Theor Appl Genet (1990) 79:401–410

THEORETICAL AND APPLIED GENETICS

Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in four South African wheats

R.P. Singh * and R.A. McIntosh

Plant Breeding Institute, The University of Sydney, P.O. Box 180, Castle Hill, NSW 2154, Australia

Received August 18, 1989; Accepted November 30, 1989
Communicated by G.S. Khush

Summary. Genes for resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* identified in four South African wheats were: *Sr6, Sr8a, Sr9e,* and *Lr13* in 'W3762'; *Sr5, Sr8a, Sr9b, Sr12, Sr24, Lr13,* and *Lr24* in 'W3760'; *Sr2, Sr24, SrC, Lr13,* and *Lr24* in 'W3751'; and *Sr7a, Sr23, Sr36,* and *Lr16* in 'W3755'. Genes *Sr2, Sr9e,* and *Sr24* also conferred adult plant resistance to the predominant pathotypes of *P. graminis tritici.* Genes *Sr7a, Sr23,* and *SrC,* when present alone, did not confer acceptable adult plant resistance, even though low seedling reactions were associated with them when tested with the same pathotypes. Genetic recombination between *Lr13* and *Sr9e* was estimated at 12.5% ± 2.3%.

Key words: *Puccinia graminis tritici* – *Puccinia recondita tritici* – Triticum aestivum – Rust resistance – Gene identification

Introduction

Wheat stem rust caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn has been controlled for over 30 years in the more disease-prone areas of Australia through the use of resistant cultivars. Initially, resistance was based on single genes that inevitably failed due to pathogenicity changes in the pathogen population. Despite this, further sources of resistance were exploited on a continual basis. As experience and knowledge accumulated, attempts were made to broaden the genetic basis of resistance by combining two or more genes, and to exploit more durable resistance genes or gene combinations (Watson 1981). The consequence of this approach has been that certain cultivars have maintained resistance for over 20 years. Nevertheless, in order to stay ahead of an evolving pathogen, there is a constant need to search for, to understand, to manipulate, and to have available for future exploitation further sources of resistance.

Mr. J. le Roux, Small Grain Center, Bethlehem, South Africa, tested various wheats of South African origin with a number of *P. graminis tritici* pathotypes at the Plant Breeding Institute, Castle Hill. Thirteen of these, displaying low infection types with an array of Australian *P. graminis tritici* pathotypes, were chosen for detailed inheritance studies. The results for four, accessioned as 'W3751,' 'W3755,' 'W3760,' and 'W3762' (W refers to the University of Sydney Wheat Accession Register), are described in this paper. Because they were also resistant to Australian cultures of *Puccinia recondita tritici,* studies of resistance to this pathogen were also undertaken.

Materials and methods

The four resistant South African wheats were:
'W3762' = Flameks*3/3/LD3982/LD3537/2/STW646;
'W3760' = T4R = T4*3/Agent (sib lines of T4 are variously known as Anza, Karamu, and WW15);
'W3751' = Heines Kolben/38MA/4/4777//Rei/Yaqui/3/Kentana/5/Yecora 70/6/Pato (B) = CM29818-M12/19-B-20-GM (?);
'W3755' = Piamontes INTA/2*Robin = CMA15773-W14-W1/10-G3.

The seedling and adult plant responses of these and certain contrasting parents chosen for genetic analyses, as well as selected controls, to pathotypes of *P. graminis tritici* and *P. recondita tritici* are listed in Tables 1 and 2, respectively.

Each culture of *Puccinia graminis tritici* maintained in the Plant Breeding Institute Collection, Castle Hill, carries a collection or culture number and a pathotype designation that de-

* Present address: CIMMYT, Apartado Postal 6-641, Delegacion Cuauhtemoc 06600, Mexico, D.F., Mexico

Table 1. Seedling infection types and adult-plant reactions of wheats inoculated with various *Puccinia graminis tritici* pathotypes and incubated at two temperature regimes. Pathotype (culture) 1 = 34-1,2,3,4,5,6,7 (74-L-1); 2 = 343-1,2,3,5,6 (73879); 3 = 222-1,2,3,5,6 (70386); 4 = 34-2,4,5,6,7,11 (72611); 5 = 98-1,2,3,5,6 (781219); 6 = 34-1,2,3,5,7,8,9 (76-L-8); 7 = 34-1,2,3,6,7,8,9 (76-L-7); 8 = 126-1,5,6,7,11 (7316); 9 = 326-1,2,3,5,6 (69822)

Wheat	Pathotype and seedling IT responses												Adult-plant reaction
	>20 °C									<20 °C			
	1	2	3	4	5	6	7	8	9	4	5	8	
'W3762'	;2=	;2=	;	;12=	;12=C	;	;	;	;	0;–	;	;2=	TR
'W3760'	2=	0;	;	;	;2=	;	;1=	;	0;	0;–	0;–	0;	0
'W3751'	2=	2=	;12=C	2–C	2–C	2=C	;12–C	;2=C	;2=	2=	;2=	;	0
;W3755'	12=	0;–	0;–	2	0;=	0;	0;=	0;	0;–	2–2	0;=	0;–	0
'Line E'	3+	3+	3+	3+	3+	3+	3+	3+	33+	3+	33+	3+	40S
'Condor'	33+	33+	33+	;X=	33+	2	X++	X++	0;	0;	;X–	;	40MS-S
'Warigo'	3+	2+C	3+C	3+	2+C	33+	X++	33+	3C	33+	;	3	TR
'W3746'	12–	12	;12–	0;	2	;12	2–2	;	2	0;	2–	0;	5MR
Controls:													
'Reliance' (*Sr5*)	3+	3+	3+	3+	3+	3+	3+	3+	0;–	3+	3+	3+	40S
'McMurachy' (*Sr6*)	3+	3+	3+	X++3	3+	3+	3+	3+	3+	0;	3+	3+	40S
'W2403' (*Sr7a*)	2+	2+	2+	3+	2+3	2+	2+	2+	2+	3+	2+	2+	30MS-S
'Mentana' (*Sr8a*)	3+	3+	3+	3+	3+	2–	3+	3+	3+	3+	3+	3+	60S
'W2402' (*Sr9b+Sr7b*)	3+	3+	3+	3+	3+	3+	2	2–N	3+	3+	2	2–N	60S
'Vernstein' (*Sr9e*)	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;	;	;	TR
'Yalta' (*Sr11*)	3+	3+	3+	3+	3+	3+	3+	;2=	3+	3+	3+	;2=	40S
'CS(Marquis3B)' (*Sr12*)	3+	3+	3+	X++	3+	33+	X++	X++	33+	;X=	X	;X=	40MS-S
'Renown' (*Sr2+Sr17+Sr7b*)	3+	3+	3+	3+	3+	3+	X++	3+	3+	3+	;X=	3+	10MR
'Exchange' (*Sr23*)	2+3CN	2+3CN	2+3CN	2+3CN	2+3CN	2+3CN	2+3CN	2+3CN	2+3CN	3+CN	3+CN	3+CN	30MS-S
'Agent' (*Sr24*)	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	;2=	TR
'CI12632' (*Sr36*)	3+	;	;	3+	;	;	;	;	;	3+	;	;	0

