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ORIGINAL PAPER

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Yr32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V

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Abstract Stripe or yellow rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, is an important disease in many wheat-growing regions of the world. A number of major genes providing resistance to stripe rust have been used in breeding, including one gene that is present in the differential tester Carstens V. The objective of this study was to locate and map a stripe rust resistance gene transferred from Carstens V to Avocet S and to use molecular tools to locate a number of genes segregating in the cross Savannah/Senat. One of the genes present in Senat was predicted to be a gene that is present in Carstens V. For this latter purpose, stripe rust response data from both seedling and field tests on a doubled haploid population consisting of 77 lines were compared to an available molecular map for the same lines using a non-parametric quantitative trait loci (QTL) analysis. Results obtained in Denmark suggested that a strong component of resistance with the specificity of Carstens V was located in chromosome arm 2AL, and this was consistent with chromosome location work undertaken in Australia. Since this gene segregated independently of *Yr1*, the only other stripe rust resistance gene known to be located in this chromosome arm, it was designated *Yr32*. Further QTLs originating from Senat were located in chromosomes 1BL, 4D, and 7DS and from Savannah on 5B, but it was not possible to characterize them as unique resistance genes in any definitive way. *Yr32* was detected in several wheats, including the North American differential tester Tres.

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Introduction

Stripe or yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), continues to be a major problem of wheat production in many parts of the world (Roelfs et al. 1992; Chen et al. 2002). Although chemical control can be very effective and is the preferred means of control in some areas, resistance remains a major objective for many breeding programs, especially in lower yielding regions and developing countries. A significant problem with resistance to *Pst* is that of variable pathogen populations. When resistant cultivars are deployed in agriculture, preexisting virulent pathotypes of low frequency may be selected on the resistant hosts, or new virulences may arise from mutation (Wellings and McIntosh 1990) or asexual recombination and reassortment of nuclei in the dikaryotic fungus (Wright and Lennard 1980). As a consequence, breeders have emphasized the need to deploy resistances based on gene combinations assuming that any changes in virulence would be step-wise and less damaging and thus, would have lower impacts on farm incomes. However, certain gene combinations have been shown to fail after widespread exposure to the *Pst* population (Wellings and McIntosh 1990; Hovmøller 2001; Chen et al. 2002).

Resistance to rust diseases may comprise genes effective at the seedling and adult growth stage, genes effective at the post-seedling and adult stages only, or combinations of both types. Genes effective at the seedling stage tend to contribute greater effects on phenotype. Adult plant resistance genes with partial and additive effects are currently favored by breeders because of their reputed durability. Seedling-effective resistance genes are nevertheless present in cultivars and breeding

populations in different parts of the world, and detailed information on these genes is essential for a complete understanding of the host-pathogen genetics of stripe rust.

Wheat lines with seedling resistance also comprise the various sets of differential genotypes used currently to assess global variability, epidemiology, and movement of *Pst*. Unfortunately, the underlying genetic bases of resistance in a number of differentials remain poorly characterized. Carstens V is a genotype employed in the differential set of Johnson et al. (1972). Virulence on seedlings of Carstens V has been common in *Pst* populations in Central and Western Europe, but has been uncommon in other areas including Southeast Asia, Australasia, Southern Europe, and the Americas (Stubbs 1985). In 1995, Wellings (unpublished) identified pathotype 104 E169A-, with increased virulence on seedlings of Carstens V, among field isolates collected in New Zealand. In Denmark, virulence for cultivars possessing Carstens V resistance was widespread in the late 1980s but declined during the early 1990s (Hovmøller 2001). In 1996, the Danish *Pst* population became extinct and since the reappearance of *Pst* in 1997 and 1998, Carstens V virulence has been absent (Hovmøller 2001; Justesen et al. 2002).

Stubbs (1985) referred to a presumed gene in Carstens V as *YrCV* and correlated studies with an array of pathotypes indicated the presence of the same gene in cultivars Caribo, Cyrano, Felix, and Okapi. Chen and Line (1993) identified three genes, *YrCV1, YrCV2*, and *YrCV3*, in Carstens V conferring resistance to pathotype PST21 (formerly CDL21), which has many unusual avirulence characteristics. It was not established if any of the latter genes corresponded with *YrCV* of Stubbs (1985).

The present work, leading to the designation of *Yr32* to what is presumed to be *YrCV*, was carried out as independent projects in Australia and Denmark. The Australian work was an attempt to isolate and characterize the gene responsible for differentiating *Pst* pathotypes. The Danish work involved a study of a doubled haploid (DH) population of a cross between the winter wheat cultivars Savannah and Senat using pathotypes characterized in the greenhouse and the prevalent *Pst* flora in the field.

