for this goal to be achieved as few varieties currently available have adequate resistance.

Compared to other diseases of wheat, there has been little progress in genetic analysis of resistance to septoria tritici blotch. Reports on the mode of inheritance of resistance to *M. graminicola* have been inconsistent with respect to the number and effects of genes involved (Kema 1996). This is partly because many experiments on progeny populations of inter-varietal crosses relied on natural infection, instead of using defined isolates (for an exception see Somasco et al. 1996), so it has not always been possible for other workers to confirm the identity of resistance genes. The use of single isolates enables geneticists and breeders to distinguish isolate-specific and non-specific resistance in the host (Parlevliet 1993). Four genes for resistance to *M. graminicola* have been identified, but they have not been mapped or located to chromosomes (Wilson 1979; Somasco et al. 1996; McIntosh et al. 1998). Only *Stb4* was identified by the use of a single pathogen isolate (Somasco et al. 1996).

The wild relatives of wheat are a valuable pool of germplasm that can be used as a source of genetic resistance to several diseases, including septoria tritici blotch (Yechilevich-Auster et al. 1983). Wheat can be hybridised with many related species, and many resistance genes have been transferred to wheat cultivars from other species and genera of the Triticeae (Cox et al. 1992). The species *Triticum tauschii* (syn. *Aegilops squarrosa*, 2n=2*x*=14) is the donor of the D genome of bread wheat (Kimber and Feldman 1987). Different accessions of this grass carry a wide range of disease resistances (Appels and Lagudah 1990; Cox et al. 1992; Villareal et al. 1994). May and Lagudah (1992) tested several accessions of *T. tauschii* and synthetic hexaploid wheats in Australia. Most accessions were resistant to infection by *M. graminicola* in tests using one pathogen isolate from Australia. May and Lagudah (1992) sug-

Communicated by F. Salamini

L.S. Arraiano (✉) · A.J. Worland · C. Ellerbrook · J.K.M. Brown
Cereals Research Department, John Innes Centre,
Norwich Research Park, Colney, Norwich, NR4 7UH, UK
e-mail: lia.arraiano@bbsrc.ac.uk

gested that the D genome of *T. tauschii* contains one dominant septoria leaf blotch resistance gene that can be successfully transferred to and expressed in synthetic hexaploid wheats. However, the chromosome carrying the gene was not identified.

Synthetic hexaploid wheats are now an important component of the breeding programme of the International Centre for Improvement of Maize and Wheat (CIMMYT), Mexico (Mujeeb-Kazi et al. 2000). A synthetic hexaploid, 'Synthetic 6*x*', generated from a cross between *Triticum dicoccoides* and *T. tauschii* (McFadden and Sears 1946; Sears 1976) carries resistance to *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*, formerly *Septoria nodorum*) (Nicholson et al. 1993). A complete set of substitution lines of chromosomes from 'Synthetic 6*x*' into the susceptible cultivar 'Chinese Spring' was developed by C.N. Law and A. J. Worland at the John Innes Centre (Nicholson et al. 1993). Homozygous, single-chromosome recombinant lines (Law 1966) for some of these substitution lines were developed by Ellerbrook et al. (1999).

Recently, Arraiano et al. (2001), studying cultivar by isolate interactions on detached seedling leaves, observed that 'Synthetic 6*x*' was completely resistant to most isolates from The Netherlands and from Portugal tested but was susceptible to isolate IPO92006 from Portugal. This paper reports, firstly, experiments conducted on the 'Chinese Spring' ('Synthetic 6*x*') chromosome substitution series (Nicholson et al. 1993) with a set of 13 *M. graminicola* isolates, to identify the chromosomal location of resistance. Secondly, experiments were conducted on homozygous single chromosome recombinant lines to map the resistance gene in 'Synthetic 6*x*'. Knowledge about the genetics of resistance in 'Synthetic 6*x*' and markers associated with the resistance gene will greatly assist breeders in introgressing this novel resistance into well-adapted germplasm, to control septoria tritici blotch.

Materials and methods

Plant material

Chromosomal location

'Chinese Spring' (CS), 'Synthetic 6*x*' (Syn; McFadden and Sears 1946; Sears 1976), and 21 substitution lines of chromosomes from Syn into CS (Nicholson et al. 1993) were used. One substitution line for each chromosome was employed in all experiments. When the substitution series was developed, all lines were checked for specific isozymes and morphological characteristics to confirm that the correct chromosome substitution was present (Nicholson et al. 1993). However these tests did not confirm the substitution for every chromosome and recent tests using microsatellite markers have shown that parts of chromosomes 2A, 4A, 7A and 6B recombined during the substitution procedure or were misidentified due to translocation differences between the CS and Syn chromosomes (A.J. Worland, unpublished results). The susceptible cultivars 'Longbow' and 'Hobbit sib' were used as controls, in detached leaf tests and polytunnel trials, respectively.

