Chromosomal location of genes encoding for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in substitution lines of wheat

M.R. Simón[1,*], A.J. Worland[2,†] and P.C. Struik[3]

[1] Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, La Plata, Argentina

[2] Cereals Research Department, John Innes Centre, Norwich, England

[3] Crop and Weed Ecology Group, Wageningen University, Wageningen, The Netherlands

† Deceased before publication

* Corresponding author (e-mail: mrsimon@ceres.agro.unlp.edu.ar)

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Abstract

Chromosomal location of resistance to *Mycosphaerella graminicola* was studied in substitution lines of resistant *Triticum* genotypes into the (susceptible) cultivar Chinese Spring (*T. aestivum*). (Moderately) resistant genotypes for which substitution lines were available were tested in a first screening. We selected a synthetic hexaploid wheat (Synthetic 6x; *T. dicoccoides* × *T. tauschii*), *T. spelta* and the wheat (*T. aestivum*) cultivars Cheyenne and Cappelle-Desprez. In a second screening the most suitable Argentinian isolates were identified. We decided to use the isolate IPO 92067 (all sets of substitution lines), IPO 93014 (substitution lines of Synthetic 6x, Cappelle-Desprez and *T. spelta*) and IPO 92064 (substitution lines of Cheyenne). In the final experiments, substitution lines of the selected genotypes into Chinese Spring were grown in two different environments and inoculated with the selected isolates at the seedling stage (lines of all four selected genotypes) or the adult stage (lines of Synthetic 6x and Cheyenne). Resistance was expressed as (reduction in) necrosis percentage or pycnidial coverage percentage; the two measures were highly (linearly) correlated. When tested in the seedling stage, all chromosomes seemed to carry genes effective against *M. graminicola*. Many genes were effective against only one isolate or in only one environment or their effects only showed in one resistance parameter. Often these effects were minor. Only chromosome 7D of Synthetic 6x was found with a major effect against both isolates tested. When tested in the adult stage, all lines but the one carrying chromosome 4B from the resistant parent seemed to show genes effective against *M. graminicola*. The line carrying chromosome 7D from Synthetic 6x showed a level of resistance similar to the resistant parent for isolate IPO 92067, but not for isolate IPO 93014. Major genes, effective against both isolates, were also found on chromosomes 5A and 5D from Synthetic 6x. Lines carrying chromosome 1B, 5D or 6D from Cheyenne showed major effects against isolate IPO 92064. For both necrosis percentage and pycnidial

coverage percentage, highly significant linear correlations were found between resistance in the seedling stage and resistance in the adult stage. However, the variance accounted for was only small (20–24%; n = 184).

Additional keywords: disease resistance, necrosis, pycnidial coverage, resistance breeding, *Triticum*

Introduction

Septoria tritici blotch caused by *Mycosphaerella graminicola* (Fuckel) Schroeter in Cohn (anamorph *Septoria tritici* Rob. ex Desm.) is an important disease in many wheat-producing areas of the world and causes significant yield losses (Eyal, 1981; Eyal *et al.*, 1987). Resistance conditioned by one or two genes was found in some materials (Narvaez & Caldwell, 1957; Rillo & Caldwell, 1966; Rosielle & Brown 1979; Lee & Cough, 1984; Brading *et al.*, 1999), whereas in other materials at least three resistance genes have been reported (Rosielle & Brown, 1979).

Although quantitative resistance has been found in different genotypes (Jlibene *et al.*, 1994; Brown *et al.*, 1999; Simón *et al.*, 2001) and most commercially grown cultivars range from moderately resistant to susceptible, indicating that minor gene effects are also present, most investigations have concentrated on the study of major gene effects. Major genes are interesting because of the high level of resistance, resulting in an almost complete absence of symptoms in the host. Partial resistance, however, is very important due to its putative durability and its expression under a broad spectrum of isolates of the pathogen. A few genes may be enough to confer resistance that will hold up in farmers' fields (Dubin & Rajaram, 1996).

Several of the components of partial resistance to *M. graminicola* may be controlled by just a few genes (Jlibene & El Bouami, 1995). The components that are genetically different could be combined into the same genetic background by crossing (Van Ginkel & Rajaram, 1999). However, significant non-additive effects were often identified (Van Ginkel & Scharen, 1987; Bruno & Nelson, 1990; Danon & Eyal, 1990; Jonsson, 1991; Jlibene *et al.*, 1994; Simón & Cordo, 1997; 1998). Heritability tends to be only moderate (Simón *et al.*, 1998), but progress in breeding for resistance may still be possible.

A few studies have been carried out to study the chromosomal location of the resistance. The increased use of molecular markers as an important tool for marker-assisted selection makes the chromosomal location more important. Once the chromosomes carrying resistance are identified, finding molecular markers linked to resistance is easier through the development of recombinant lines for those specific chromosomes.

The aim of this work was:

1. To identify resistant materials in a set of accessions of *Triticum* spp. that are parents of substitution and monosomic substitution lines;
2. To determine the chromosomal location of major and important minor genes controlling resistance against septoria tritici blotch of some of the resistant materials

found, using chromosome substitution lines of the susceptible *Triticum aestivum* cultivar Chinese Spring;
3. To assess to what extent resistance is isolate specific;
4. To assess how well resistance recorded as necrosis percentage is correlated with resistance recorded as pycnidial coverage percentage over a diverse set of genotypes and isolates;
5. To assess whether resistance in the seedling stage is related to resistance in the adult stage.

