STUDIES ON THE PATHOGENICITY OF *PUCCINIA GRAMINIS f. sp. TRITICI* AND THE NATURE OF HOST RESISTANCE IN WHEAT

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ABSTRACT

This study was undertaken to investigate the pathogenic variation of *Puccinia graminis* f.sp. *tritici* in South Africa and to determine the nature of host resistance to this fungus. Seventeen pathotypes of wheat stem rust were identified in 1981 and only one during 1982 and 1983. The 1984 and 1985 surveys provided six and nine pathotypes respectively. These included two new pathotypes 2SA100 and 2SA101, both virulent for *Sr24*. No avirulence was detected for wheat stocks having *Sr7a*, *8a*,*10*,*11*,*14*,*16*, *18*,*19*,*20* and *28*. No virulence was found on wheats with *Sr8b*,*13*,*21*,*22*,*25*,*26*,*27*,*29*,*31*,*32*,*35*,*dp2* and *Tt3*. Data suggested that the pathogenicity pattern in South Africa is strongly influenced by the resistance genes present in wheat cultivars. Pathogenic studies of pathotypes 2SA100 and 2SA101 demonstrated that both possessed increased virulence for *Sr24* and appeared to be mutations of earlier types. Seedling and adult plant response studies indicated genetic vulnerability in 60% of the locally cultivated cultivars. Nine of the 23 recommended cultivars possess *Sr24*, either alone or in combination with other resistance factors. Pathotypes 2SA100 and 2SA101, or races similar to these, constitute a major threat to wheat production in Southern Africa since they combine increased virulence with aggressiveness and good survival ability.

Genetic studies showed that resistance was conferred by one dominant gene in each of Belinda (<u>Sr5</u>), SST33 (<u>Sr9e</u>), SST66 (<u>Sr9e</u>), SST102(<u>Sr24</u>), Gamka (<u>Sr24</u>), Gamtoos (<u>Sr31</u>), Karee and Betta. Two partially dominant genes were identified in Wilge, Gouritz(<u>Sr36</u>+) and SST23 (<u>Sr24</u>+). Resistance in Palmiet was conferred by one dominant, (<u>Sr24</u>) and one recessive gene, whereas one recessive and one dominant gene operated in Molen. Tugela carried three partially dominant genes, one of which was positively identified as <u>Sr5</u>. Palala also has three unidentified dominant genes. The study determined that inheritance of resistance in 15 South African cultivars was relatively simple with little genetic diversity. Three spring wheat cultivars, SST44,Palmiet and Gamtoos were used to study the quantitative aspects of specific resistance to <u>P. graminis tritici</u>. Averaged over three cultivars, latent period was extended by 57% and uredinium density was reduced by 29% relative to the susceptible check Morocco. Latent period appeared to be a race-nonspecific resistant component of all three cultivars. This study indicated that the variation in uredinium density associated with specific resistance was similar to that reported by other workers for compatible host pathogen interactions.

DECLARATION

I, JACOBUS LE ROUX, hereby declare that this thesis is my own original research and has not been submitted, in part or as a whole for a degree at any other university.

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CHAPTER 1

OCCURRENCE AND PATHOGENICITY OF PUCCINIA GRAMINIS f. sp. TRITICI IN SOUTH AFRICA DURING THE PERIOD 1981-1985*

1.1 ABSTRACT

Seventeen pathotypes of wheat stem rust(Puccinia graminis f.sp. tritici) were identified in 1981. The most frequent pathotypes were 2SA6 and 2SA45, making up 32% and 31% respectively of isolates evaluated. Pathotype 2SA6 differed from 2SA45 only in virulence for Sr9g. Pathotype 2SA48 constituted 14% of the isolates. Due to extreme drought, only six collections were obtained during 1982 and 1983, all being pathotype 2SA48. The 1984 and 1985 surveys provided six and nine pathotypes respectively. These included two new pathotypes, 2SA100 and 2SA101, virulent for Sr24. Pathotype 2SA100 was most frequently isolated, constituting 58% of collections in 1984 and 55% in 1985. Pathotype 2SA101 differing from 2SA100 in possessing virulence for Sr9g, constituted 4% of collections in both years. The second most frequently found pathotype in 1984 and 1985 was

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*The chapters contained in this dissertation were prepared as papers for publication in scientific journals. Therefore American spelling and punctuation have been used and certain experimental procedures etc. are inevitably repeated in different chapters.

2SA4 constituting 20% of all isolates. Characterized by virulence for Sr9e, 2SA4 was almost exclusively isolated from SST66 and SST33, both having Sr9e as a major resistance gene. No virulence was found on wheats with Sr8b,13,21,22,25,26,27,29,31,32,35,dp2 and Tt3. No avirulence was detected for wheat stocks having Sr7a, 8a,10,11,14,16,18,19,20 and 28. The data suggested that the pathogenicity pattern in South Africa is strongly influenced by the resistance genes that are present in wheat cultivars.

1.2 INTRODUCTION

The importance in South Africa of stem rust, caused by *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn. on wheat(*Triticum aestivum* L.), is self evident from persistently incurred yield losses(23). Initially, resistant cultivar and germplasm development was accomplished by the crossing of lines selected for resistance under conditions of both natural infection and field inoculation. In the absence of local information on pathogen variation, the products of such breeding were planted on a multilocational basis across the wheat-producing areas of South Africa, in an effort to expose them to the widest possible array of pathotypes. However, this method of screening for resistance proved to be successful only in the short term, since newly released cultivars soon succumbed to either an

undetected, or a mutated stem rust pathotype. To select for effective resistance, conditioned by a single gene or a combination of genes, continual monitoring of pathogenic variation is imperative. Early pathotype surveys were based on the Stakman(55) host differential set(8,65,66,67). However, data obtained by means of these surveys proved inadequate, because the host resistance genes being monitored in the differential set were generally different from those present in the wheat breeders population(23).