Mapping of resistance gene

One hundred and one homozygous single-chromosome recombinant lines (SCRs) of chromosome 7D of Syn substituted into a CS background, consisting of whole chromosome recombinants or recombinants for individual arms (A.J. Worland, unpublished results), were used to locate the septoria tritici blotch resistance gene. The SCRs were developed by crossing the individual chromosome substitution line to CS euploid (or ditelocentric), then backcrossing the F_1 to the corresponding monosomic from the CS monosomic series. Monosomic plants were then selected in the backcrossed F_1 progeny and selfed to extract disomics (Law et al. 1987). CS, Syn and CS (Syn7D) were included in the tests, along with the cultivars 'Longbow' and 'Baldus' as susceptible controls.

Disease tests

Disease tests were conducted on detached seedling leaves by the method of Arraiano et al. (2001). To determine the chromosomal location of resistance, seven isolates of *M. graminicola* from Portugal, numbered IPO92001 to IPO92007, and six isolates from the Netherlands, IPO001, IPO290, IPO323, IPO89011, IPO94265 and IPO94269, all provided by Dr. Gert Kema, Plant Research International, The Netherlands, were used. IPO323 and IPO94269 were employed in experiments to map the resistance gene. These two isolates were selected because they have been used in genetic analysis of the fungus (Kema et al. 2000).

Adult plants were tested in a polytunnel. Seeds were pre-germinated (Arraiano et al 2001), sown individually in 7×7 cm pots containing modified John Innes No. 2 compost and subsequently transplanted to 9×9 cm pots (GS 16, Zadoks et al. 1974). Only isolates IPO323 and IPO94269 were used for tests of both chromosomal location and mapping within chromosome 7D. The plants were inoculated at the beginning of anthesis (GS 60, Zadoks et al. 1974) using a hand-pumped sprayer (3.3×10^{12} spores ha^{-1}). In this way, fully developed flag leaves were sprayed, so avoiding the confounding effects of height and flowering time on disease progression (Arama et al. 1994). Inoculation was done in the evening so that moisture was retained on the leaf surface overnight, promoting infection. Trials were inoculated twice at an interval of 7 days and scored 4 weeks after the second inoculation.

Inoculum for both laboratory and polytunnel tests was produced from sporulating cultures of *M. graminicola* grown on potato dextrose agar (PDA) plates for 7 days under near-ultra violet light (Philips TL 20 W/05) for 16 h per day at 15°C. Cultures were flooded with sterile distilled water and scraped to release conidia. The concentration of the conidial suspension was adjusted to 10^7 spores ml^{-1} and polyoxyethylene-sorbitan monolaurate (Tween 20; Sigma) was added to 0.15% v/v.

Experimental design

All experiments were conducted in randomised complete blocks in layouts generated with the Experimental Design Generator and Randomiser (EDGAR) (Brown 1997). Experiments with detached seedling leaves of the 21 substitution lines, CS and Syn, were conducted on seven dates. Three or four different isolates were tested on each date. Isolates IPO323 and IPO94269 were tested separately on two different dates on all the lines. Substitution lines CS (Syn1B), CS (Syn3D), CS (Syn5D) and CS (Syn7D), and CS and Syn were tested a second time on a different date with all 13 isolates to obtain results for the line CS (Syn5D), which was not available for testing previously. Leaves were placed in clear polystyrene boxes with three boxes per block. Each box contained one leaf each of eight to ten different lines and was inoculated with a single isolate. There were four replicate leaves of each plant line per isolate. The susceptible control 'Longbow' was present in every box.

Six tests were conducted on different dates on detached seedling leaves of 101 SCRs, CS, Syn and CS (Syn7D). There were six to seven boxes per block, depending on the number of lines tested, and each box had one leaf each of nine different lines, inoculated

with a single isolate. At least four replicate leaves were tested of each plant line with each isolate, but most combinations of line and isolate were tested with six replicate leaves. The susceptible controls 'Baldus' and 'Longbow' were included in every box.

Adult plants were tested in two trials in 1999 and 2000 for the 21 substitution lines and in one trial in 2000 for 62 of the 101 SCRs. In each trial, there were two blocks per isolate and five randomised plots per block. Each plant line was represented once in each plot.