Materials and methods

Preliminary screening

Two preliminary screenings were carried out to select the sets of substitution lines and the isolates of the fungus to be used. The first screening included 15 parents of a monosomic series of substitution lines and the susceptible cultivar Shafir as a tester. It was carried out at the former research institute IPO-DLO, The Netherlands, in 1995. Genotypes were the *T. aestivum* cultivars Bezostaya, Cappelle-Desprez, Cheyenne, Chinese Spring, Favorits, Hobbit Sib, Hope, Lutescens, Mara, Poros, Shafir, Sinvalocho, Timstein, the synthetic hexaploid Synthetic 6x [(*Triticum dicoccoides* × *T. tauschii* (Sears, 1976)], *T. macha* and *T. spelta*.

The first preliminary screening was done in small pots in a growth chamber at 20–22 °C and 85–90% relative humidity in a complete randomized design with two replications (pots). Five to ten seeds were sown per genotype per replication. Plants were vernalized for one week at 4–8 °C because of the cold requirements of some genotypes. Plants were inoculated at the 1-leaf stage. Seven isolates from Argentina (IPO 86068, IPO 92061, IPO 92064, IPO 92065, IPO 92066, IPO 92067 and IPO 93014) and three from the Netherlands (IPO 001, IPO 290 and IPO 323) were grown in petri dishes on V8 juice agar for 3 days and transferred to yeast-glucose liquid medium. Flasks were shaken for 5 days at 18 °C. Spores were suspended in distilled water and the conidial suspension was adjusted to 10^7 spores ml^{-1}. One ml of Tween 20 per litre was added as a surfactant. After inoculating the plants by spraying the suspension, plants were covered with transparent polythene to maintain high humidity levels. Necrosis and pycnidial coverage were scored 21–22 days after inoculation and their relationship was assessed. Data were arcsine transformed and analysed with ANOVA.

The second preliminary screening included 5 genotypes (Cappelle-Desprez, Cheyenne, Synthetic 6x, *T. spelta* and Chinese Spring) and 4 isolates (IPO 92064, IPO 92065, IPO 92067 and IPO 93014). They were planted at the Department of Plant Sciences, Wageningen University, The Netherlands, in 1999, in a growth chamber with conditions and experimental design similar to the ones in the first preliminary screening. Genotypes and isolates were selected according to their performance in the first preliminary screening and taking into account the availability of substitution lines. Vernalization, inoculation and evaluation in the seedling stage were performed as described for the first screening experiment. At tillering, the plants were transplant-

ed to 10-litre pots in a greenhouse at 14–17 °C and 75% relative humidity after an adaptation period of 3 days at 12 °C. The plants were inoculated at boot stage (GS 49; Zadoks *et al.*, 1974) for evaluation at the adult stage by spraying suspensions of the same isolates as in the seedling stage. After inoculation, plants were covered with a transparent polythene tent to maintain humidity at very high levels for 72 h. After that, conditions in the greenhouse were 18–22 °C and 80–85% humidity, the latter being maintained by means of a humidifier. Necrosis percentage and pycnidial coverage percentage were scored 24–25 days after inoculation and their relation was assessed. Data were arcsine transformed and analysed by a combined ANOVA for the growth chamber (seedling stage) and greenhouse (adult stage) environments. A protected LSD test ($P = 0.05$) was used for means separation.

From these screenings, four sets of substitution lines and two isolates for each of them were selected for final experimentation in the seedling stage. Parents of these sets showed differences in resistance to septoria tritici blotch with the selected isolates at seedling stage. We selected the substitution lines of Synthetic 6x, Cheyenne, Cappelle-Desprez and *T. spelta*, and decided to use the isolate IPO 92067 (all sets of substitution lines), IPO 93014 (substitution lines of Synthetic 6x, Cappelle-Desprez and *T. spelta*) and IPO 92064 (substitution lines of Cheyenne). Furthermore, two sets were selected for evaluating resistance in the adult stage: lines of Synthetic 6x and lines of Cheyenne.

Substitution lines used were developed by C.N. Law and A.J. Worland at the John Innes Centre, Norwich, UK, and by Rosalind Morris, University of Nebraska, USA.

Final experiments

Seedling stage

Two final experiments were carried out with four sets of substitution lines of the 21 chromosomes of Synthetic 6x, Cheyenne, Cappelle-Desprez and *T. spelta* as resistant parents in the susceptible Chinese Spring. The first experiment was planted in a growth chamber at the Department of Plant Sciences, Wageningen University, The Netherlands on 27 July 1999. The second was planted in the outdoor experimental facilities of the Facultad de Ciencias Agrarias y Forestales, La Plata, Argentina on 13 July 2000.

In both environments, the four sets of substitution lines were sown together with the parents in 10-litre pots in a randomized block design with two replications for each isolate. In each pot 6 to 8 seeds were sown. Genotypes were vernalized for 3 weeks at 4–8 °C. In 1999, the seeds were vernalized after sowing (in the growth chamber) and in 2000 in a growth chamber before sowing in pots outdoors.

The sets with Synthetic 6x, Cappelle-Desprez and *T. spelta* were inoculated with the Argentinian isolates named IPO 92067 and IPO 93014 by the former IPO-DLO, Wageningen, The Netherlands. The set with Cheyenne was inoculated with the Argentinian isolates named IPO 92067 and IPO 92064. Isolate IPO 92064 was used instead of IPO 93014 because it gave better discrimination between the parents of the Cheyenne set.