Table 2. Seedling infection types and adult-plant reactions of wheats inoculated with various *Puccinia recondita tritici* pathotypes and incubated at two temperature regimes. Pathotype (culture) 1 = 10-1,2,3,4 (72469); 2 = 26-1,3 (67028), 3 = 76-0 (63666); 4 = 104-2,3,6, (7) (76694); 5 = 122-2,3,4 (76348); 6 = 122-2,3,6,7 (81502); 7 = 135-1,2,3,4,5 (64-L-3)

Wheat	Pathotype and seedling IT responses									Adult-plant reaction
	>20 °C							<20 °C		
	1	2	3	4	5	6	7	3	7	
'W3762'	X−	X−	X	X−	X	X	X	X+X++	X	TR
'W3760'	0;	0;	;	;	0;	;	0;	;1=	;	TR
'W3751'	;	;	;	;	;	;	;	;1=	;1=	TR
'W3755'	2++3−	2++3−	22+	2+3−	22+	3−	2+3−	3−	2+3−	30MS
'Line E'	3+	3+	3+	3+	3+	3+	3+	3+	3+	80S
'Condor'	3+	3+	3+	3+	3+	3+	3+	33+	33+	60MS-S
'Warigo'	3+	3+	X	3+	3+	3+	X−	X−	33+	60S
Controls:										
'Chris' (*Lr13*)	X−	;11+	X−	X−	X−	X−	X−	X++	X+	TR
'Selkirk' (*Lr16, Lr14a*)	2++	2+3−	X−[a]	2+	2	2++3−	2+	X[a]	2+	30MS
'Agent' (*Sr24*)	0;	0;	;	;	0;	;	0;	;	;	0

[a] Pathotype 76-0 is avirulent for *Lr14a*

scribes the pathogenicity phenotype. Pathotype designations include a 'standard race' number determined on the key of Stakman et al. (1962), followed by a series of numbers indicating individual virulences on an ordered sequence of 13 supplementary testers. These include 12 testers described by Watson and Luig (1963) and McIntosh et al. (1983), together with triticale 'Satu' as the 13th tester.

A similar system applies to the pathotype designations for cultures of *Puccinia recondita tritici*. A 'standard race' designation based on the key of Johnston and Levine (1955) is followed by the number designations of those of ten supplementary testers for which the particular culture is virulent. In addition to the five supplementary testers listed by Watson and Luig (1961), testers 6 = Gatcher (*Lr27+Lr31*), 7 = Songlen (*Lr17*) and 8 = CS 2M/2D 4/2 (*Lr28*), 9 = Benno (*Lr26*), and 10 = Egret (*Lr13*) are also used.

Crosses of the South African wheats with 'Line E,' 'Condor,' and 'Warigo' were used for inheritance studies. Although 'Condor' possessed stem rust resistance genes *Sr5, Sr8a,* and *Sr12*, it was susceptible to *P. graminis tritici* pathotypes 34-1,2,3,4,5,6,7; 343-1,2,3,5,6; 98-1,2,3,5,6; and 222-1,2,3,5,6 in seedling tests and displayed a high reaction in the field (Table 1). 'Warigo' possessed *Sr7b* and *Sr17* and displayed high seedling reactions to certain pathotypes (Table 1). However, the cross with 'Warigo' was not useful for adult plant inheritance studies because 'Warigo' also carried *Sr2,* conferring adult plant resistance (Hare and McIntosh 1979). A cross of 'W3755' with 'W3746' was also studied to test for allelism of *Sr7a*. 'W3746' possesses *Sr7a* and *Sr12* (Singh and McIntosh 1987).

Seedling populations were sown and inoculated by standard procedures. Seedling infection type (IT) scores were based on the 0 (immune), ; (fleck), X (mesothetic), 1–4 scale proposed by Stakman et al. (1962), with 3+ and 4 representing susceptible responses. Symbols +, −, C, and N were used to denote more, less, chlorosis, and necrosis, respectively. Seedlings with different infection types were tagged with different colored wire rings and transplanted in the field to evaluate their adult plant reactions. The mature plants were harvested and threshed individually to derive F_3 lines.

Rust epidemics for adult-plant tests in the field were created by spraying the experimental plots with urediospore-oil suspensions using an ultra-low volume sprayer. Infection of spreader rows and susceptible checks indicated infection of the entire nursery. The predominant *P. graminis tritici* pathotypes in the field were 34-1,2,3,7,8,9 and 98-1,2,3,5,6, whereas the *P. recondita tritici* population was largely composed of pathotype 104-2,3,6,(7). The adult plant scores were based on the modified Cobb scale comprising percent area affected (including 0 and T = trace) and a response description (e.g., R = resistant, S = susceptible).

Results

Resistance to stem rust and leaf rust in 'W3762'

Crosses of 'W3762' (IT";2=") with 'Condor' (IT"33+"), 'Warigo' (IT"3+"), and 'Line E' (IT"3+4") resulted in F_1 seedlings with ITs ";2=," ";2=," and "2=," respectively, with pathotype 34-1,2,3,4,5,6,7, indicating that resistance was dominant. With the same pathotype, the F_2 segregation for individual crosses and for all three crosses when pooled (382 resistant:121 susceptible seedlings) conformed with a monogenic ratio ($\chi^2_{3:1} = 0.24$). The F_3 line distributions individually and pooled (31 nonsegregating resistant: 60 segregating:33 nonsegregating susceptible) also conformed with segregation at a single locus ($\chi^2_{1:2:1} = 0.19$).