Materials and Methods

Australia

Chromosome location of Yr32

A homozygous resistant F_3 selection, CRW380, from Carstens V/3*Avocet S was chosen for monosomic analysis using a partially synthesized Avocet R monosomic series as the female parents. When the study of stripe rust-infected F_2 populations derived from monosomic F_1 plants failed to indicate a candidate chromosome, a study was undertaken by the second author to confirm the identities of the monosomic hybrids by a series of testcrosses with the corresponding ditelosomics and/or double ditelosomics of Chinese Spring. In addition further rust tests were performed on additional F_2 and various F_3, F_4 and F_5 homozygous resistant progenies of various monosomic plants. The testcrosses with the ditelosomics and double ditelosomics showed that some of the monosomic populations were incorrectly designated (Afshari 2000). The work to be reported here will be confined to the monosomic 2A population that was correctly designated.

Linkage of Yr32 and Yr1

A disomic plant (FA99.12216.5) from family 8 of Avocet R mono-2A/CRW380 was crossed with a near-isogenic line Chinese 166/6*Avocet S possessing *Yr1*. The genotypes of 80 F_2 plants derived from four F_1 plants were determined by testing their F_3 progeny separately with pathotypes 110 E143 A+ (PBI accession number 861725; avirulent on Carstens V and Chinese 166) and 111 E143 A+ (881732; avirulent on Carstens V and virulent on Chinese 166). Pathotype nomenclature was described by Wellings and McIntosh (1990). A+ and A- pathotypes were differentiated on seedlings of Avocet R, whereas Avocet S was susceptible to both pathotypes.

Postulation of Yr32 in the United States differential Tres

Tres, which was released as a stripe rust-resistant, soft white club wheat in North America in 1984, was initially thought to derive its resistance from *Triticum spelta*, a component of the pedigree (Chen and Line 1992). Virulence on Tres seedlings in the United States was first indicated in 1988 (Chen and Line 1992), although the occurrence of races PST51 and PST52 with virulence for Tres was not reported until 1992 (Chen et al. 2002). As part of an ongoing program, Tres was used as a resistance donor in an Australian backcrossing program using the recurrent susceptible parent Avocet S, which is devoid of genes for both seedling and adult plant resistances to most *Pst* populations. Tres, Carstens V, *T. spelta album*, Avocet S, and certain backcross derivatives and differential control cultivars were tested with *Pst* cultures with contrasting virulence combinations on seedlings of Carstens V and *T. spelta album*.

Seedling rust tests

Seedlings were grown in 9 cm diameter pots and were planted as populations of 15–20 seedlings or as three to four clumps, each of 15–20 seedlings. Inoculations were performed at the two-leaf stage using uredospores suspended in light mineral oil. The inoculated seedlings were placed in a dew chamber at 9°C for 12–20 h before being placed on greenhouse benches at 17–18°C. Stripe rust responses were determined after 14–16 days using a 0, (fleck), 1 to 4 infection type (IT) scale with ITs 3 and 4 being regarded as susceptible (Wellings and McIntosh 1990).

Denmark

The study in Denmark was part of a broader analysis of stripe rust resistance in 77 of the 106 DH derivatives of a cross between the winter wheats Savannah (Riband/Brigadier) and Senat (Ritmo/SJ7830), described previously in Eriksen et al. (2003). Both parents possess combinations of known and unknown seedling and, possibly adult plant, resistances. The presence of one or more genes from Carstens V in Senat was confirmed by the infection type patterns using 13 *Pst* isolates of diverse origin and virulence spectrum (M. S. Hovmøller, personal communication). The parents and DH lines were tested as seedlings with four pathotypes in the greenhouse and as adult plants in the field. These pathotypes were represented by isolates 71/93, 72/94, 08/97 (Justesen et al. 2002) and 70/99. The predominant pathotypes in the field were probably similar to isolates 08/97 and 70/99.

Seedling and adult plant rust tests

In the field, each parent and DH line was represented by a plot of six rows of 1 m. Naturally occurring stripe rust was assessed on the upper leaves (flag leaf and flag −1) using a 0–9 scale where 0 was fully resistant and 9 susceptible. Seeds for seedling tests were sown in mini-cap trays, five seeds per parent and DH line and 36 lines in each tray. Seedlings were inoculated when second leaves were 2/3 emerged by shaking a pot of the susceptible cultivar Bredo infected with the appropriate isolate, over the trays. The trays were kept in the dark at 10–12°C and high humidity for 24 h and subsequently incubated in a temperature-controlled greenhouse room at 17–19°C. After 17–19 days of incubation, ITs on seedlings were assessed on the following 0–9 scale (McNeal et al. 1971): 0 no visible symptoms, 1–2 necrotic or chlorotic flecks or blotches without sporulation, 3–6 necrotic or chlorotic blotches with increasing sporulation, 7–8 stripes without chlorosis and with moderate to abundant sporulation and 9 stripes with profuse sporulation. The seedling tests were performed in two replications.