Data analysis

In the detached seedling leaf experiments, the percentage leaf area covered by lesions bearing pycnidia was scored four to five times at intervals of 2–4 days during a period of 19 to 28 days after inoculation. All assessments were carried out using a dissecting microscope at 40× magnification. Disease scores were summarised as the area under the disease progress curve (AUDPC) (Shaner and Finney 1977), calculated as the area under the graph of observed disease level plotted against time, from the first to last scoring. The controls, mock inoculated with water only, always had AUDPC scores of zero. These data were not included in the statistical analysis, to avoid underestimating the variance between replicates. In the polytunnel trials of adult plants, disease was scored visually on flag leaves as the percentage leaf area covered by lesions bearing pycnidia.

Data were analysed by generalised linear mixed modelling of binomial proportions (GLMM) (Welham 1993). 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 detached seedlings was the AUDPC as a proportion of the maximum possible AUDPC for each experiment (i.e. a score of 100% at each time of scoring).

In the detached-leaf tests of the chromosomal location of resistance, median tetrad analysis was used to identify specific resistance or susceptibility of lines to particular isolates (Brown 2001; Brown et al. 2001). The analysis was done on logit-transformed data. The analyses were performed using the statistical package Genstat for Windows Release 4.1, 4th Edition (Numerical Algorithms Group Ltd., Oxford).

Linkage analysis

A genetic linkage map for chromosome 7D of Syn was previously developed to locate a gene for resistance to stagonospora nodorum blotch and to locate genes for resistance to greenbug and Russian aphids (C. Ellerbrook, unpublished results). The map presented in this paper includes four microsatellite markers, *Xgwm44*, *Xgwm111*, *Xgwm121* and *Xgwm437* (Röder et al 1998), an RFLP marker, *Xpsr490* (Harcourt 1992), and the anthocyanin pigment gene *Rc3* (red coleoptile; Rowland and Kerber 1974). The septoria tritici blotch resistance gene *Stb5* was mapped using JoinMap version 2.0 (Stam and Van Ooijen 1995). All markers were linked to at least one other marker with a log-likelihood (LOD) score of at least 2.0 (actual minimum 2.4). All recombination fractions between pairs of markers less than 0.48 were used to construct the map and the Kosambi (1944) mapping function was used to convert recombination data to map distances. The map was drawn using the program DrawMap Version 1.1 (J.W. Van Ooijen, Plant Research International, The Netherlands).

Results

Chromosomal location

In detached seedling-leaf tests, the isolate and line main effects and the line by isolate interaction were large and highly significant (Table 1). To identify resistances in the detached leaf tests it is necessary to consider each line's response to the complete set of isolates and the general level of aggressiveness of each isolate. Syn and the CS (Syn7D) line were resistant to all isolates tested except the Portuguese isolate IPO92006. Median tetrad analysis indicated that they were specifically susceptible to IPO92006 (Table 2). The other substitution lines were susceptible to all the isolates from Portugal and to isolates IPO001, IPO89011, IPO94265 and IPO94269 from The Netherlands, but moderately resistant to IPO290 (Table 2). Isolate IPO323 caused relatively low levels of disease severity on all lines except Longbow (Table 2).

Table 1 Generalised linear mixed modelling of septoria tritici blotch (*M. graminicola*) on plants of the 'Chinese Spring' ('Synthetic 6*x*') substitution series. a) The variate analysed was the area under the disease progress curve of lesions bearing pycnidia of 13 isolates in detached seedling leaf tests. b) The variate analysed was percentage of leaf area with lesions bearing pycnidia of isolates IPO323 and IPO94269 in polytunnel trials of adult plants

Factor	*df*[a]	Wald statistic	*dd*[b]	P[c]
a) Detached leaf tests				
Isolate	12	160.8	92	***
Line	23	284.7	1,085	***
Line.Isolate	275	850.7	1,085	***
Mean deviance		0.43		
b) Polytunnel trials				
Isolate	1	113.1	36	***
Line	23	464.3	844	***
Line.Isolate	23	238.9	844	***
Mean deviance		0.45		

[a] *df*, treatment degrees of freedom
[b] *dd*, denominator degrees of freedom
[c] *P*, *F*-test probability of Wald statistic (Elston 1998) with *df* and *dd* degrees of freedom, *** P=0.001

In the polytunnel trials of adult plants, the isolate and line main effects and the line by isolate interaction were large and highly significant (Table 1). The polytunnel trial confirmed the resistance of Syn and CS (Syn7D) to isolate IPO94269 (Table 3). The substitution lines CS (Syn3A), CS (Syn4D) and CS (Syn6A) had significantly greater levels of disease with isolate IPO94269 than CS did (Table 3). All lines had much less disease with IPO323 than with IPO94269 (Table 3).