In 1999, the isolates were grown as described for the preliminary screening experi-

ments. In 2000, the isolates were grown in petri dishes on agar potato medium and transferred onto malt extract agar. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionized water. The conidial suspension was adjusted in both experiments to 10^7 spores ml^{-1} and 1 ml of Tween 20 per litre was added as a surfactant. Plants were inoculated at the 1-leaf stage. After the inoculation, both experiments were covered with transparent polythene to maintain high humidity conditions for 48 hours. During 1999 (growth chamber experiment), conditions after inoculation were 20–22 °C and 85–90% relative humidity. During 2000 (outdoor experiment), the average conditions after the first 48 h until evaluations were: mean temperature 12.6 °C, mean relative humidity 75% and 45 mm of rainfall distributed over 10 days.

Plants were scored 21–22 days after inoculation. Necrosis (%) and pycnidial coverage (%) were recorded. Data were arcsine transformed and analysed by a combined ANOVA for both environments, but separately for each set of lines by isolate combination. The protected LSD test ($P = 0.05$) was used for means separation. Linear correlation between necrosis and pycnidial coverage was also performed.

Adult stage

Synthetic 6x/Chinese Spring and Cheyenne/Chinese Spring sets were also inoculated at the flag leaf stage (GS 49; Zadoks *et al.*, 1974) with isolates IPO 92067 and IPO 93014 for the Synthetic 6x set, and with isolates IPO 92067 and IPO 92064 for the Cheyenne set. Inoculum was prepared and conditions immediately after inoculation were as described previously. Conditions after inoculation in 1999 (growth chamber experiment) were similar to those for the seedling testing. In 2000 (outdoor pot experiment), mean temperature after the first 48 h until evaluation was 16.9 °C, mean relative humidity 89.4% and rainfall 66.5 mm distributed over 14 days.

Twenty-five days after inoculation, necrosis and pycnidial coverage were scored on the two upper leaves of each plant. Averages of the two leaves were arcsine transformed and analysed in a combined ANOVA for both environments, but separately for each set of lines by isolate combination. The protected LSD test ($P = 0.05$) was used for means separation. Linear correlation between necrosis and pycnidial coverage in the adult stage and between resistance scores of the two development stages was also assessed.

Results

Preliminary screening

The first preliminary screening (1995) in the seedling stage showed a very close, linear correlation between the values for necrosis percentage and those for pycnidial coverage percentage across genotypes and isolates ($R^2 = 0.790$; n = 160), although *T. spelta* showed lower pycnidial coverage than expected on the basis of necrosis values (data set without *T. spelta*: $R^2 = 0.856$; n = 150). We therefore only show the data on pycnidial coverage percentage (Table 1).

Table 1. Mean percentages of pycnidial coverage (untransformed values) in the first screening (1995) in the seedling stage of 16 *Triticum* genotypes exposed to 7 Argentinian and 3 Dutch isolates of *Mycosphaerella graminicola*. Genotype by isolate combinations in bold were also used in the second screening.

Genotype	Argentinian isolates							Dutch isolates			Average of genotype
	IPO 86068	IPO 92061	**IPO 92064**	**IPO 92065**	IPO 92066	**IPO 92067**	**IPO 93014**	IPO 001	IPO 290	IPO 323	
Synthetic 6x	3ab[1]	0a	**3a**	**1ab**	0a	**0a**	**1a**	0a	0a	0a	1a
Mara	0a	7ab	3a	16abc	9abcd	17abc	14abc	0a	5ab	11a	8ab
Cappelle-Desprez	0a	0a	**12ab**	**3ab**	5abc	**15ab**	**31bcde**	0a	27abcd	46b	14bc
T. spelta	2ab	1a	**1a**	**0a**	0a	**0a**	**0a**	58d	34cd	56d	15bcd
Hobbit Sib	0a	13ab	25abc	14abc	5abc	12ab	8ab	1ab	30bcd	53c	16bcd
Sinvalocho	13abc	22abcd	35bcd	29bcd	37def	44cdef	13abc	4abc	0a	1a	20cd
Bezostaya	4ab	46cdefg	28abc	73g	1ab	5a	24abcd	0a	20abc	0a	20cd
T. macha	11abc	50defg	14ab	16abc	3ab	38bcde	50defg	3abc	15abc	1a	20cd
Cheyenne	0a	61g	**3a**	**63eg**	13abcd	**1a**	**58efg**	3abc	38cd	1a	24de
Chinese Spring	19abc	20abc	**36bcd**	**51defg**	29bcde	**64efg**	**46defg**	14abc	43cde	0a	32ef
Favorits	30bc	35bcdefg	61def	67fg	32cde	25abcd	42defg	6abc	36cd	0a	33ef
Poros	35c	42cdefg	53cdef	36cde	48ef	57e	37cdef	29c	37cd	0a	37fg
Shafir	24abc	55fg	66ef	56defg	44ef	51defg	67g	16abc	54de	0a	44gh
Timstein	20abc	51efg	45cde	54defg	63f	75g	58efg	25abc	43cd	3a	44gh
Lutescens	35c	26abcde	75f	68fg	37def	68fg	61fg	9abc	54de	50b	48h
Hope	29bc	43cdefg	73ef	49defg	43ef	60efg	70g	29bc	71e	57d	52h
Average of isolate	14a	29cd	3de	37e	23bc	33de	36d	12a	32d	17ab	27

[1] Means in the same column for genotypes within each isolate of the fungus and for the average of genotypes, followed by the same letter are not statistically different ($P = 0.05$). Means in the same row for the averages of isolates of the fungus, followed by the same letter are not statistically different ($P = 0.05$).