All F_2 seedlings with ITs ";2=" to "2=" in all three crosses were resistant as adult plants and gave nonsegregating resistant or segregating F_3 seedling progenies. F_2 seedlings with ITs "33+"–"3+" in crosses involving 'Condor' and 'Line E' were moderately susceptible or susceptible as adult plants, and gave nonsegregating susceptible F_3 seedling progenies. In the 'W3762'/'Warigo' cross, 3 of the 12 seedlings with ITs "33+"–"3+" were classified resistant – moderately resistant as adult plants,

indicating the presence of plants homozygous for the recessive gene *Sr2*, presumably coming from 'Warigo'. The occurrence of 9 moderately susceptible – susceptible plants in this group confirmed the absence of *Sr2* in 'W3762', otherwise all susceptible seedlings would have displayed adult-plant resistance. The results indicated that seedling resistance to pathotype 34-1,2,3,4,5,6,7 and field resistance to the predominent pathotypes 98-1,2,3,5,6 and 34-1,2,3,7,8,9 were due to the same dominant resistance allele.

During the test, one IT "3+" pustule was observed on an otherwise resistant F_3 seedling. Inoculum from this pustule was isolated and increased on a susceptible wheat. When tested on a full differential series, the resultant culture was identified as pathotype 40-1,2,3,4,5,6,7, and was similar to pathotype 34-1,2,3,4,5,6,7 but possessed added virulence for the tester carrying *Sr9e*. Presumably, the single pustule originated as a mutant in the test culture of pathotype 34-1,2,3,4,5,6,7. These results suggested that the gene identified in tests with pathotype 34-1,2,3,4,5,6,7 was *Sr9e*. Subsequent tests with additional *Sr9e*-virulent pathotypes, viz., 40-1,2,3,4,5,6,7,11 and 40-1,2,3,4,(5),6,7 (data not presented), confirmed this postulate since 'W3762' displayed IT "3".

When F_3 lines from the three crosses were tested with *P. recondita tritici* pathotype 104-2,3,6,(7), the F_3 line distributions for all three crosses individually and when pooled (36 nonsegregating resistant: 57 segregating: 30 nonsegregating susceptible) accorded with segregation for a single locus ($\chi^2_{1:2:1} = 1.24$). The IT responses with other pathotypes (Table 2) led to the postulation that the resistance allele was *Lr13*.

Because both *Sr9e* and *Lr13* are located in chromosome *2B* (McIntosh 1983) and were expected to exhibit genetic linkage, a test of genetic independence was performed. The observed ratio deviated significantly from that expected for segregation at two independent loci (Table 3). Recombination of 12.5% ± 2.3% was estimated using the method of maximum likelihood (Allard 1956). This provided additional evidence that the leaf rust gene was *Lr13*.

Resistance to stem rust and leaf rust in 'W3760' and 'W3751'

'W3760'. F_1 seedlings in crosses of 'W3760' (IT"2=") with 'Condor' (IT"33+"), 'Warigo' (IT"3+"), and 'Line E' (IT"3+4") displayed ITs "2–," "2–," and "2–2," respectively, when tested with pathotype 34-1,2,3,4,5,6,7. The F_2 seedling frequencies for crosses individually and when pooled (364 resistant: 140 susceptible) accorded with a 3:1 ratio ($\chi^2_{3:1} = 2.07$). Furthermore, F_3 line distributions individually and overall (26 nonsegregating resistant: 70 segregating: 33 nonsegregating susceptible) also conformed satisfactory with monogenic segregation ($\chi^2_{1:2:1} = 1.70$). All F_2 seedlings with IT "2=" in all crosses were resistant as adult plants, and gave either nonsegregating resistant or segregating F_3 seedling progenies. Seedlings with IT "3+" in crosses involving 'Condor' and 'Line E' were susceptible as adult plants and gave nonsegregating susceptible F_3 seedling progenies. In 'W3760'/'Warigo,' 3 of 11 seedlings with IT "33+" were scored as 'resistant – moderately resistant' as adult plants, whereas the other 8 were rated 'moderately susceptible – susceptible'. The progenies of all 11 plants were classified in the seedling stage as nonsegregating susceptible. Since 'Warigo' possessed *Sr2*, segregation for adult-plant response in 'W3760'/'Warigo' indicated the absence of *Sr2* in 'W3760'. The correlation between seedling reaction and adult-plant response and F_3 line behaviour indicated the ease and precision with which the phenotypes could be scored, and that the seedling resistance to pathotype 34-1,2,3,4,5,6,7 and adult-plant resistance to pathotypes 343-1,2,3,5,6 and 98-1,2,3,5,6 were conferred by the same dominant gene.

Table 3. Pooled frequencies of F_2 genotypes for crosses involving 'W3762'

Genotype		Frequency	χ^2-analysis		
			Components	*df*	χ^2 value
Sr9eSr9e	*Lr13Lr13*	24	*Sr9e* vs *sr9e*	2	0.22
	Lr13lr13	5	*Lr13* vs *lr13*	2	1.24
	lr13lr13	1	Deviation	4	96.78 *
Sr9esr9e	*Lr13Lr13*	11	Independence	8	98.24 *
	Lr13lr13	43			
	lr13lr13	6			
sr9esr9e	*Lr13Lr13*	1			
	Lr13lr13	9			
	lr13lr13	23			
Total		123			

* Significant at $P = 0.01$

Seedlings of F_3 lines from 'W3760'/'Condor' were also tested with pathotype 343-1,2,3,5,6. Results similar to those obtained with pathotype 34-1,2,3,4,5,6,7 further confirmed that the same gene conferred resistance to both pathotypes. This allele was postulated to be *Sr24*, since cv 'Agent' with *Sr24* (McIntosh et al. 1977) occurred in the pedigree of 'W3760'.