Mapping the resistance gene

The single chromosome recombinant lines were tested for resistance to IPO323 and IPO94269 in both detached seedling leaf tests and a polytunnel trial of adult plants. In the polytunnel, disease severity with IPO323 was low, as in the trial of the substitution lines, so the data for this isolate were not included in the analysis. There were highly significant differences between lines in the disease severity caused by IPO94269 (Table 4). The correlation of the lines' means between detached leaf tests and the polytunnel trial was high and there was a clear

Table 2 Percent leaf area of detached leaves of 'Chinese Spring' ('Synthetic 6*x*') substitution lines covered by lesions bearing pycnidia of *M. graminicola* isolates

Line	Isolates from Portugal							Isolates from The Netherlands						Line mean[a]
	IPO 92001	IPO 92002	IPO 92003	IPO 92004	IPO 92005	IPO 92006	IPO 92007	IPO 001	IPO 290	IPO 323	IPO 89011	IPO 94265	IPO 94269	
IA	25[b]	47	63	44	62	76	77	31	11	6	29	27	21	38
1B	29	49	44	57	39	65	79	62	19	3	43	42	42	44
1D	29	76	43	74	65	90	85	54	25	5	39	46	33	54
2A	37	66	59	49	83	83	69	66	23	9	33	45	50	55
2B	36	25	44	58	78	88	74	50	10	6	24	38	7	39
2D	59	60	29	44	44	86	59	29	21	2	39	40	28	44
3A	52	53	65	79	68	92	78	76	16	21	57	48	36	60
3B	53	45	36	61	51	60	56	56	13	8	37	34	41	43
3D	46	32	15	72	49	56	71	43	23	6	32	31	33	38
4A	59	45	57	70	74	76	96	75	28	21	71	67	50	62
4B	20	53	24	39	48	86	88	73	11	11	56	32	32	42
4D	31	45	60	75	69	81	87	36	23	11	61	50	42	53
5A	17	47	21	56	28	80	74	41	17	3	46	43	29	37
5B	42	48	17	60	69	64	80	35	11	4	55	22	35	40
5D	61	28	50	71	52	42	70	40	20	4	28	–	28	41
6A	28	52	36	66	34	75	82	24	38	2	62	45	36	45
6B	51	37	25	39	42	65	53	34	31	6	50	49	31	41
6D	47	32	21	59	47	92	90	50	18	3	44	45	32	45
7A	32	25	46	66	60	80	66	28	7	2	55	20	26	38
7B	62	47	45	55	57	84	84	39	27	5	50	63	46	53
7D	2	0	1	0	4	50(1)[c]	3	0	0	0	0	1	0	0
CS	47	46	43	63	62	75	83	49	33	11	41	36	54	50
Syn	1	1	0	11	1	37(2)	2	9	1	0	0	0	0	1
Longbow	38	48	19	40	27	82	61	60	30	67(9)	71	40	28	44
Isolate mean[d]	40	45	38	59	55	78	78	48	19	6	46	41	33	

[a] Line means excluding isolates IPO92006 and IPO323
[b] Means over leaves and dates estimated by generalised linear mixed modelling
[c] In brackets, expected means in the absence of cultivar by isolate interaction, where the median tetrad was significant ($P \leq 0.05$)
[d] Isolate means excluding lines 7D and Syn

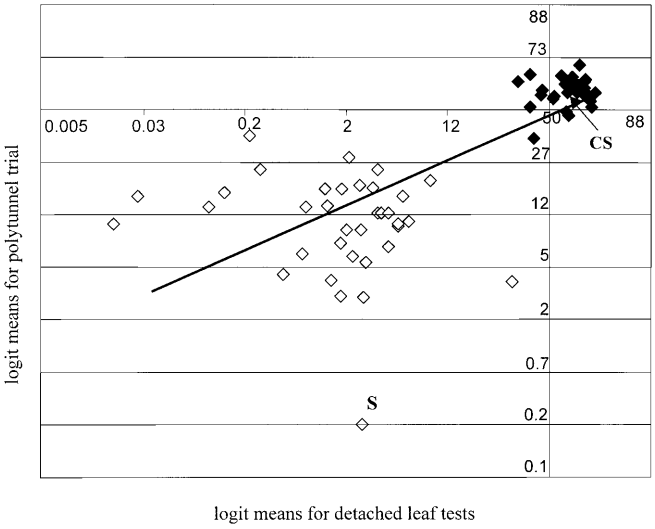

Fig. 1 Mean percent leaf area of 'Chinese Spring' ('Synthetic 6*x*' 7D) single-chromosome recombinant lines covered by lesions bearing pycnidia of *M. graminicola* isolate IPO94269 in detached seedling leaf tests and polytunnel trials of adult plants (correlation coefficient, r^2=0.60). *Open diamonds* resistant lines. *Closed diamonds* susceptible lines. *CS* 'Chinese Spring'. *S* 'Synthetic 6*x*'. Axes are logit-scaled