Table 2. Mean percentages of necrosis (untransformed data) in the second preliminary screening (1999) of 5 *Triticum* genotypes exposed to four Argentinian isolates of *Mycosphaerella graminicola* in the seedling and adult stages. Genotype by isolate combinations with values in bold are used in the final experiments.

Genotype	*Mycosphaerella* isolate								Average of genotype	
	IPO 92064		IPO 92065		**IPO 92067**		**IPO 93014**			
Stage:	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult
Synthetic 6x	4a[1]	10a	4a	24a	**1a**	**5a**	**3a**	**24a**	3a	16a
Cheyenne	**19ab**	**16a**	52c	22a	**5a**	**7a**	51b	26a	32c	18a
T. spelta	5a	35b	6a	33a	**5a**	8a	**4a**	46a	5a	30b
Cappelle-Desprez	26b	50b	23b	68b	**12a**	17a	**15a**	34a	19b	42c
Chinese Spring	80c	49b	90d	32a	90b	42b	91c	32a	88d	39c

[1] Means within the same column, followed by the same letter are not statistically different ($P = 0.05$).

Differences between genotypes, isolates and interactions between genotypes and isolates were all statistically significant. Some materials such as the synthetic hexaploid (Synthetic 6x) was very resistant to all of the 10 isolates of *M. graminicola* tested at the seedling stage. Chinese Spring proved to be susceptible or moderately susceptible to all isolates, except to IPO 323, to which it was resistant. For all materials, except for Hope, high levels of resistance (less than 20% of necrosis, data not shown; below 10% pycnidial coverage, Table 1) were found with at least one of the isolates.

For the second preliminary screening, the four selected genotypes were Synthetic 6x, *T. spelta* and the *T. aestivum* cultivars Cheyenne and Cappelle-Desprez, whereas the selected isolates were IPO 92064, IPO 92065, IPO 92067 and IPO 93014; see bold combinations in Table 1. These combinations showed acceptable levels of resistance. We also selected Chinese Spring for this screening as chromosome substitution lines of the four genotypes into Chinese Spring were available.

Also for the second preliminary screening, the linear correlation between necrosis and pycnidial coverage was very high. We only show the necrosis data (Table 2). In the seedling stage, Chinese Spring was susceptible to all four isolates, Synthetic 6x, *T. spelta* and Cappelle-Desprez were resistant or moderately resistant to all isolates, but in Cheyenne results depended on the isolate. In the adult stage, Synthetic 6x and Cheyenne were resistant or moderately resistant to all isolates, Chinese Spring was susceptible or moderately susceptible and for Cappelle-Desprez and *T. spelta* results depended on the isolate (Table 3).

Based on the results of both preliminary screenings, isolates IPO 92067 and IPO 93014 were selected for the inoculation of Synthetic 6x, Cappelle-Desprez and *T. spelta* and isolates IPO 92064 and IPO 92067 for the inoculation of Cheyenne in the seedling stage. Furthermore, isolates IPO 92067 and IPO 93014 were selected for the inoculation of Synthetic 6x and IPO 92064 and IPO 92067 for the inoculation of Cheyenne in the adult stage. See bold genotype by isolate combinations in Table 2.

Table 3. Results of the ANOVA four sets of substitution lines grown in two environments (years) in the seedling stage. Given are the mean squares for necrosis percentage and pycnidial coverage percentage (after transformation), exposed to different virulent isolates of *Mycosphaerella graminicola*.

		Sets of substitution lines							
		Synthetic 6x / Chinese Spring		Cheyenne / Chinese Spring		Cappelle-Desprez / Chinese Spring		*T. spelta* / Chinese Spring	
	Isolate:	IPO 92067	IPO 93014	IPO 92067	IPO 92064	IPO 92067	IPO 93014	IPO 92067	IPO 93014
Necrosis percentage									
Source of variation	df[1]								
Line	22	946 (0.000)[2]	973 (0.000)	594 (0.000)	652 (0.000)	509 (0.000)	570 (0.000)	629 (0.000)	435 (0.000)
Environment	1	14277 (0.000)	4250 (0.000)	14239 (0.000)	4599 (0.000)	33622 (0.000)	6515 (0.000)	25064 (0.000)	3967 (0.000)
Environment × Line	22	292 (0.010)	307 (0.327)	111 (0.145)	201 (0.265)	112 (0.225)	68 (0.492)	257 (0.000)	78 (0.000)
Error	45	130	264	77	164	86	77	60	62
Pycnidial coverage percentage									
Source of variation	df								
Line	22	772 (0.000)	809 (0.001)	600 (0.000)	410 (0.003)	483 (0.000)	344 (0.000)	501 (0.000)	303 (0.000)
Environment	1	21231 (0.000)	8129 (0.000)	23342 (0.000)	3081 (0.000)	28196 (0.000)	6925 (0.000)	24205 (0.000)	7632 (0.000)
Environment × Line	22	338 (0.001)	285 (0.440)	175 (0.267)	142 (0.582)	88 (0.480)	89 (0.366)	183 (0.013)	163 (0.000)
Error	45	90	274	142	156	88	79	50	31

[1] df = degrees of freedom.

[2] Mean with probability in parentheses.

Final experiments

Seedling stage

There were statistically significant differences for necrosis and pycnidial coverage percentages between lines and between environments (1999 and 2000) for the four sets of substitution lines in the seedling stage. There was also a statistically significant environment × line interaction for the set Synthetic 6x/Chinese Spring with the isolate IPO 92067 and for the set *T. spelta*/Chinese Spring with isolates IPO 92067 and IPO 93014. This was observed for both resistance parameters (Table 3).