To further test this postulation, F_3 lines were classified for seedling leaf rust response. *Lr24*, characterized by IT ";", is known to be completely linked in coupling with *Sr24* (McIntosh et al. 1977). With *P. recondita tritici* pathotype 104-2,3,6,(7), segregation at two loci was apparent. The first resistance gene conferred ITs ";" to ";1=," whereas the second conferred ITs in the "X" category. Based on infection types, these two genes were postulated to be *Lr24 and Lr13*, respectively. Ho-

mozygosity for *Lr24Lr24* precluded determination of the status of the *Lr13/lr13* locus. Also, when *Lr24* was heterozygous, the frequencies of *Lr13Lr13* and *Lr13lr13* genotypes were pooled, because only small populations of 15–20 seedlings were tested. The pooled genotype distribution of 26 *Lr24Lr24–*:29 *Lr24lr24 Lr13-*:19 *Lr24lr24 lr13lr13*:7 *lr24lr24 Lr13Lr13*:13 *lr24lr24 Lr13lr13*:10 *lr24lr24 lr13lr13* accorded with that expected for segregation at two independent loci ($\chi^2_{4:6:2:1:2:1}=2.86$). No recombinant genotype with respect to *Sr24* and *Lr24* was obtained in a population of 124 lines, strongly indicating that the dominant allele for stem rust resistance in 'W3760' was *Sr24*.

A gene for red grain color is known to be linked with *Sr24*. Grains from all resistant and from 25 of 33 susceptible F_2 plants, in all three crosses, were red in color. All eight F_2 segregates with white grain color gave nonsegregating susceptible F_3 progenies when tested with stem rust. Because 'Condor,' 'Warigo,' and 'Line E' are white seeded, it was postulated that 'W3760' possessed an additional independent gen for red grain color [$\chi^2_{15:1}$ (128 red : 8 white) = 0.00]. Among non-segregating susceptible plants, grain color segregation was 25 red : 8 white.

'W3751'. F_1 seedlings in crosses of 'W3751' (IT";2=") with 'Condor' (IT"33+"), 'Warigo' (IT"3+"), and 'Line E' (IT"3+4") displayed ITs ";2–," ";2–," and ";2–2," respectively, when tested with pathotype 34-1,2,3,4,5,6,7. With the same pathotype, F_2 seedlings were classified in three IT classes, viz., ";2="–"2=" (resistant), "22+"–"2++" (intermediate), and "33+"–"3+" (susceptible) in crosses involving 'Condor' and 'Warigo'; and in IT classes ";2="–"2–" (resistant), "2++3"–"3" (intermediate), and "3+"–"3+4" (susceptible) in the cross involving 'Line E'. The F_2 segregations for individual crosses and when pooled (218 resistant : 59 intermediate : 24 susceptible) conformed satisfactorily with a ratio of 12:3:1 expected for segregation at two indpendent loci ($\chi^2_{12:3:1}=2.86$). Based on the IT responses with a number of pathotypes (Table 1), the gene conferring ITs ";2="–"2–" was postulated to be *Sr24*. For convenience, the gene conferring the intermediate response was designated *SrC*.

The joint distributions of F_2 genotypes in the three crosses are presented in Table 4. With homozygosity for *Sr24Sr24*, genotypes for the *SrC/srC* locus could not be determined and, among *Sr24sr24* plants, the frequencies of *SrCSrC* and *SrCsrC* were pooled because only small populations in each progeny were tested. The genotypic distributions for individual crosses and when pooled (140 lines) accorded with those expected for segregation at two independent loci (Table 4).

When F_3 lines from 'W3751'/'Condor' were tested with pathotype 343-1,2,3,5,6, the results were similar to those obtained using pathotype 34-1,2,3,4,5,6,7, indicating that the same genes conferred resistance to both pathotypes.

In order to test a postulation that *SrC* was *Sr7a*, a gene giving similar response with the above cultures (Singh and McIntosh 1987), the ten *sr24sr24* segregates in 'W3751'/'Warigo' (Table 4) were tested with pathotype 34-2,4,5,6,7,11, which is virulent for *Sr7a* (Table 1). Similar responses to those obtained with pathotype 34-1,2,3,4,5,6,7, which is avirulent for *Sr7a* (i.e., four nonsegregating resistant : four segregating : two nonsegregating susceptible), indicated that *SrC* conferred resistance to both pathotypes. Clearly, *SrC* was different from *Sr7a*.

To further test the *Sr24* postulation, F_3 lines were tested with *P. recondita tritici* pathotype 104-2,3,6,(7). As with 'W3760' crosses, segregation at two loci was apparent. Again, the second factor appeared to be *Lr13* and the distributions of F_2 genotypes in individual crosses, and overall (32 *Lr24Lr24–*:52 *Lr24lr24 Lr13–*:16 *Lr24lr24 lr13lr13*:9 *lr24lr24 Lr13Lr13*:22 *lr24lr24 Lr13lr13*:6*lr24lr24 lr13lr13*), conformed satisfactorily with those expected for segregation at two independent loci ($\chi^2_{4:6:2:1:2:1}=2.41$). No recombinant genotype with respect to *Sr24* and *Lr24* was obtained in the population of 137 F_2 plants. Again, association with red grain color was indicated, since grains from all F_2 plants postulated to be genotypes *Sr24Sr24* and *Sr24sr24* were red, and all

Table 4. Frequencies of F_2 genotypes in crosses involving 'W3751'

Cross with	F_2 genotype and frequency						χ^2 4:6:2:1:2:1 (5 *df*)
	Sr24Sr24	*Sr24sr2*		*sr24sr24*			
	– –	*SrC–*	*srCsrC*	*SrCSrC*	*SrCsrC*	*srCsrC*	
'Condor'	6	19	4	4	8	2	5.06
'Warigo'	16	16	6	4	4	2	2.89
'Line E'	10	20	5	2	6	6	3.95
Total	32	55	15	10	18	10	1.11
							Heterogeneity (10 *df*) 10.79

Table 5. F_2 adult-plant reactions of *Sr24/sr24* genotypes in various crosses involving 'W3751'

Cross with	F_2 genotype and reaction								
	Sr24Sr24			*Sr24sr24*			*sr24sr24*		
	R	MR	MS-S	R	MR	MS-S	R	MR	MS-S
'Condor'	6	0	0	23	0	0	0	4	10
'Line E'	10	0	0	25	0	0	0	5	9
'Warigo'	16	0	0	22	0	0	0	10	0