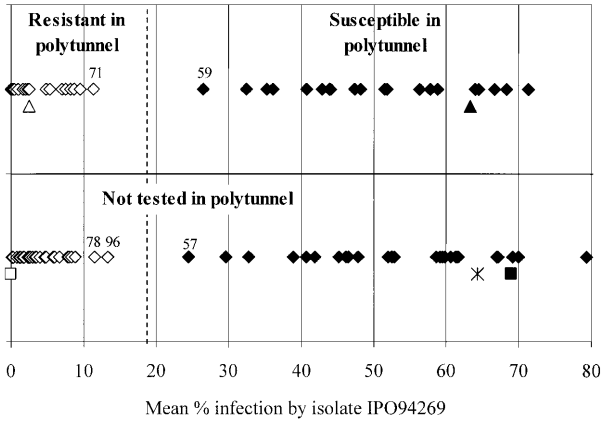

Fig. 2 Mean percent leaf area of 'Chinese Spring' ('Synthetic 6*x*' 7D) single-chromosome recombinant lines covered by lesions bearing pycnidia of *M. graminicola* isolate IPO94269 in detached seedling-leaf tests. Top set of data: lines tested in the polytunnel trial of adult plants. Bottom set of data: lines not tested in the polytunnel. *Vertical dashed line* divides the resistant and the susceptible groups of lines according to the Fig. 1 classification. *Open diamonds* resistant lines. *Closed diamonds* susceptible lines. *Open triangle* 'Synthetic 6*x*'. *Closed triangle* 'Chinese Spring'. *Open square* 'Chinese Spring' ('Synthetic 6*x*' 7D). *Star* Baldus. *Close square* 'Longbow'

Table 3 Mean percent leaf area of 'Chinese Spring' ('Synthetic 6*x*') substitution lines covered by lesions bearing pycnidia of *M. graminicola* isolates IPO323 and IPO94269 in polytunnel trials of adult plants

Line	Isolate		
	IPO323	IPO94269	Line mean
CS (Syn1A)	1[a]	43	7
CS (Syn1B)	1	35	8
CS (Syn1D)	2	46	12
CS (Syn2A)	1	42	10
CS (Syn2B)	0	40	5
CS (Syn2D)	1	50	11
CS (Syn3A)	2	61*	16
CS (Syn3B)	2	44	11
CS (Syn3D)	1	28	6
CS (Syn4A)	2	39	11
CS (Syn4B)	1	26	5
CS (Syn4D)	8	64*	29
CS (Syn5A)	1	37	5
CS (Syn5B)	0	44	5
CS (Syn5D)	1	24	4
CS (Syn6A)	3	60*	18
CS (Syn6B)	2	36	10
CS (Syn6D)	1	30	6
CS (Syn7A)	2	52	12
CS (Syn7B)	1	56	11
CS (Syn7D)	0	0	0
CS	1	44	6
Syn	0	0	0
Hobbit sib	58	62	60
Isolate mean	1	32	

* Lines with a significantly greater level of disease than CS for isolate IPO94269 (P=0.01, Student's t-test)
[a] Means over replicate plants and dates estimated by generalised linear mixed modelling

Table 4 Generalised linear mixed modelling of septoria tritici blotch (*M. graminicola*) on plants of 'Chinese Spring' ('Synthetic 6*x*' 7D) single-chromosome recombinant lines. a) The variate analysed was the area under the disease progress curve of lesions bearing pycnidia of isolates IPO323 and IPO94269 in detached seedling leaf tests. b) The variate analysed was the percentage of leaf area with lesions bearing pycnidia of isolate IPO94269 in polytunnel trials of adult plants

Factor	df[a]	Wald statistic	dd[b]	P[c]
a) Detached leaf tests				
Isolate	1	63.9	17	***
Line	105	1,474.0	981	***
Line.Isolate	105	448.6	981	***
Mean deviance		1.20		
b) Polytunnel trials				
Line	63	592.6	517	***
Mean deviance		0.31		

[a] df, treatment degrees of freedom
[b] dd, denominator degrees of freedom
[c] P, F-test probability of Wald statistic (Elston 1998) with df and dd degrees of freedom, *** P=0.001

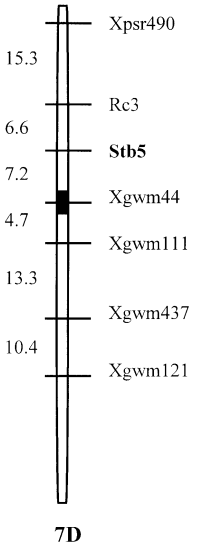

Fig. 3 Map location of the septoria tritici blotch gene, *Stb5*, on chromosome 7D of the 'Chinese Spring' ('Synthetic 6*x*' 7D) homozygous single-chromosome recombinant lines population. Distances on the left of the chromosome are in Kosambi cM. The short arm of chromosome 7D is at the top. The approximate position of the centromere (Röder et al. 1998) is represented by the *closed rectangle*

distinction between two groups of lines, susceptible and resistant (Fig. 1).