The linear correlation (across genotypes, isolates, years and substitution lines) between necrosis and pycnidial coverage in the seedling stage was highly significant ($R^2 = 0.763$; n = 368; when data for *T. spelta* were excluded: $R^2 = 0.797$; n = 276; correlation for *T. spelta*: $R^2 = 0.700$; n = 92). We therefore only show data on pycnidia coverage.

Necrosis (data not shown) and pycnidial coverage percentages (Tables 4 and 5) were higher in 1999 than in 2000. This was caused by the fact that the conditions in the 1999 growth chamber experiment were more suitable for the development of the disease than the outdoor conditions in 2000.

When tested in the seedling stage and taken into account both resistance parameters, all chromosomes seemed to carry genes effective against *M. graminicola*. This phenomenon is partly illustrated by the bold values in Tables 4 and 5. Most genes were effective against only one isolate or in only one environment or its effect only showed in one resistance parameter. Only chromosome 7D of Synthetic 6x was found with a major effect against both isolates tested (Table 4).

For the set Cheyenne/Chinese Spring, the line carrying chromosome 1B (average of both environments) for the isolate IPO 92067 showed higher levels of resistance (expressed as reduction in necrosis percentage (data not shown) and pycnidial coverage (Table 4)) than the susceptible parent but not as high as the resistant one, suggesting the presence of partial resistance.

For the Cappelle-Desprez/Chinese Spring set, the average of both environments showed three lines (those carrying chromosome 2B, 3A or 3B; bold values in Table 5) with higher levels of resistance than Chinese Spring. This was visible in both the necrosis percentage (data not shown) and the pycnidial coverage percentage (Table 5) for the isolate IPO 92067. No chromosomes conferring higher resistance than Chinese Spring (expressed as reduction in pycnidial coverage) could be detected for the average of isolate IPO 93014 over both environments, although some effects were present in only one of the environments (Table 5).

For the *T. spelta*/Chinese Spring set, the line carrying chromosome 7D showed similar levels of resistance expressed as a reduction in the two resistance components (only pycnidial coverage shown, see Table 5) compared with the resistant parent or at least higher than the susceptible parent in both environments and for the average of them for isolate IPO 92067. Lines carrying some other chromosomes such as 2D, 5A and 6D showed better resistance expressed as reduction in necrosis percentage than the susceptible parent (data not shown) or even similar to *T. spelta* also in the two environments and for the average of them. For pycnidial coverage (Table 5) minor resist-

Table 4. Mean percentages pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of Synthetic 6x, the *T. aestivum* cultivar Cheyenne and the sets of 21 chromosome substitution lines of these genotypes in Chinese Spring. Values in bold are lines that are significantly more resistant than the susceptible parent.

	Sets of substitution lines											
	Synthetic 6x / Chinese Spring						Cheyenne / Chinese Spring					
Isolate:	IPO 92067		IPO 93014	IPO 92067			IPO 92064					
Year:	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	89bcdef[1]	51ef	70bcd	47b	29bcd	38b	97ef	48def	72d	89defg	40abc	65bcdefg
1B	78bcde	25abcde	51b	70bcd	40cdef	55bcd	**37b**	30bcde	**34b**	63bc	45abcd	54bcd
1D	87bcdef	42bcde	64bcd	78bcd	35cde	56bcd	90cde	53ef	71cd	70bcd	42abc	56bcd
2A	78bcdef	42bcde	60bcd	71bcd	41cdef	56bcd	96def	56f	76d	88efg	71ef	80fg
2B	83bcdef	77f	80d	59bc	58efg	59bcd	86cde	50ef	68cd	96g	50bcd	73defg
2D	88bcdef	33bcde	61bcd	84cde	65g	75d	87cde	36bcdef	62cd	60bc	42abc	51bc
3A	75bcd	46cde	60bcd	72bcd	69g	70bcd	74cd	35bcdef	54bcd	51b	37ab	44ab
3B	90bcdef	27bcde	59bcd	80cde	20bc	50bcd	93def	33bcdef	63cd	94fg	68cdef	81g
3D	81bcdef	22abcde	51b	68bcd	38cdef	53bcd	92cdef	43cdef	67cd	76bcde	68cdef	72bcdefg
4A	100g	34bcde	67cd	83cde	46cdef	65bcd	94def	29bcd	61cd	89defg	62bcde	75cdefg
4B	87bcdef	39bcde	63bcd	88de	56defg	72cd	73c	22bcd	47bc	66bc	68cdef	67bcdefg
4D	88bcdef	28bcde	58bcd	59bc	30cd	45bc	92cdef	48def	70cd	77bcde	45abcd	61bcdef
5A	75bcd	28bcde	51b	78bcde	38cdef	58bcd	88cde	39bcdef	64cd	70bcd	63bcde	66bcdefg
5B	72bc	33bcde	52b	99e	26bc	63bcd	96def	49def	72d	91efg	66bcde	79defg
5D	88bcdef	30bcde	59bcd	60bc	55defg	57bcd	92cdef	31bcde	61cd	72bcde	70def	71bcdefg
6A	97fg	16ab	57bcd	87cde	18bc	53bcd	84cde	42cdef	63cd	74bcde	85f	80efg
6B	89bcdef	17abc	53b	73bcd	37cde	55bcd	70c	34bcdef	52bcd	69bcd	66bcde	68bcdefg
6D	95efg	38bcde	66bcd	59bc	52defg	56bcd	91cdef	14ab	53bcd	54b	38ab	46ab
7A	**64b**	48de	56bcd	73bcd	35cde	54bcd	78cd	33bcdef	55bcd	79cdef	48abcd	64bcdefg
7B	92defg	20abcd	56bcd	81cde	45cdef	63bcd	99f	39bcdef	69cd	85cdef	49abcd	67bcdefg
7D	**1a**	**6a**	**3a**	**0a**	**5a**	**3a**	92cdef	20abc	56cd	77cdef	63bcde	70bcdefg
Chinese Spring	92cdefg	30bcde	61bcd	69bcd	39cdef	54bcd	92cdef	30bcde	61cd	69bcd	60bcde	64bcdefg
Resistant parent	1a	6a	3a	0a	7ab	4a	0a	4a	2a	15a	22a	18a

[1] Means within the same column, followed by the same letter are not statistically different ($P = 0.05$).