Data analysis

A molecular linkage map of SSR (simple sequence repeat) and AFLP (amplified fragment length polymorphism) markers (Eriksen et al. 2003) was employed to map the stripe rust resistance phenotypes. SSR markers were assigned to chromosomes based on data from Röder et al. (1998), Chalmers et al. (2001), Gupta et al. (2002) and M. J. Christiansen (unpublished). The SSR markers were developed by the Wheat Microsatellite Consortium (*Xwmc*) and by Röder et al. (1998) (*Xgwm*). AFLP markers were named, MNN/PNN-###, where M = *Mse*I restriction enzyme, P = *Pst*I restriction enzyme, NN = primer number (primer sequences are found at: http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html) and ### = length of amplified fragment in bp. For the present study, the following modifications were made to the map: chromosomes 2A and 5B each consisted of two linkage groups which were joined, 5 SSR markers were added, and 7 AFLP markers were removed. In addition, the locus encoding ω-secalins (*Sec-1*) on rye chromosome 1RS of the 1BL/1RS translocation (Singh et al. 1990) present in Savannah was added to the map of 1B. The *Sec-1* locus was mapped using PCR primers developed from the sequence of the *Sec-1b* allele (Froidmont 1998). The location of the *Xwmc85* locus on 1BL is uncertain as two loci, on 1BS and on 1B close to the centromere, have been reported (M. J. Christiansen, unpublished). The allele sizes of Savannah and Senat matched the locus on 1BS. However, a 1BS location would have meant that the 1BL/1RS translocation was no longer intact, as recombination between *Sec-1* and *Xwmc85* (and *M48/P38–361*) was observed. This was considered unlikely and *Xwmc85* was placed on 1BL. After these modifications the linkage map included 72 SSR markers and 135 AFLP markers mapping to 17 of the 21 wheat chromosomes covering 1,770 cM.

Quantitative trait loci (QTL) analysis was applied to map the stripe rust resistance genes segregating in the population, using the software MAPQTL (Van Ooijen and Maliepaard 1996). A non-parametric Kruskal-Wallis analysis was performed due to non-normal distribution of the data. The Kruskal-Wallis (K) statistic is chi-square-distributed with one degree of freedom. Due to multiple tests, a more stringent significance threshold than $P \leq 0.05$ has to be applied. In this study a threshold of $P \leq 0.005$, equivalent to a K statistic of $K \geq 7.8$, was assumed. The approximate effect on disease resistance of a marker locus was estimated as the numerical difference in mean disease score between the Senat and the Savannah marker classes. For the seedling data, the magnitude of the QTL effects differed between the two replicated tests, but the location of the putative QTLs detected were the same. Therefore, only analysis of the average disease scores of the two replications is shown.

Results

Australia

Confirmation of the location of Yr32 in chromosome 2A

The cytological status, rust responses and mature heights of individual plants in F_3 lines derived from 16 F_2 plants from a monosomic F_1 of cross Avocet R monosomic 2A/CRW380 are summarized in Table 1. Six F_2 plants were disomic and 10 were monosomic. The disomic plants were homozygous resistant to stripe rust and were uniform in height equal to that of Avocet R. All monosomic plants gave low numbers of susceptible progeny with the appearance of nullisomy. This low

Table 1 Seedling rust responses, relative plant heights and mitotic chromosome numbers for F_3 families of Avocet R mono-2A/CRW380

Family	Number of F_3 seedlings with		Status of F_2 plant	Seedling stripe rust response (R:S[a])	Adult plant height	
	2*n*=42	2*n*=41			Equal to Avocet	Shorter than Avocet
5	6	0	Disomic	23:0	23	0
6	4	0	Disomic	22:0	19	0
7	7	0	Disomic	20:0	21	0
8	7	0	Disomic	23:0	24	0
14	7	0	Disomic	24:0	26	0
15	6	0	Disomic	19:0	25	0
1	1	2	Monosomic	22:1	6	17
2	1	3	Monosomic	24:1	7	20
3	0	3	Monosomic	19:2	4	15
4	2	3	Monosomic	24:2	7	22
9	1	3	Monosomic	18:1	5	16
10	2	5	Monosomic	19:2	6	17
11	1	3	Monosomic	22:1	4	15
12	1	3	Monosomic	20:1	5	16
13	1	3	Monosomic	21:2	7	22
16	1	2	Monosomic	22:1	6	18
Total for monosomics	11	30		211:14	57	178

[a] Segregating for very short plants with ITs 3+, presumably nullisomic

proportion of putative nullisomics (6.2%) was characteristic of what is expected for the breeding behavior of monosomics (Sears 1954). Six of the 14 susceptible plants survived transplantation in the field. All were weak and sterile, typical of nullisomics, as well as being susceptible to stripe rust. Finally, the variation in height in the monosomic populations was typical of that expected in homoeologous group 2 semidwarfs. Cytological studies had earlier confirmed that the monosomic chromosome was 2A.