R – Resistant, MR – Moderately resistant, MS – Moderately susceptible, S – Susceptible

Table 6. F_2 and F_3 adult-plant responses and genotypes for an unidentified locus (*SrC/srC*) in segregates with genotype *sr24sr24* in crosses of 'W3751' with 'Condor' and 'Warigo'

Cross with	F_2 reaction	F_3 response	Genotype and frequency			Total
			SrCSrC	*SrCsrC*	*srCsrC*	
Condor	MR	Nonseg. Res.	1	3	0	4
	MS-S	Seg.	2	4	1	7
		Nonseg. Sus.	1	1	1	3
Warigo	TR-MR	Nonseg. Res.	4	4	2	10

Nonseg. Res. – Nonsegregating resistant, Seg. – Segregating, Nonseg. Sus. – Nonsegregating susceptible

eight white-seeded F_2 segregates were classified *sr24sr24*. The presence of two independent genes for red grain color was again evident [$\chi^2_{15:1}$ (132:8) = 0.07].

The relationships of F_2 adult-plant reactions and *Sr24/sr24* genotypes in crosses involving 'W3751' are presented in Table 5. Plants scored as 'R' in all three crosses comprised genotypes *Sr24Sr24* or *Sr24sr24*. Four of 14 plants in the 'Condor' cross, 5 of 14 in the 'Line E' cross, and all 10 in the 'Warigo' cross, with genotype *sr24sr24*, were scored as 'MR' and the remainder were 'MS-S'.

Progenies of all 14 F_2 plants with genotype *sr24sr24* in the 'Condor' cross and of ten plants in the 'Warigo' cross were also classified for adult-plant response. The relationship between F_2 adult-plant reaction and F_3 adult-plant responses and the distribution of *SrC/srC* genotypes are presented in Table 6. In the 'Condor' cross, all four F_2 plants classified as 'MR' were nonsegregating resistant as adult plants, whereas plants scored as 'MS-S' either segregated or gave nonsegregating susceptible progenies. The absence of correlation between F_3 responses and *SrC/srC* genotypes indicated that the *SrC* gene did not confer discernable adult-plant resistance. The F_3 line distribution of 4 nonsegregating resistant: 7 segregating: 3 nonsegregating susceptible was in accordance with a 1:2:1 ratio ($P > 0.9$).

Because 'W3751' displayed the pseudo-black chaff phenotype known to be associated with *Sr2* (Hare and McIntosh 1979), and all ten *sr24sr24* segregates in 'W3751'/'Warigo gave nonsegregating resistant F_3 lines for adult-plant reaction, it was concluded that 'W3751' possessed *Sr2*.

'W3760'/'W3751'. With pathotype 34-1,2,3,4,5,6,7, F_1 seedlings displayed IT ";2=" and all 183 F_2 seedlings were resistant with ITs ";2="–"2=". All 38 F_3 lines were classified nonsegregating resistant, with similar infection types to those of the parents. These results confirmed that both 'W3760' and 'W3751' possessed a common gene, presumably *Sr24*.

Resistance to stem rust and leaf rust in 'W3755'

F_1 seedlings in crosses of 'W3755' (IT"12=") with 'Condor' (IT"33+"), 'Warigo' (IT"3+"), and 'Line E' (IT"3+4") displayed ITs "2++3–," "2+3–," and "3+," respectively, when tested with pathotype 34-1,2,3,4,5,6,7. These results indicated that the resistance showed variable dominance in crosses with susceptible wheats. In F_2, variable responses were obtained; these included seedlings with ITs ranging from "2" to "3," "33+CN" to "3+CN," and "3+" to "3+4". Classification was difficult, especially for seedlings with ITs "33+CN" or "3+CN" versus "3+."

The ratio of seedlings considered resistant (ITs "2"–"3") versus susceptible (ITs "33+CN"–"3+4") for all three crosses, individually and when pooled (167 resistant: 54 susceptible), conformed satisfactorily with those expected for segregation at a single locus ($\chi^2_{3:1} = 0.04$). The distribution of F_3 lines for the individual crosses and when pooled (32 nonsegregating resistant: 58 segregating: 36 nonsegregating susceptible) also accorded with a monogenic ratio ($\chi^2_{1:2:1} = 1.05$). Possible segregation for a second resistance gene conferring ITs "33+CN" or "3+CN" was indicated, but seedlings with these ITs were pooled with those displaying ITs "33+" to "3+4."

F_1 seedlings in the cross 'W3755'/'W3746' displayed IT "2–" when tested with pathotype 34-1,2,3,4,5,6,7. All 272 F_2 seedlings were scored as resistant with ITs ";12–"–"23=." All 31 F_3 lines were nonsegregating resistant, displaying a similar range of low infection types. Since 'W3746' carried *Sr7a* (Singh and McIntosh 1987), the allele determining low infection type in 'W3755' was also presumed to be *Sr7a*.

When F_3 lines in 'W3755'/'Condor' and 'W3755'/'Line E' were tested with pathotype 343-1,2,3,5,6, and those in 'W3755'/'Warigo,' with pathotype 222-1,2,3,5,6, segregation of an additional gene determining ITs "0;"–";1–" was obtained. A gene conferring this response and for which these pathotypes were avirulent and pathotype 34-1,2,3,4,5,6,7 virulent is *Sr36*. The distributions of the

Table 7. Distributions of *Sr36/sr36* F_2 genotypes in crosses involving 'W3755,' when tested with two *P. graminis tritici* pathotypes

Cross with	Pathotype	F_2 genotypes			χ^2 1:2:1
		Sr36Sr36	*Sr36sr36*	*sr36sr36*	
'Condor'	343-1,2,3,5,6	13	21	3	6.08*
'Line E'	343-1,2,3,5,6	15	20	6	3.98
'Warigo'	222-1,2,3,5,6	22	19	8	10.17**
Total		50	60	17	17.54**
					Heterogeneity (4 *df*) 2.99

* Significant at $P=0.05$
** Significant at $P=0.01$

Table 8. F_2 adult-plant stem rust reaction and distributions of F_2 genotypes in crosses involving 'W3755'