Table 5. Mean percentages pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of the *T. aestivum* cultivar Cappelle-Desprez, *T. spelta* and the sets of 21 chromosome substitution lines of these genotypes in Chinese Spring. Values in bold are lines that are significantly more resistant than the susceptible parent.

	Sets of substitution lines											
	Cappelle-Desprez / Chinese Spring						*T. spelta* / Chinese Spring					
Isolate:	IPO 92067			IPO 93014			IPO 92067			IPO 93014		
Year:	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	80bcd[1]	40e	60bcd	76def	45cd	60def	70d	45d	58fgh	92h	46b	69i
1B	91d	**16bcd**	54bcd	66bcde	**14ab**	40bc	83de	30bcd	56fgh	60cde	35ab	48cdef
1D	87bcd	35de	61bcd	**48b**	40c	44bcde	90e	30bcd	60h	72defg	35ab	53defgh
2A	91d	27cde	59bcd	72cdef	63d	67f	80de	13ab	46defg	84gh	45b	65hi
2B	79bcd	**15bc**	**47b**	69bcde	**15a**	42bcde	82de	14ab	48defg	75efg	31ab	53defgh
2D	89bcd	30de	59bcd	74def	30bc	52bcdef	60bcd	10ab	**35cd**	80gh	36ab	58fgh
3A	**71b**	**15bc**	**43b**	64bcde	25bc	45bcde	83de	30bcd	56fgh	75efg	30ab	53cdefgh
3B	**74bc**	**20bcd**	**47b**	60bcde	35c	47bcdef	90e	28bcd	59h	85gh	34ab	60ghi
3D	87bcd	35de	61cd	66bcde	45cd	56bcdef	90e	20abcd	55gh	55bcd	35ab	45cdefg
4A	90cd	**25bc**	57bcd	58bcd	33bc	46bcde	71d	33bcd	52defg	70def	30ab	50cdefgh
4B	83bcd	**15bc**	49bc	80ef	45cd	63ef	90e	20abcd	55gh	55bcd	33ab	44cde
4D	89cd	35de	62cd	86f	45cd	65ef	73de	13ab	43cdef	47bc	33ab	40c
5A	87bcd	35de	61bcd	73def	45cd	59def	66cd	16abcd	41cdef	72defg	33ab	52cdefg
5B	88bcd	35de	61cd	80ef	43cd	61ef	80de	16abc	48defg	44bcd	30ab	37c
5D	83bcd	**23bc**	53bcd	72cdef	45cd	59cdef	68d	13ab	**40cde**	78fgh	35ab	57fgh
6A	92d	35de	63bcd	57bcd	45cd	51bcdef	60bcd	20abcd	**40cde**	72defg	35ab	54defgh
6B	**73bc**	30de	51bcd	79ef	40c	60def	69d	43cd	56fgh	62cdef	35ab	48cdefg
6D	86bcd	35de	61bcd	54bcd	45cd	50bcdef	**35bc**	10ab	**23bc**	**38b**	**15a**	**26b**
7A	80bcd	35de	57bcd	70cde	45cd	57cdef	90e	20abcd	55gh	75efg	34ab	54defgh
7B	82bcd	35de	58bcd	68cde	40c	54bcdef	69d	25bcd	47defg	58bcde	45b	52cdefg
7D	84bcd	28cde	56bcd	53bc	28bc	40bcd	**30b**	**4a**	**17ab**	82gh	30ab	56fgh
Chinese Spring	92d	43e	67cd	69cde	43cd	56bcdef	79de	30bcd	54fgh	60cde	43b	51cdefg
Resistant parent	1a	0a	1a	4a	7a	6a	3a	6a	4a	0a	25a	12a

[1] Means within the same column, followed by the same letter are not statistically different ($P = 0.05$).

ance genes seem to be present in chromosomes 2D, 5D, 6A and 6D. For isolate IPO 93014, lines carrying chromosome 6D, 7B or 4B showed higher levels of resistance expressed as reduction in necrosis percentage than the susceptible parent did for both isolates in both environments and for the average of them (data not shown). For pycnidial coverage only lines with chromosome 7D (for isolate IPO 92067) or 6D (for isolate IPO 93014) showed higher resistance than the susceptible parent for each environment and for the average of them.

Adult stage

There were statistically significant differences for necrosis and pycnidial coverage percentages between lines and environments (1999 and 2000) for the two sets of substitution lines in the adult stage. The environment × line interactions were not statistically significant (Table 6).

The linear correlation (across genotypes, isolates, years and substitution lines) between necrosis en pycnidial coverage in the seedling stage was highly statistically significant ($R^2 = 0.812$; n = 184). We therefore only show data on pycnidia coverage.