In detached leaf tests, the line and line by isolate interaction effects were large and highly significant (Table 4). The dispersion was high, as in the analysis of the CS (Syn) substitution lines (Table 4). Although the disease levels for isolate IPO323 were higher in detached leaf tests than in polytunnel trials they were still comparatively low, so only data for isolate IPO94269 was used to classify the lines into resistant and susceptible groups. Of the lines that had been scored in both the polytunnel and detached leaf trials, the resistant line with the highest level of disease in the polytunnel was line 71 (11%) and the susceptible line with the lowest level of disease in the polytunnel was line 59 (26%) (Fig. 2). Initially, all lines scored only in the detached leaf test were recorded as resistant if they had lower disease levels than line 71, or as susceptible if they had higher disease levels than line 59. Three lines tested only in detached leaf tests were within this interval but two of the lines, 78 and 96, appeared to be resistant and one line, 57, appeared to be susceptible (Fig. 2). The order of the markers in the linkage group was the same whether or not these three lines were included in the analysis, so they were retained in the data set. The segregation of resistance and susceptibility to septoria tritici blotch, 52 and 49 lines respectively, was not significantly different from a 1:1 ratio (χ^2=0.09, P=0.8), consistent with resistance being controlled by a single gene.

The septoria tritici blotch resistance gene, named *Stb5*, was mapped to the short arm of the Syn 7D chromosome, near the centromere. *Stb5* is flanked by *Xgwm44*, which is close to the centromere (Röder et al. 1998), and by *Rc3*. Both markers are approximately 7 cM from *Stb5* (Fig. 3).

Discussion

Stb5 is the first gene for resistance to septoria tritici blotch, currently the major foliar disease of wheat worldwide, to be located to a chromosome and to be mapped, and is only the second gene to be identified using a defined pathogen isolate. Disease testing of 101 CS (Syn7D) single-chromosome recombinant lines and the use of a molecular-marker linkage map allowed *Stb5* to be located in the short arm of the 7D chromosome of Syn (Fig. 3). *Stb5* is closely linked to the gene for red coleoptile, *Rc3* (Rowland and Kerber 1974; Worland et al. 1988), and to the microsatellite marker *Xgwm44* (Röder et al. 1998) (Fig. 3). The close linkage of microsatellite marker *Xgwm44* to the centromere and the location of markers *Xgwm111*, *Xgwm437* and *Xgwm121* to the long arm of chromosome 7D (Fig. 3) are consistent with the results of Röder et al. (1998).

The resistance of Syn to a wide range of *M. graminicola* isolates has been reported previously (Arraiano et al. 2001). Here we show by analysis of the CS (Syn) substitution series, that a gene or genes on Syn chromosome 7D (Tables 2,3) control all these resistances. Both Syn and CS (Syn7D) were susceptible only to one isolate from Portugal, IPO92006 (Table 2), indicating that *Stb5* is not effective against this isolate.

The specific resistance of CS to IPO323, i.e. CS has a high level of disease with all isolates tested except IPO323, whereas Longbow (Table 2) and Hs (Table 3) are susceptible to all isolates, was identified previously in field trials and detached leaf tests (Arraiano et al. 2001; Brown et al. 2001). The use of the CS (Syn) substitution series should have allowed us to identify a chromosome carrying the specific resistance of CS to IPO323 if one substitution line had been significantly more susceptible than CS to that isolate. As this was not the case, we conclude that, either the specific resistance of CS to IPO323 is controlled by genes on more than one chromosome or, if there is only one resistance gene in CS, the susceptibility allele of that gene in Syn is masked by genes conferring partial resistance.

Four genes for resistance to *M. graminicola* have been identified previously, but *Stb1*, *Stb2* and *Stb3* were identified in field trials which became naturally infected by *M. graminicola*, which means that it may not be possible to reproduce the identification of these resistance genes, as the pathogen genotypes to which they were resistant were not isolated (Wilson 1979; Eyal 1999). *Stb4* was identified with the isolate CA 30 but was not mapped or located to a chromosome (Somasco et al. 1996).