Independent segregation of Yr32 and Yr1

Eighty F_2 plants derived from four F_1 plants were genotyped by testing their F_3 progeny separately with *Pst* pathotypes 110 E143 A+ and 111 E143 A+. The controls Chinese 166/6*Avocet S (Yrl) , Carstens V and Avocet S gave ITs O_i, iN and 3+ with pathotype 110 E143 A+ and 3+, iN and 3+ with pathotype 111 E143 A+. The *Yr32* locus was fully phenotyped using the second pathotype. However, within the *Yr32Yr32* genotypes, it was not possible to distinguish *Yr1Yr1* and *Yr1yr1* genotypes due to constraints of overlapping phenotypes (between 0; and ;N) and low seed numbers for certain lines. The two genotypes were pooled for the purpose of analysis. As the results for four F_1 families were homogeneous, data were pooled to obtain the following array of F_2 genotypes:

Yr32Yr32Yr1	12	*Yr32yr32Yr1Yr1*	10	*yr32yr32Yr1Yr1*	4
		Yr32yr32Yr1yr1	19	*yr32yr32Yr1yr1*	11
Yr32Yr32yr1yr1	3	*Yr32yr32yr1yr1*	15	*yr32yr32yr1yr1*	6

Yr32 and *Yr1* were genetically independent ($\chi^2_{3:1:2:4:2:1:2:1} = 4.45$, 7*df*, $P > 0.3$)

Postulation of Yr32 in the United States differential Tres

The data in Table 2 indicate that *Pst* pathotype 104 E169 A- produced a distinctly higher infection type on Carstens V (*Yr32*) compared to pathotypes 110 E143 A+ and 360 E137 A-. Distinctly different responses were recorded for the differential cultivars Lee (*Yr7*), Heines Kolben (*Yr6*) and *T. spelta album* (*Yr5*). The identical specific interaction between these pathotypes on Carstens V, Tres, and their respective backcross derivatives in Avocet S provided evidence for *Yr32* in these materials. However, there was evidence in this experiment of further resistance in Carstens V and Tres to the *Yr32*-virulent pathotype.

Denmark

Parental responses

Savannah was susceptible to the isolates 08/97 and 70/99 (Table 3). The IT 1–2 phenotypes with isolate 72/94 could be due to the presence of *Yr4*, *Yr17* or both genes, and in the case of the 71/93 isolate, *Yr9* should also be effective. The ITs produced on Senat can be explained by the

Table 2 Seedling infection type responses produced on selected wheat lines infected with contrasting Australian *Pst* pathotypes

Cultivar	Genotype	*Pst* pathotype 110 E143 A+ (861725)[a]	*Pst* pathotype 104 E169 A- (951501)[a]	*Pst* pathotype 360 E137 A- (841924)[a]
Carstens V	*Yr32*	;C	2+C	;C
Tres	*Yr32*	;	1=CN	0;
Carstens V/6*Avocet S	*Yr32*	1-C	2+3	;C
Tres/6*Avocet S	*Yr32*	;	3-	;
Lee	*Yr7*	33+	;N1-	;N1
Heines Kolben	*Yr6*	3+	1+	1++
Triticum spelta album	*Yr5*	0;	0;	3+
Avocet S		3+	3+	3+

[a] PBI accession number

Table 3 Seedling infection types and field reactions of Savannah and Senat with predicted resistance genes conferring low ITs in *parentheses*

Cultivar	Postulated resistance genes	*Pst* isolate used on seedlings and virulence phenotype[a]				Field[c]
		08/97 *Yr1,2,3,4,9,17,Sd*	70/99 *Yr1,2,3,4,6,9,17,Sd*	72/94	71/93 *Yr1,2,3,32,Sd*	
Savannah	*Yr1,2,3,4,9,17* [b]	7–8	8	1–2 (*Yr4,17*)	1–2 (*Yr4,9,17*)	6
Senat	*Yr3,32,+* [a]	1–2 (*Yr32,+*)	2 (*Yr32,+*)	1 (*Yr32,+*)	5–6 (*Yr+*)	0