Table 6. Results of the ANOVA for two sets of substitution lines grown in two environments (years) in the adult stage. Given are the mean squares for necrosis percentage and pycnidial coverage percentage (after transformation), exposed to two virulent isolates of *Mycosphaerella graminicola*.

		Sets of substitution lines			
		Synthetic 6x / Chinese Spring		Cheyenne / Chinese Spring	
	Isolate:	IPO 92067	IPO 93014	IPO 92067	IPO 93014
Necrosis percentage					
Source of variation	df[1]				
Line	22	398 (0.002)[2]	532 (0.001)	434 (0.000)	564 (0.000)
Environment	1	363 (0.125)	7440 (0.000)	2821 (0.000)	7922 (0.000)
Environment × Line	22	199 (0.199)	163 (0.606)	134 (0.337)	248 (0.172)
Error	45	149	183	117	186
Pycnidial coverage percentage					
Source of variation	df				
Line	22	538 (0.000)	568 (0.000)	519 (0.000)	744 (0.000)
Environment	1	291 (0.195)	2386 (0.000)	4054 (0.000)	6958 (0.000)
Environment × Line	22	151 (0.599)	200 (0.384)	129 (0.332)	211 (0.267)
Error	45	168	183	112	171

[1] df = degrees of freedom.

[2] Mean with probability in parentheses.

When tested in the adult stage and taken into account both resistance parameters, all lines but the one carrying chromosome 4B from the resistant parent seemed to show genes effective against *M. graminicola*. The line carrying chromosome 7D from Synthetic 6x showed a level of resistance similar to the resistant parent for isolate IPO 92067 (necrosis percentage data not shown; for pycnidial coverage see Table 7). Major genes effective against both isolates were also found on chromosomes 5A and 5D from Synthetic 6x (Table 7). Some other small effects against isolate IPO 92067 were not consistent over environments. For isolate IPO 93014 lines carrying chromosome 4A, 5A, 5D, 6D, 7A or 7B showed higher levels of resistance than Chinese Spring or even a resistance similar to Synthetic 6x (expressed as reduction in necrosis percentage in the two environments; data not shown). Lines carrying chromosome 5A, 5D or 6D also showed to carry resistance (expressed as reduction in pycnidial coverage; Table 7). Also for this isolate some other chromosomes showed small effects in one environment only (Table 7).

For the Cheyenne set, the line carrying chromosome 1B showed similar levels of resistance as the resistance parent with isolate IPO 92067, whereas the lines with chromosome 2B or 5D showed higher levels of resistance than the susceptible parent (expressed as necrosis percentage for the average of the environments; data not shown). When both environments were considered separately only the line with chromosome 1B showed a similar level of resistance compared with the resistant parent. Lines with some other chromosomes showed some resistance in one of the two environments, but not for pycnidial coverage (except for line with chromosome 1B; Table 7). With the isolate IPO 92064, lines carrying chromosome 1B, 5D or 6D showed similar levels of resistance as the resistant parent or a resistance higher than the susceptible parent (expressed as reduction in pycnidial coverage in both environments and usually also for the averages of them; Table 7), whereas lines with chromosome 4A or 4D showed to carry some resistance expressed as reduction in necrosis percentage or pycnidial coverage (Table 7). Lines with some other chromosomes only showed some levels of resistance in one of the two environments.

Correlation between resistance in seedling stage and adult stage

For two sets of substitution lines, resistance was assessed both in the seedling stage and in the adult stage. For necrosis percentage there was a highly significant linear correlation between the resistance in these two stages ($R^2 = 0.242$; $n = 184$), but the variance in resistance in the adult stage accounted for by the levels in the seedling stage was low. This was also true for the resistance measured as the reduction in pycnidial coverage ($R^2 = 0.200$; $n = 184$).

Discussion

In the preliminary screening experiments, despite similar controlled environmental conditions, some differences in level of resistance were observed. These might be attributed to the duration of vernalization. In general, higher levels of resistance were found in the resistant parents in the second preliminary screening, where vernalization was longer.

Table 7. Mean percentages pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the adult stage of Synthetic 6x, the *T. aestivum* cultivar Cheyenne and the sets of 21 chromosome substitution lines of these genotypes in Chinese Spring. Values in bold are lines that are significantly more resistant than the susceptible parent.