Cytogenetic analysis has proved to be effective for the location of other disease resistance genes (Worland and Law 1986; Worland et al. 1988; Ellerbrook et al. 1999). The accurate location of genes is greatly facilitated by the use of linkage maps and the use of microsatellite and RFLP markers which are largely chromosome-specific (Röder et al. 1998; Sourdille et al. 1999). The Synthetic 6*x* line used in this paper also carries resistance to *Phaeosphaeria nodorum*, the stagonospora nodorum blotch pathogen, on several chromosomes derived from both parents (Nicholson et al. 1993) and one resistance gene, *Srb3*, was mapped on chromosome 5D of Syn (Ellerbrook et al. 1999); this implies that Syn can be used as a source of resistance to both diseases. Several accessions of *Triticum tauschii*, the donor of the D genome in Synthetic 6*x*, carry genes for resistance to leaf rust, powdery mildew, greenbug, Russian wheat aphid, Hessian fly, soil-borne mosaic virus, and stagonospora nodorum blotch (Kerber and Dyck 1969; Gill et al. 1986; Cox et al. 1992; Murphy et al. 2000; Smith et al. 2000). May and Lagudah (1992) showed that several accessions of *T. tauschii* contained at least one dominant gene for resistance to *M. graminicola*. *T. tauschi* is therefore a valuable genetic resource for breeding wheat for disease resistance.

The markers linked to *Stb5*, *Rc3* and *Xgwm44* may be useful in marker-assisted selection for resistance to septoria tritici blotch, which is variable in its expression in the field and therefore difficult and expensive to select in wheat breeding programmes. Markers may also be used in constructing resistant wheat genotypes by pyramiding different sources of resistance (Sourdille et al. 1999). The knowledge of the genetics of the septoria tritici blotch resistance in 'Synthetic 6*x*' and the linkage of *Stb5* to markers may be of value to wheat breeders in improving resistance to this very important disease.

Acknowledgements This research was funded by PRAXIS XXI – Fundação para a Ciência e Tecnologia, Portugal, the Biotechnology and Biological Sciences Research Council and the Ministry of Agriculture, Fisheries and Food for England.

References

Appels R, Lagudah ES (1990) Manipulation of chromosomal segments from wild wheat for the improvement of bread wheat. Aust J Plant Physiol 17:253–266

Arama PF, Parlevliet JE, van Silfhout CH (1994) Effect of plant height and days to heading on the expression of resistance in *Triticum aestivum* to *Septoria tritici* in Kenya. In: Arseniuk E, Goral T, Czembor P (eds) Proc 4th Int Septoria of Cereals Workshop, The Plant Breeding and Acclimatization Institute, Radzików, Poland, pp 153–157

Arraiano LS, Brading PA, Brown JKM (2001) A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. Plant Pathol 50:339–346

Brown JKM (1997) EDGAR-experimental design generator and randomiser. *Internet [http://www.jic.bbsrc.ac.uk/services/statistics/edgar.htm]*

Brown JKM (2001) Procedure MEDIANTETRAD. In: Genstat Release 4.1 Procedure Library Manual PL12. Numerical Algorithms Group, Oxford (in press)

Brown JKM (2000) MEDIANTETRAD. In: Payne RW, Arnold GW (eds) Genstat Release 4.2 Reference Manual. Part 3: Procedure Library PL 12. VSN International Ltd, Oxford, UK, pp 213–233

Brown JKM, Kema GHJ, Forrer H-F, Verstappen ECP, Arraiano LS, Brading PA, Foster EM, Fried PM, Jenny E (2001) Resistance of wheat cultivars and breeding lines to septoria tritici leaf blotch caused by isolates of *Mycosphaerella graminicola* in field trials. Plant Pathol 50:325–338

Cook RJ (1999) Management by chemicals. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a study of pathosystems. CABI Publishing, Oxon, pp 286–298

Cox TS, Raupp WJ, Wilson DL, Gill BS, Leath S, Bockus WW, Browder LE (1992) Resistance to foliar diseases in a collection of *Triticum tauschii* germplasm. Plant Dis 76:1061–1064

Ellerbrook CM, Korzun V, Worland AJ (1999) Using precise genetic stocks to investigate the control of *Stagonospora nodorum* resistance in wheat. In: Van Ginkel M, McNab A, Krupinsky J (eds) *Septoria* and *Stagonospora* diseases of cereals: a compilation of global research. CIMMYT, Mexico DF, pp 134–139

Elston DA (1998) Estimation of denominator degrees of freedom of *F*-Distributions for assessing Wald statistics for fixed-effect factors in unbalanced mixed models. Biometrics 54:1085–1096

Eyal Z (1999) Breeding for resistance to *Septoria* and *Stagonospora* diseases of wheat. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a study of pathosystems. CABI Publishing, Oxon, pp 332–344

Genstat 5 Committee (1993) Genstat 5, release 3, reference manual. Oxford University Press, Oxford

Gill BS, Raupp WJ, Sharma HC, Browder LE, Hatchett JH, Harvey TL, Moseman JG, Waines JG (1986) Resistance in *Aegilops squarrosa* to wheat leaf rust, wheat powdery mildew, greenbug, and Hessian fly. Plant Dis 70:553–556