Cross with	F_2 reaction	F_2 genotypes			
		Sr36 –	*sr36sr36*		
		– –	*Sr7aSr7a*	*Sr7asr7a*	*sr7asr7a*
'Condor'	R	34	0	0	0
	MS-S	0	1	2	0
'Line E'	R	35	0	0	0
	MS-S	0	2	3	1
'Warigo'	R	41	0	0	0
	MR	0	1	2	1
	MS-S	0	1	1	2

postulated *Sr36/sr36* F_2 genotypes in the three crosses are presented in Table 7. In 'W3755'/'Line E,' the distribution of the three genotypes conformed with a 1:2:1 ratio, whereas in 'W3755'/'Condor' a poor fit ($P<0.05$) to this ratio was obtained. The distribution in 'W3755'/'Warigo' and the pooled distribution (involving 127 F_3 lines) deviated significantly from monogenic ratios with bias against genotype *sr36sr36*. These results were not unexpected, since the *Sr36* allele is known to be differentially inherited (Nyquist 1962). The lack of heterogeneity in the data from the three crosses indicated that the results were not significantly different.

When eight F_3 lines, nonsegregating susceptible (genotype *sr7asr7a*) with pathotype 34-1,2,3,4,5,6,7 in 'W3755'/'Condor,' were tested with pathotype 34-2,4,5,6,7,11 at higher temperatures (>25 °C), a distribution of three nonsegregating resistant:four segregating:one nonsegregating susceptible was obtained. The resistant seedlings displayed ITs "3=CN"–"3CN."

When tested with a range of *P. recondita tritici* pathotypes (Table 2), 'W3755' gave similar responses to "Selkirk" with *Lr16* (Anderson 1961). This indicated that 'W3755' may carry *Lr16*. Since *Lr16* is completely

Table 9. Distribution of postulated *Sr5/sr5*, *Sr8a/sr8a*, *Sr9b/sr9b*, and *Sr12/sr12* F_2 genotypes, when F_3 lines nonsegregating susceptible to pathotype 343-1,2,3,5,6 from crosses involving four South African wheats with Condor of Line E were tested with four pathotypes of *P. graminis tritici* at two temperature regimes

Cross	Pathotype												Postulated gene(s) in South African parent
	326-1,2,3,5,6 Temperature >25 °C		34-1,2,3,5,7,8,9 Temperature >25 °C			126-1,5,6,7,11 Temperature >25 °C			34-1,2,3,6,7,8,9 and 126-1,5,6,7,11 Temperature <20 °C				
	Genotype and frequency												
	Sr5Sr5	*Sr5sr5*	*sr5sr5*	*Sr8aSr8a*	*Sr8asr8a*	*sr8asr8a*	*Sr9bSr9b*	*Sr9bsr9b*	*sr9bsr9b*	*Sr12Sr12*	*Sr12sr12*	*sr12sr12*	
'W3751/Condor'	1	2	2	2	1	2	0	0	5	2	2	1	–
'W3755/Line E'	0	0	8	0	0	8	0	0	7	0	0	8	–
'W3760/Condor'	8	0	0	8	0	0	2	3	3	8	0	0	*Sr5, Sr8a, Sr9b, Sr12*
'W3762/Condor'	3	3	2	8	0	0	0	0	8	2	3	3	*Sr8a*

linked in coupling with *Sr23* (McIntosh et al. 1974), the stem rust resistance allele clearly detected with *P. graminis tritici* pathotype 34-2,4,5,6,7,11 was postulated to be *Sr23*. Segregation of this allele was noted in tests with other *P. graminis tritici* pathotypes, but no classification was attempted due to the very high reactions. This gene is also known to be more effective at higher temperatures.

F_2 adult-plant reactions to *P. graminis tritici* and genotypes of the plants derived from seedling classifications are summarized in Table 8. As expected, because both predominant field pathotypes were avirulent for *Sr36*, all F_2 plants scored as adult-resistant were either *Sr36Sr36* or *Sr36sr36*. All nine adult plants classified 'MS-S' in crosses involving 'Condor' and 'Line E' were genotype *sr36sr36*. Of these, three were homozygous *Sr7aSr7a*, five heterozygous, and one was homozygous *sr7asr7a*, indicating that *Sr7a* did not confer resistance to adult plants. A similar result was indicated in the cross involving 'Warigo'. Moreover, *Sr2* must be absent in 'W3755,' because four F_2 plants in this cross were scored 'MS-S.' 'W3755' also did not display the pseudo-black chaff phenotype usually associated with *Sr2* (Hare and McIntosh 1979).

Identification of Sr genes, ineffective to pathotypes 343-1,2,3,5,6 and 222-1,2,3,5,6 in 'W3762,' 'W3760,' 'W3751,' and 'W3755'

F_2 populations of crosses involving 'Condor' and 'Warigo' or 'Line E' were inoculated with pathotypes 343-1,2,3,5,6 and 222-1,2,3,5,6, respectively. Seedlings with ITs "33+"–"3+" were transplanted, and progenies (F_3 lines) were tested to confirm the absence of resistance alleles for which the above pathotypes were avirulent.

Usually, seven or eight nonsegregating susceptible F_3 lines, together with controls, were tested with selected cultures of known pathogenicity (pathotypes). The genotypes of F_2 plants were postulated by comparing F_3 seedling infection types with those of the controls. The results are presented in Tables 9 (for crosses involving 'Condor' or 'Line E') and 10 ('Warigo' or 'Line E').

The various genes in 'Condor' (*Sr5, Sr8a*, and *Sr12*) and 'Warigo' (*Sr17*) were used in tests of allelism. Appropriate pathotypes and temperature conditions were chosen such that usually only one resistance gene was expressed in a particular test. The presence of a particular gene in a South African parent was confirmed when all F_3 lines proved nonsegregating resistant, because the probability of all seven or eight lines being homozygous for a resistance allele from the second parent was $(1/4)^7$ or $(1/4)^8$, i.e., <0.001. Similarly, the probability of all seven or eight lines being homozygous for two resistance genes located at independent loci, but determining similar ITs was $(7/16)^7$ or $(7/16)^8$, i.e., <0.01. The presence of genes other than those in 'Condor' and 'Warigo' was indicated by segregation for resistance. In populations from crosses involving 'Line E,' the presence of a particular gene was indicated by segregation.