	Sets of substitution lines											
	Synthetic 6x / Chinese Spring						Cheyenne / Chinese Spring					
Isolate:	IPO 92067			IPO 93014			IPO 92067			IPO 92064		
Year:	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	44de[1]	31defg	37cde	74fgh	56g	65gh	62efg	27bcd	45efgh	40cd	**29d**	35bcde
1B	58efg	41efg	49def	41bcd	38defgh	40bcdefgh	30bc	19bc	**24b**	**15b**	**10bc**	**12b**
1D	71fg	40ef	55ef	79ghi	33cdef	56efgh	50def	45def	48gh	48cdef	38defg	43cdef
2A	76g	29defg	52def	56def	35cdefg	46cdefgh	55defg	35cde	45efgh	73ghijkl	**13bcd**	43cdef
2B	35bcde	28defg	32cdef	30bc	**13ab**	21abc	29bc	29bcd	29bc	35c	30defg	33bcde
2D	55efg	65h	60ef	48cde	63h	55efgh	38bc	38cdef	38bcdef	91l	38defg	64f
3A	**18b**	47fgh	32cde	51def	**15bc**	33bcdef	49cde	31bcdef	40cdefg	87jkl	40defg	63f
3B	48de	31defg	40cde	38bcd	60h	49cdefgh	47cde	36cdef	42cdefg	57cdefg	52g	54def
3D	**20bc**	49gh	35cde	59def	43cdef	51defgh	57defg	32bcde	45efgh	68hijkl	44efg	56ef
4A	35cde	31defg	33cde	46cde	**15bc**	31bcdef	31bc	31bcd	31bcde	48cde	**23bcd**	35bcde
4B	70g	66h	68f	66efg	38defgh	52defgh	56defg	49f	52gh	80l	44efg	62f
4D	53ef	46fgh	50def	91i	33cdef	62gh	64fg	39cdef	51gh	58defgh	**8b**	33bcd
5A	**27bcd**	**23bcd**	25bcd	**24b**	**23bcde**	23abcd	68g	34cdef	51gh	60defgh	28cdef	44cdef
5B	43de	50gh	46def	53def	38defgh	45cdefgh	67g	42def	55h	86kl	36defg	61ef
5D	**3a**	**11bc**	**7ab**	**13ab**	**13ab**	**13ab**	27bc	33bcde	30bcd	**9b**	**10bc**	**9ab**
6A	69fg	**25bcde**	47def	83hi	43efgh	63h	46cd	37cdef	42cdefg	72ghijkl	40defg	56def
6B	44de	28cdef	36cde	59def	53fgh	56efgh	36bc	26bc	31bcde	68fghijk	**15bc**	41cdef
6D	**32bcd**	42ef	37cde	**18ab**	**29bcde**	23abcd	49cde	46ef	47gh	**15b**	**24bcd**	19bc
7A	**40d**	49fgh	45def	53def	**18bcd**	35bcdefg	46cd	31bcde	38bcdef	74ijkl	43defg	58ef
7B	41cde	49gh	45def	48cd	**15bc**	32bcdef	58defg	28bcd	43cdefg	62efghi	40defg	51def
7D	**21b**	**7ab**	**14abc**	31bc	33cdef	33bcdef	51def	40def	46fgh	80l	30defg	55def
Chinese Spring	60ef	42fg	51def	49cde	50fgh	49cdefgh	49cdef	38cdef	44cdefg	49cdef	46fg	48cdef
Resistant parent	5a	0a	2a	3a	11a	7a	6a	0a	3a	0a	0a	0a

[1] Means within the same column, followed by the same letter are not statistically different ($P = 0.05$).

Similarly to the results found in these experiments but with different isolates, Arraiano *et al.* (2001) observed on detached seedling leaves, that Synthetic 6x was completely resistant to most isolates from the Netherlands and from Portugal, except IPO 92006 from Portugal, whereas Chinese Spring was susceptible to all isolates except IPO 323, to which it was moderately resistant. We observed a high level of specificity in resistance against certain isolates and sometimes a significant genotype × environment interaction.

Information about chromosomal location of resistance to *M. graminicola* is scarce. In our results, resistance found in lines with chromosome 7D of Synthetic 6x to both isolates was almost complete, indicating that probably only one gene confers resistance to these isolates. In line with these results, Arraiano *et al.* (2001), using the substitution Synthetic 6x/Chinese Spring tested with the Dutch isolate IPO 94269, mapped a gene on the short arm of chromosome 7D, named *Stb5*, near the centromere. This may indicate that this gene is also effective to IPO 92067, IPO 93014 and IPO 94269. No information from other researchers is available about the other sets of chromosome substitution lines involved in this study.

Our results show that complete resistance and partial resistance are present in the pathosystem *M. graminicola*/*T. aestivum*. All chromosomes seemed to contribute to some extent to the resistance in the seedling stage, whereas in the adult stage only chromosome 4B failed to show any major or minor resistance genes.

Chromosome 7D from Synthetic 6x carries a major gene that confers resistance to some isolates in the seedling stage. In the adult stage, resistance conferred by Synthetic 6x was not as high as in the seedling stage. However, chromosomes 7D, 5A and 5D showed to carry major genes providing resistance to at least one isolate. Arraiano *et al.* (2001) found complete resistance in the adult stage in chromosome 7D from Synthetic 6x with isolate IPO 94269.

In the seedling stage, resistance in Cheyenne and Cappelle seems to be conferred by minor genes. In the adult stage the highest levels of resistance were conferred by chromosome 1B from Cheyenne for both isolates and by 6D and 5D for one isolate, suggesting the presence of major gene effects besides some minor ones.

T. spelta showed a high level of resistance in the seedling stage to isolate IPO 92067 in chromosome 7D besides minor gene effects in some other chromosomes.

In spite of some differences in the chromosomes conditioning resistance expressed as necrosis percentage and as pycnidial coverage percentage, the tendency was similar for both resistance components and they were highly correlated in the seedling stage and the adult stage. *T. spelta*, however, deviated from the general trend, thus reducing the overall correlation. Other researchers carried out experiments under optimal environmental conditions (Eyal *et al.*, 1987; Brown *et al.*, 1999) and also found high correlation coefficients between both resistance components. Necrosis without pycnidia formation is mostly expressed under sub-optimal environmental conditions by resistant cultivars (Brokenshire, 1975; Eyal *et al.*, 1987).

Resistance in the seedling stage was significantly correlated with resistance in the adult stage, but the predictive value of assessments in the seedling stage for the susceptibility in the adult stage was relatively low.

Location of the genes through the use of molecular markers will be the next step to

incorporate this resistance in commercial materials. At present we are investigating resistance in the seedling stage in chromosome 7D of *T. spelta*. Pyramidization of genes conditioning incomplete resistance is an important tool to obtain lines with durable resistance.

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