Harcourt RL (1992) DNA sequence polymorphisms in *Triticeae* species. PhD thesis,Cambridge University, UK

Kema GHJ (1996) *Mycosphaerella graminicola* on wheat, genetic variation and histopathology. PhD thesis, DLO-Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands

Kema GHJ Verstappen ECP, Waalwijk C (2000) Avirulence in the wheat septoria tritici blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. Mol Plant- Microbe Interact 13:1375–1379

Kerber ER, Dyck PL (1969) Inheritance in hexaploid wheat of leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can J Genet Cytol 11:639–647

Kimber G, Feldman M (1987) Wild wheat – an introduction. Special Report 353 College of Agriculture, University of Missouri, Columbia, USA

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Law CN (1966) The location of genetic factors affecting a quantitative character in wheat. Genetics 53:487–498

Law CN, Snape JW, Worland AJ (1987) Aneuploidy in wheat and its uses in genetic analysis. In: Lupton FGH (ed) Wheat breeding: its scientific basis. Chapman and Hall, London, pp 71–108

May CE, Lagudah ES (1992) Inheritance in hexaploid wheat of *Septoria tritici* blotch resistance and other characteristics derived from *Triticum tauschii*. Aust J Agric Res 43:433–442

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89, 107–116

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols of wheat. In: Proc 9th Int Wheat Genet Symp, University Extension Press, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, vol 5, p 129

Mujeeb-Kazi A, Gilchrist LI, Villareal RL, Delgado R (2000) Registration of ten wheat germplasms resistant to *Septoria tritici* leaf blotch. Crop Sci 40:590–591

Murphy NEA, Loughman R, Wilson R, Lagudah ES, Appels R, Jones MGK (2000) Resistance to septoria nodorum blotch in the *Aegilops tauschii* accession RL5271 is controlled by a single gene. Euphytica 113:227–233

Nicholson P, Rezanoor HN, Worland AJ (1993) Chromosomal location of resistance to *Septoria nodorum* in a synthetic hexaploid wheat determined by the study of chromosomal substitution lines in 'Chinese Spring' wheat. Plant Breed 110:177–184

Parlevliet JE (1993) What is durable resistance, a general outline. In: Jacobs Th, Parlevliet JE (eds) Durability of disease resistance. Kluwer Academic Publishers, The Netherlands, pp 23–39

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rowland GG, Kerber ER (1974) Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can J Genet Cytol 16:137–144

Scharen AL (1999) Biology of the Septoria/Stagonospora pathogens: an overview. In: Van Ginkel M, McNab A, Krupinsky J (eds) Septoria and Stagonospora diseases of cereals: a compilation of global research. CIMMYT, Mexico DF, pp 19–22

Sears ER (1976) A synthetic hexaploid wheat with fragile rachis. Wheat Inf Serv 41–42:31–32

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67:1051–1056

Smith CM, Liu X, Flinn M, Harvey TL, Gill BS, Tolmay V (2000) Molecular markers in wheat linked to genes expressing resistance to greenbug and the Russian wheat aphid. XXI Int Congress of Entomology, Brazil, p 980

Somasco OA, Qualset CO, Gilchrist DG (1996) Single gene resistance to *Septoria tritici* blotch in the spring wheat cultivar 'Tadinia'. Plant Breed 115:261–267

Sourdille P, Robe P, Tixier M-H, Doussinault G, Pavoine M-T, Bernard M (1999) Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. Euphytica 110:193–198

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands

Villareal RL, Mujeeb-Kazi A, del Toro E, Crossa J, Rajaram S (1994) Agronomic variability in selected *Triticum turgidum*×*T. tauschii* synthetic hexaploid wheats. J Agron Crop Sci 173: 307–317

Welham SJ (1993) Procedure GLMM. In: R. W. Payne, G. M. Arnold and G. W. Morgan (eds) Genstat 5 procedure library manual release 3 [1], Numerical Algorithms Group, Oxford, pp 187–92

Wilson RE (1979) Resistance to *Septoria tritici* in two wheat cultivars, determined by independent, single dominant genes. Aust J Plant Pathol 8:16–18

Worland AJ, Law CN (1986) Genetic analysis of chromosome 2D of wheat. I. The location of genes affecting height, day lenght insensitivity, hybrid dwarfism and yellow-rust resistance. Z Pflanzenzüchtg 96:331–345

Worland AJ, Law CN, Hollins TW, Koebner RDM, Giura A (1988) Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. Plant Breed 101:43–51

Yechilevich- Auster M, Levi E, Eyal Z (1983) Assessment of interactions between cultivated and wild wheats and *Septoria tritici*. Phytopathology 73:1077–1083

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421