[a] Justesen et al. (2002); M. S. Hovmøller (personal communication); *Sd* = Strubes Dickkopf
[b] J Clarkson (personal communication)
[c] Natural infection, presumed to be with predominating pathotypes similar to isolates 08/97 and 70/99

Table 4 Kruskal-Wallis (*K*) statistics for stripe rust resistance in the Savannah/Senat population, at all markers on chromosomes with a significant effect on resistance. $K \geq 7.8$ indicates significance at the $P \leq 0.005$ level

cM[a]	Marker[b]	*Pst* isolate used on seedlings				Field
		08/97	70/99	72/94	71/93	
Chromosome 1B						
0.0	*Sec-1*	2.5	7.5	0.0	3.5	2.4
2.2	*M48/P38–361*	5.9	11.0	0.0	6.3	5.8
3.2	*Xwmc85–1BL**	7.3	13.1	0.2	5.0	7.6
6.9	*M48/P19–324*	7.7	13.1	0.1	4.9	5.5
11.1	*M49/P14–234*	9.0	15.3	0.4	5.0	7.2
21.6	*Xwmc156–1BL*	7.6	17.8	0.1	7.8	6.1
30.5	*M47/P32–283*	9.6	16.3	0.3	6.4	7.5
43.8	*M60/P10–287*	8.9	13.3	0.1	3.2	10.0
Chromosome 2A						
0.0	*Xwmc382–2AS**	0.6	0.6	11.1	23.9	0.0
6.3	*M61/P19–209*	0.4	0.3	17.1	32.4	0.1
9.7	*M61/P32–320*	0.7	0.5	17.8	39.3	0.1
15.4	*Xwmc25**	0.2	0.3	14.1	32.2	0.1
23.5	*Xwmc177–2AS*	0.0	1.3	18.6	36.7	0.6
58.3	*Xwmc522–2AS*	4.0	0.4	0.8	4.0	6.0
62.2	*Xgwm95–2AC*	3.6	0.7	0.1	6.5	5.4
94.1	*Xwmc170–2AL*	50.7	34.7	23.9	0.0	47.3
98.9	*Xwmc198-2AL*	52.0	34.2	26.2	0.3	48.4
100.8	*M62/P19–156*	56.7	38.1	29.3	0.0	52.1
104.3	*M59/P37–375*	55.8	37.6	28.6	0.0	50.9
121.6	*Xwmc181–2AL*	20.2	15.0	10.0	0.0	17.0
141.8	*M61/P11–400*	5.1	7.7	0.5	1.1	5.2
154.8	*M62/P35–247*	6.6	9.6	1.3	0.1	2.4
178.5	*M48/P10–344*	0.4	1.0	1.3	3.4	0.1
188.3	*M60/P10–378*	2.6	3.3	0.7	3.3	0.2
204.9	*M62/P35–130*	0.1	0.0	1.9	0.9	1.9
Chromosome 4D						
0.0	*M49/P41–233*	0.0	0.7	4.6	10.0	1.5
1.0	*Xwmc262*	0.2	1.2	4.9	9.4	1.7
12.0	*M48/P32–448*	0.6	3.8	2.8	2.0	2.7
41.7	*Xwmc89–4DC*	1.5	0.3	0.7	0.3	3.0
44.8	*Xwmc457–4DC*	2.0	0.2	0.7	0.6	2.3
Chromosome 5B						
0.0	*M61/P15–277*	3.0	2.2	0.3	1.1	0.2
2.8	*M50/P38–253*	3.1	2.1	0.8	2.7	0.1
6.3	*M59/P37–384*	4.0	3.2	0.0	1.7	0.1
21.4	*Xwmc85**	2.4	2.0	0.4	6.0	0.4
24.2	*Xwmc73–5BC*	2.4	1.8	0.4	4.4	0.5
39.0	*M49P41–388*	1.9	1.4	0.5	4.6	0.7
50.3	*M59/P32–192*	2.9	1.6	0.5	10.9	1.5
58.0	*M59/P19–290*	4.2	2.9	0.4	10.3	0.9
62.8	*M47/P35–247*	4.0	2.7	0.6	8.0	1.3
102.0	*M62/P38–364*	0.0	1.5	0.3	1.8	0.0
130.0	*Xwmc160–5BL*	0.1	0.0	1.4	1.7	0.3
134.1	*M48/P40–184*	0.1	0.0	0.9	1.8	0.3
136.1	*M49/P41–210*	0.1	0.0	1.4	1.7	0.4
140.9	*Xwmc118–5BL*	0.2	0.9	0.1	0.1	0.5
143.8	*M59/P19–378*	0.0	0.1	0.7	0.3	0.5
145.8	*M59/P37–371*	0.0	0.3	0.6	0.4	0.6
165.4	*M61/P38–54*	0.7	0.2	0.9	0.1	0.3
168.1	*M61/P11–55*	0.5	0.5	0.1	0.7	0.0
Chromosome 7D [c]						
0.0	*M60/P37–126*	8.7	10.9	0.0	7.6	3.3
3.4	*M59/P19–202*	7.5	9.3	0.0	7.2	2.9
8.2	*Xwmc506–7DS*	9.5	11.4	0.1	6.6	3.9
0.0	*Xwmc436–7DS*	0.0	0.1	0.1	0.0	0.2
30.5	*Xwmc221–7DL*	1.0	0.2	0.0	1.5	3.3
0.0	*M60/P37–348*	0.1	1.1	0.0	1.1	0.0
2.1	*M48/P19–365*	0.0	0.8	0.0	1.8	0.0
13.6	*Xwmc157–7DL*	0.8	1.2	0.6	0.3	0.7
0.0	*Xwmc14–7DL*	1.4	0.0	0.0	0.4	0.8
8.8	*M62/P37–161*	0.0	3.6	0.5	0.8	0.0