Sr5: crosses with 'Condor' (genotype Sr5Sr5) and 'Line E.' The 'Reliance' control with *Sr5* displayed ITs "0;–" and "3+" with pathotypes 326-1,2,3,5,6 and 343-1,2,3,5,6, respectively. The presence of *Sr5* in 'W3760' was indicated, since all F_3 lines in the Condor cross were homozygous for IT "0;" with pathotype 326-1,2,3,5,6. *Sr5* was absent in 'W3751,' 'W3762,' and 'W3755,' since segregation occurred in the crosses of 'W3751' and 'W3762' with 'Condor,' and all eight lines were nonsegregation susceptible in the cross of 'W3755' with 'Line E' (Table 9).

Table 10. Distribution of postulated *Sr6/sr6, Sr9b/sr9b, Sr11/sr11, Sr12/sr12*, and *Sr17/sr17* genotypes, when F_3 lines nonsegregating susceptible to phathotype 222-1,2,3,5,6 from crosses involving four South African wheats with 'Warigo' and 'Line E' were tested with three pathotypes of *P. graminis tritici* at two temperature regimes

Cross	*Puccinia graminis tritici*								
	34-2,4,5,6,7,11; 126-1,5,6,7,11 & 34-1,2,3,6,7,8,9 Temperature <20 °C								
	Genotype and frequency								
	Sr6Sr6	*Sr6sr6*	*sr6sr6*	*Sr12Sr12*	*Sr12sr12*	*sr12sr12*	*Sr17Sr17*	*Sr17sr17*	*sr17sr17*
'W3751/Warigo'	0	0	6	0	0	6	2	3	1
'W3755/Warigo'	0	0	8	0	0	8	3	4	1
'W3760/Warigo'	0	0	8	2	4	2	4	3	1
'W3762/Warigo'	3	5	0	0	0	8	3	2	3
'W3762/Line E'	4	7	3	–	–	–	–	–	–

Sr6: crosses with 'Warigo' and 'Line E'. At low temperatures (<20 °C), 'McMurachy' with *Sr6* displayed IT "0;" with pathotype 34-2,4,5,6,7,11, and IT "3+" with pathotypes 126-1,5,6,7,11 and 34-1,2,3,6,7,8,9. In the presence of *Sr12,* identification of *Sr6* was difficult because firstly, appropriate pathotypes with avirulence for *Sr6* and virulence for *Sr12* were not available in the Plant Breeding Institute culture collection. Secondly, both genes were temperature sensitive, the low IT responses for both can vary from "0;" to "X" or higher. However, the recessive *Sr12* had a greater tendency to confer IT "X" compared with the dominant *Sr6*, which usually conferred IT "0;" at the prevalent test conditions. Therefore, F_3 lines were scored with three pathotypes to test specificity, and when the presence of *Sr6* was indicated in 'Warigo' crosses, larger populations in corresponding 'Line E' crosses were also classified to confirm the 'Warigo' results. On this basis, the presence of *Sr6* was indicated in 'W3762' (Table 10).

Sr8a: crosses with 'Condor' (genotype Sr8aSr8a). 'Mentana' with *Sr8a* displayed ITs "2−" and "3+" with pathotypes 34-1,2,3,5,7,8,9 and 34-1,2,3,6,7,8,9, respectively. Although at low temperatures (<20 °C) *Sr12* determined Its ";X−"−"X" with these pathotypes, considerably higher ITs "X++3+"−"3+" were obtained for this gene at higher temperatures (>25 °C). The presence of *Sr8a* was indicated in 'W3760' and 'W3762' because all F_3 lines in 'Condor' crosses were nonsegregating resistant with ITs "2−"−"2" (Table 9). Gene *Sr8a* was absent in 'W3751,' because the cross with 'Condor' segregated. The lack of segregation in 'W3755'/'Line E' indicated the absence of *Sr8a* (Table 9).

Sr9b: crosses with 'Condor,' 'Warigo,' and 'Line E.' 'W2402', with *Sr9b* displayed a very characteristic IT "2-N" with pathotype 126-1,5,6,7,11, and IT "2" with 34-2,4,5,6,7,11. Segregation for these ITs in the cross of 'W3760' with 'Condor' indicated the presence of *Sr9b* (Table 9), and this was further confirmed by segregation in the corresponding 'Warigo' cross (Table 10).

Sr11: crosses with 'Warigo' and 'Line E.' 'Yalta' with *Sr11* displayed ITs ";2=" and "33+" with pathotypes 126-1,5,6,7,11 and 34-2,4,5,6,7,11, respectively, at >25 °C. The absence of this dominant gene was shown by the lack of segregation for IT ";2=" in crosses with 'Warigo' using the former pathotype (Table 10). This resistance was not apparent in tests with the latter. On the other hand, segregation for the hypostatic *Sr9b* gene was evident with both pathotypes in the cross 'W3760'/'Warigo.'

Sr12: crosses with 'Condor' (genotype Sr12Sr12), 'Warigo,' and 'Line E.' In crosses involving 'Condor,' the presence of *Sr12* was postulated in 'W3760' since all F_3 lines were nonsegregating resistant with ITs ";"−"X" to pathotypes 34-1,2,3,6,7,8,9 and 126-1,5,6,7,11 at low temperatures (Table 9). Segregation for this recessive resistance allele in crosses of 'W3751' and 'W3762' with 'Condor' and the lack of segregation in 'W3755'/'Line E' (Table 9) indicated the absence of *Sr12* in 'W3751,' 'W3755,' and 'W3762.'

In 'Warigo' crosses, the genotypes of F_3 lines were postulated from tests with pathotypes 34-1,2,3,6,7,8,9; 34-2,4,5,6,7,11; and 126-1,5,6,7,11. The conclusions from these tests (Table 10) were similar to those obtained from the 'Condor' and 'Line E' crosses.

Sr17: crosses with 'Warigo' (genotype Sr17Sr17). 'Renown' and 'Warigo' with *Sr17* displayed IT ";" with pathotype 34-1,2,3,6,7,8,9 and IT "33+" with pathotypes 34-2,4,5,6,7,11 and 126-1,5,6,7,11 at lower temper-

Table 10. Continued

Pathotype						Postulated gene(s) in South African
126-1,5,6,7,11 & 34-2,4,5,6,7,11 Temperature >25 °C						
Genotype and frequency						
Sr9bSr9b	*Sr9bsr9b*	*sr9bsr9b*	*Sr11Sr11*	*Sr11sr11*	*sr11sr11*	
0	0	6	0	0	6	–
0	0	8	0	0	8	–
2	4	2	0	0	8	*Sr9b, Sr12*
0	0	8	0	0	8	} *Sr6*
–	–	–	–	–	–	

atures. Segregation for this recessive resistance allele in all crosses indicated its absence in all four South African wheats (Table 10).

Discussion

The four South African wheats were selected for inheritance studies because of their low seedling reactions and adult-plant resistance to most Australian isolates of *P. graminis tritici*. The main objective of the inheritance studies was to identify the gene(s) conferring resistance to economically important pathotypes such as 34-1,2,3,4,5,6,7 and 343-1,2,3,5,6. A secondary objective was to demonstrate the presence or absence of other well-known resistance genes that are widely distributed among wheats selected for stem rust resistance. The gene(s) conferring low seedling reactions to either or both of the above pathotypes were: *Sr9e* in 'W3762'; *Sr24* in 'W3760'; *Sr24* and an unidentified gene, *SrC*, in 'W3751;' and *Sr7a*, *Sr23*, and *Sr36* in 'W3755.' Genes *Sr9e*, *Sr24*, and *Sr36* also conferred high levels of adult plant resistance to the predominant field pathotypes, whereas genes *Sr7a*, *Sr23*, and *SrC* on their own did not confer readily detectable levels of resistance. The relative ineffectiveness of *Sr7a* in other wheats was reported elsewhere (Singh and McIntosh 1986, 1987).

In order to test for the presence of additional genes, about eight F_3 lines, nonsegregating susceptible to pathotypes 343-1,2,3,5,6 or 222-1,2,3,5,6, were chosen and evaluated with several pathotypes, each avirulent for one or two genes for which the above pathotypes were virulent. Thus, genes *Sr6* and *Sr8a* were identified in 'W3762,' and *Sr5*, *Sr8a*, *Sr9b*, and *Sr12* were found in 'W3760.' This approach is satisfactory in the absence of genetic linkage with the genes identified in the primary analysis. Because of the small numbers of F_3 lines used for the secondary analysis, close genetic linkage could result in failure to detect the presence of a resistance gene for which the assay was designed. However, the procedure adopted here can be recommended for those situations where following the genetic investigations of resistance to currently important pathotypes, a more complete genotype for a particular host can be ascertained. Moreover, this can be achieved even when the alternative parent used in crossing carries resistance factors. In this case, the test is for genetic homozygosity rather than for segregation.

Crosses of the South African wheats with 'Warigo' were made especially to test for *Sr2*, which cannot be detected in seedling tests (Hare and McIntosh 1979). *Sr2* was present only in 'W3751.' This study also demonstrated that the presence of *Sr2* could be more easily confirmed by testing for homozygosity.

Tests with various pathotypes of *P. recondita tritici* and limited genetic analyses indicated the presence of genes *Lr13* in 'W3762;' *Lr13* and *Lr24* in 'W3760' and 'W3751;' and *Lr16* in 'W3755.' *Lr24* was completely linked with *Sr24*. Both are known to be located in an alien chromosome segment (McIntosh et al. 1977) that also carries a gene for red grain color. In 'W3762,' *Sr9e* and *Lr13* were linked with recombination 12.5% ±2.3%. This value was similar to that of 17.6% ±3.1% for *Sr9b* and *Lr13* reported by Singh and McIntosh (1986), and to many other estimates made by W. H. Hawthorn and R. A. McIntosh (unpublished data).

Acknowledgements. This work constituted part of a Ph.D. program undertaken by the senior author while a recipient of a University of Sydney postgraduate studentship. Support from the Wheat Research Council of Australia is gratefully acknowledged.

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heridity. Hilgardia 24:235–277

Anderson RG (1961) The inheritance of leaf-rust resistance in seven varieties of common wheat. Can J Plant Sci 41:342–359

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistance in 'Hope' and related cultivars to wheat rusts. Z Pflanzenzuecht 83:350–367

Johnston CO, Levine MN (1955) Fifth revision of the international register of physiologic races of *Puccinia rubigo-vera* (DC) Wint. f sp. *tritici* (Eriks) Carleton. Plant Dis Rep, ARS-USDA, Suppl. 233

McIntosh RA (1983) A catologue of gene symbols for wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genetics Symp, Kyoto, Japan, pp 1299–1309

McIntosh RA, Dyck PL, Green GJ (1974) Inheritance of reaction to stem rust and leaf rust in wheat cultivar Etoile de Choicy. Can J Genet Cytol 16:571–577

McIntosh RA, Dyck PL, Green GJ (1977) Inheritance of leaf rust and stem rust resistance in wheat cultivars Agent and Agatha. Aust J Agric Res 24:37–45

McIntosh RA, Luig NH, Milne DL, Cusick J (1983) Vulnerability of triticales to wheat stem rust. Can J Plant Pathol 5:61–69

Nyquist WE (1962) Differential fertilization in the inheritance of stem rust resistance in hybrids involving a common wheat strain derived from *Triticum timopheevi*. Genetics 47:1109–1124

Singh RP, McIntosh RA (1986) Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in Kenya Plume wheat. Euphytica 35:245–256

Singh RP, McIntosh RA (1987) Genetics of resistance to *Puccinia graminis tritici* in 'Chris' and 'W3746' wheats. Theor Appl Genet 73:846–855

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var *tritici*. USDA, ARS E617 (revised)

Watson IA (1981) Wheat and its rust parasites in Australia. In: Evans LT, Peacock WJ (eds) Wheat science – today and tomorrow. Cambridge University Press, Cambridge, pp 129–147

Watson IA, Luig NH (1961) Leaf rust on wheat in Australia: a systematic scheme for the classification of strains. Proc Linn Soc NSW 86:241–250

Watson IA, Luig NH (1963) The classification of *Puccinia graminis* var. *tritici* in relation to breeding resistant varieties. Proc Linn Soc NSW 88:235–258