[a] Map distance from the short to long chromosome arm in centiMorgans
[b] C: near centromere, * SSR marker with multiple loci in Savannah/Senat
[c] Chromosome consists of multiple linkage groups

Table 5 Putative QTL positions for stripe rust resistance detected in the Savannah/Senat population, with Kruskal-Wallis statistics (*K*) and effect on the disease scores. When Senat contributes the resistance allele the effect is *underlined*. *Bold figures* indicate significance at $P \leq 0.005$ level

cM[a]	Marker[b]	*Pst* isolate used on seedlings and virulence phenotype								Field	
		08/97 *Yr1,2,3,4,9,17,Sd*		70/99 *Yr1,2,3,4,6,9,17,Sd*		72/94 *Yr1,2,3,9,Sd*		71/93 *Yr1,2,3,32,Sd*		K	Effect[c]
		K	Effect[c]	K	Effect[c]	K	Effect[c]	K	Effect[c]		
Chromosome 1B											
21.6	*Xwmc156–1BL*	7.6	1.6	**17.8**	1.7	0.1	0.0	**7.8**	1.6	6.1	1.5
30.5	*M47/P32–283*(1BL)	**9.6**	1.7	**16.3**	1.6	0.3	0.2	6.4	1.5	7.5	1.7
43.8	*M60/P10–287*(1BL)	**8.9**	1.7	**13.3**	1.5	0.1	0.0	3.2	1.0	**10.0**	1.9
Chromosome 2A											
9.7	*M61/P32–320*(2AS)	0.7	0.5	0.5	0.4	**17.8**	2.3	**39.3**	3.7	0.1	0.2
100.8	*M62/P19–156*(2AL)	**56.7**	4.2	**38.1**	2.6	**29.3**	2.7	0.0	0.2	**52.1**	4.5
Chromosome 4D											
0.0	*M49/P41–233*(4D)	0.0	0.3	0.7	0.5	4.6	0.9	**10.0**	1.9	1.5	0.7
Chromosome 5B											
50.3	*M59/P32–192*(5B)	2.9	0.9	1.6	0.5	0.5	0.5	**10.9**	1.7	1.5	0.9
Chromosome 7D											
8.2	*Xwmc506–7DS*	**9.5**	1.8	**11.4**	1.4	0.1	0.1	6.6	1.5	3.9	1.2

[a] Map distance from the short to long chromosome arm in centiMorgans
[b] The estimated chromosomal locations of AFLP markers are shown in *parenthesis*
[c] The effect is the numerical difference in disease score between the Savannah and Senat marker classes

presence of *Yr32* in the case of isolates 08/97, 70/99 and 72/94. The possibility of a further unknown gene in Senat is suggested by the intermediate IT of 5–6 with isolate 71/93. Since *Yr3* is postulated to be present in both parents and all four isolates are virulent for this gene, no effects attributable to interactions involving this gene were expected. Senat was highly resistant in the field, probably due to the presence of *Yr32*, whereas Savannah gave an intermediate response of 6, indicating the presence of a moderate to low level of adult plant resistance (APR). Because of the possible segregation of up to four known genes, individual segregation ratios were difficult to interpret and hence QTL analysis was used to map the genes responsible for the low stripe rust responses.

Confirmation of the location of Yr32 in chromosome 2AL of Senat

The highest significance and the largest effects on disease response in the Savannah/Senat population were associated with markers in chromosome arm 2AL (Tables 4, 5). These effects were obtained in the field and in all seedling tests, except with culture 71/93, which is known to be virulent on Carstens V. In order for a candidate QTL to comply with a prediction of *Yr32*, it should meet the following criteria: (1) the resistance allele should be from Senat, (2) it should be effective against isolates 08/97, 70/99, and 72/94 and ineffective to isolate 71/93, and (3) it should be effective in the field, as *Yr32* is currently effective in Denmark (Hovmøller and Jensen 2003). The only QTL fulfilling these criteria was located on chromosome 2AL near the AFLP marker *M62/P19–156* (Table 4). This locus gave the highest effect on resistance on seedlings in the greenhouse and on adult plants in the field, reducing the disease score by 2.6 to 4.2 units on seedlings and by 4.5 units on adult plants (Table 5).

Additional stripe rust QTLs segregating in Savannah/Senat

In addition to 2AL, chromosomes 1BL, 2AS, 4D, 5B, and 7DS carried QTLs for resistance to stripe rust (Tables 4, 5). QTLs from Senat on 1BL and 7DS provided resistance to isolates 08/97 and 70/99, reducing the disease score by 1.4 to 1.8. On 2AS, a highly significant QTL from Savannah provided resistance to 72/94 and 71/93, with an effect on disease score of 2.3 and 3.7 units. QTLs on 4D from Senat and on 5B from Savannah provided resistance to one isolate, 71/93, with effects of 1.9 and 1.7, respectively. A marginally significant QTL from Savannah on 1BL gave resistance to 71/93. In the field *Yr32* provided the major part of the resistance, but a smaller effect from Senat was detected on chromosome 1BL, reducing the disease score by 1.9 units. None of the other QTLs detected as seedling responses had any effect in the field.

Discussion

Designation of gene *Yr32* for resistance to stripe rust

The combined results of monosomic and linkage analysis in Australia and a genetic marker analysis in Denmark provide adequate evidence for the location of a new specific seedling resistance gene in chromosome 2AL. This gene is designated *Yr32*. The only other named gene shown clearly to be located in this chromosome arm is *Yr1*, which is distally located and closely linked (2.0 cM) with the *Pm4* locus associated with resistance to powdery mildew (McIntosh and Arts 1996). The genes *Yr1* and *Yr32* showed independent segregation. Although the position of the centromere is not clear in the linkage map of Savannah/Senat (Fig. 1), the map does suggest that the marker *M62/P19–156*, with the strongest association with *Yr32,* should be linked with the centromere whereas earlier work by The et al. (1979) indicated that *Pm4*, and hence *Yr1*, were distally located. Unfortunately, none of the markers with close linkage to *Pm4* (Ma et al. 1994) was used for the present study. However, a map of the ITMI population (M. J. Christiansen, unpublished) shows a map distance of 35 cM from *Xwmc198* to *Xcdo678* [co-segregating with *Pm4* (Ma et al. 1994)], suggesting a distance from the most likely position of *Yr32* to *Yr1* of about 33 cM (Table 4; Fig. 1). Furthermore, a combination of the map distance *M62/P19–156* to *Xwmc181* of about 21 cM and the distance *Xwmc181* to *Xbcd292* [closely linked with *Pm4* (Ma et al. 1994)] of 14 cM (Gupta et al. 2002) suggest a genetic distance between *Yr32* and *Yr1* of about 35 cM. A genetic distance of 33–35 cM is consistent with the genetic independence obtained for *Yr1*and *Yr32.*

The seedling stripe rust resistance of Carstens V was reported to be complex (Chen and Line 1993; Calonnec et al. 2002). The pathotypes used in both these studies were mainly chosen to reveal the maximum numbers of resistance genes whereas the present studies focused on the important component of resistance to current pathotypes. According to Calonnec et al. (2002), Carstens V carried three genes for seedling resistance. One of the genes was probably *Yr25*, which was also present in Strubes Dickkopf, and this gene was effective against a pathotype of non-European origin. One of the other two genes detected with pathotype 43 E138 was undoubtedly *Yr32.*

Additional stripe rust resistance in Savannah and Senat

Stripe rust resistance genes often interact in an additive manner when present in combinations. The resistant ITs of 1–2 for Savannah with isolates 72/94 and 71/93 are thus slightly higher than expected for the combination of 2–3 effective genes. Moreover, the expected ITs produced by *Yr4* and *Yr9* are often more incompatible than those observed on Savannah (McIntosh et al. 1995). This discrepancy must be attributed to an effect of the genetic background in Savannah.

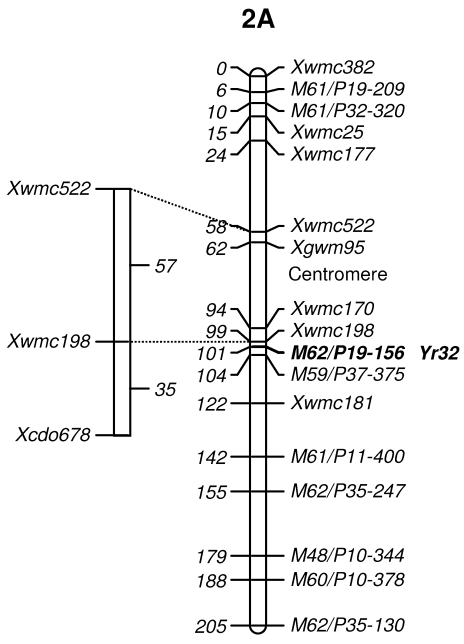

Fig. 1 Linkage map of chromosome 2A of the cross Savannah/Senat, with the approximate position of the centromere indicated. Marker positions (*left*) are in centiMorgans, with the short arm at the *top*. A short section of a map of the ITMI population aligned at *Xwmc198* (M. J. Christiansen, unpublished) is shown on the *left*. Only markers relevant to this study are shown on the ITMI map, indicating the map distance from the most likely position of *Yr32* at *M62/P19–156* to *Xcdo678* which is closely linked with *Yr1*

The significant effects from Savannah in chromosome 2AS on resistance to isolates 72/94 and 71/93, avirulent for *Yr17* and *Yr4*, provides evidence for the effect of *Yr17* which is known to be located in chromosome 2AS (Bariana and McIntosh 1993). No other effects from Savannah uniquely associated with these two isolates were obtained, suggesting that if *Yr4* is present in Savannah it is located in chromosome 2AS and its effects are confounded with those of *Yr17*, or it is located in part of the genome not covered by the linkage map. Evidence reported by Worland suggested that *Yr4* was located on chromosome 3B (McIntosh et al 1995). The *Yr9* gene located on the 1BL/1RS translocation (Singh et al. 1990) in Savannah should have produced an effect associated with *Sec-1* from Savannah (null allele in Senat) against isolate 71/93, which was avirulent for *Yr9*. However, only a small and non-significant effect was observed (Table 4).

The effect associated with the 1BL chromosome from Senat could be caused by a number of *Yr* genes but, due to the incomplete linkage map and limited pedigree information, none seem obvious. There was no compelling evidence to associate the 4D and 7DS effects from Senat with *Yr28* (chromosome 4DS) derived from *Aegilops tauschii* or *Yr18* (chromosome 7DS) (Singh et al. 2000). One problem was that only a fraction of chromosomes 4D and 7D were covered with markers when compared to the full chromosome lengths obtained in other studies (Röder

et al. 1998; Gupta et al. 2002). Therefore, some significant effects on rust response might have escaped detection with the markers that were used in the study. The effects from Savannah against culture 71/93 on chromosomes 5B and 1BL also could not be related to any known genes.

Yr32 in Tres and certain European cultivars

Infection type data confirmed the conclusion of Chen and Line (1992) that *Yr5* was probably not present in Tres. Chen et al. (1995) determined that resistance in Tres was conferred by two genes, one of which was dominant (*YrT1* on chromosome 6D) and the second (*YrT2* on chromosome 3A) dominant or recessive, depending on genetic background. The present data indicate that Tres and its backcross derivative carry *Yr32* and this appears to be unrelated to *YrT1* and *YrT2*. The source of *Yr32* in the pedigree of Tres (CI 12666/6*Omar/3/*T. spelta*/Coastal/3*Omar, Allan et al. 1986) is not clear. The data also indicate the possibility of further gene(s) for resistance in Tres to Australian pathotypes, although the relationship of this resistance and *Yr32* with *YrT1* and *YrT2* (Chen and Line 1992) are unresolved. *Yr32* appears to be deployed in a range of European wheats, including Consort, Hereward and possibly Oxbow (Bayles et al. 2002), Hereward and Kraka (Hovmøller 2001), and Consort, Danis, and Vivant (Pathan and Wellings, unpublished). In Denmark, currently more than 1/3 of the wheat area is grown with cultivars (Solist, Senat, Stakado, and Wasmo) likely to possess *Yr32* (Hovmøller and Jensen 2003). Virulence for *Yr32* in *Pst* populations in the United Kingdom fluctuated from an unpredicted high frequency in 1994 to low frequencies in the late 1990s, and a more recent resurgence in 2001 (Bayles et al. 2002). These changes in pathotype composition over time possibly reflect the deployment of cultivars carrying *Yr32*. In contrast, virulence for *Yr32* in Danish *Pst* populations was relatively low from 1993 to 1999, although it was present in several pathotypes (Hovmøller 2001).

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