Lombard(22) and Lombard and Lombard(24) showed that the standard P. graminis tritici races 21 and 34(55) comprised diverse pathotypes that could be distinguished using additional host testers. These studies resulted in the use of various additional testers, the composition and number of which were modified as more information became available(23).

With the resolution of the Department of Agriculture, that stem rust resistance would be mandatory for cultivars released from 1980 onwards, increased emphasis was applied to resistance breeding. Consequently, pathotype surveys are now conducted on an annual and more intensive basis.

The objectives of the surveys are :(a) the development of a host tester series capable of differentiating the existing P. graminis tritici pathotypes in South Africa

;(b) to timeously detect new pathotypes originating either by means of mutation or by introduction from neighbouring countries ;(c) to identify and manipulate resistance genes singly or in combination for use in the wheat breeding program ;(d) to identify pathotypes and maintain an appropriate pathotype collection in order to expedite genetic studies of the host and (e) to enhance the knowledge of host and pathogen and thereby to anticipate future pathogen variation.

This paper reports on pathogenic variation of P. graminis tritici during the period 1981-1985.

1.3 MATERIALS AND METHODS

Stem rust collections were submitted by personnel of the Department of Agriculture, universities and other co-operators throughout South Africa and the neighbouring countries of Lesotho, Swaziland and Botswana. Such samples were supplemented by an annual survey covering the major wheat production areas of the western, southern and eastern Cape Province, Natal, Orange Free State and Transvaal. During the annual survey, stops were made at randomly selected commercial fields. Special attention was given to volunteer wheat plants and adjacent grasses.

Additional inspections were carried out on experimental farms to survey cultivar trials, breeder material, nurseries and rust trap plots. At least one collection, consisting of rusted stems from a single plant bearing well spaced, young, erupted uredinia, was made at each site. Collections from co-operators were sent to the rust laboratory at Bethlehem(Orange Free State) in pre-addressed envelopes with the following information: cultivar from which collected, date collected, production region, growth stage of crop and name and address of sender.

On receipt of samples, urediniospores were harvested from each accession using a cyclone collector(57). The collected spores, suspended in light-weight mineral oil (Soltrol 130 ®), were applied to the first seedling leaves of wheat, cv. McNair 701 which had been previously treated with maleic hydrazide to enhance urediniospore production. Urediniospores collected from grasses, or from a host which could not,with certainty, be identified as wheat, were applied to one pot containing seedlings of each of wheat(cv. McNair 701), barley(cv. Clipper), oats(cv.Heros) and rye(cv. SSR1). Inoculated seedlings were placed in an incubation chamber, held at 100% relative humidity for a 14h dark period, followed by a 3h gradual drying period at a light intensity of 10 000 lux. Temperature inside the chamber was maintained at 20$\pm$2C. Plants were then moved

to a greenhouse where natural daylight was supplemented with illumination from cool-white fluorescent tubes for 14h each day and where temperature was controlled at 22±2C.

After 10 days, urediniospores were collected from a single uredinium per accession to provide isolates for the 1981 survey. During 1984 and 1985, two single uredinium isolates per accession were established in a similar way. Isolates were increased on 7-day old McNair 701 seedlings to produce sufficient urediniospores to inoculate the primary leaves of the host differential series. For this period the host differential series consisted of three groups. The standard Stakman series(55) consisting of Little Club (SrLC), Marquis(Sr7b, 18, 19, 20, x), Reliance(Sr5, 16, 18, 20), Kota(7b, 18, 28, kt2), Arnautka(Sr9d), Mindum (Sr9d), Spelmar(Sr9d), Kubanka(Sr9d), Acme(Sr9g), Einkorn(Sr21), Vernal(Sr9e) and Khapli (Sr13, 14). The second group included 10 additional wheats, each having a special resistance gene viz. ISr5Ra(Sr5), ISr6Ra (Sr6), ISr7bRa(Sr7b), Mentana (Sr8a), W2691 + Sr9b, Yalta(Sr11), Renown(Sr7b + Sr17), BtSr24 Ag(Sr24), Festiquay(Sr30) and W2691 + SrTt1(Sr36).

A bulked urediniospore sample, representing each pathotype and each wheat production region, was used to inoculate a South African series consisting of the fol-

lowing: Barleta Benvenuto(Sr8b), Triticum aestivum derv.(Sr22), Agent(Sr24), Agatha(Sr25), Eagle(Sr26), WRT238-5(Sr27), Etiole de Choisy(Sr29), Veery#3(Sr31), CSSr32T5-2(Sr32), Medea Ap9d (Srdp-2) and the South African cultivars SST44, Gamka, Wilge, Karee and SST102.

Inoculated sets were exposed to the same incubation procedures as described for McNair 701 seedlings. Infection types were recorded 10-12 days later. Data were grouped into six ecological areas(Fig.1.1): Area 1: western Cape Province, hard red spring wheats, cultivated under winter rainfall conditions, mainly dryland; Area 2: southern Cape Province, hard red spring wheats, winter rainfall region with limited summer precipitation; Area 3: eastern Cape Province, hard red spring wheats, winter rainfall; Area 4: Orange Free State, hard red winter wheats, summer rainfall; Area 5: Natal, hard red spring wheats, predominantly irrigated, summer rainfall; Area 6:Transvaal, hard red spring wheats, summer rainfall, dryland and irrigation.

1.4 RESULTS

The characteristically low seedling infection types produced by 31 wheat genotypes carrying designated genes for resistance to *P. graminis* f.sp. *tritici* are presented in Table 1.1.

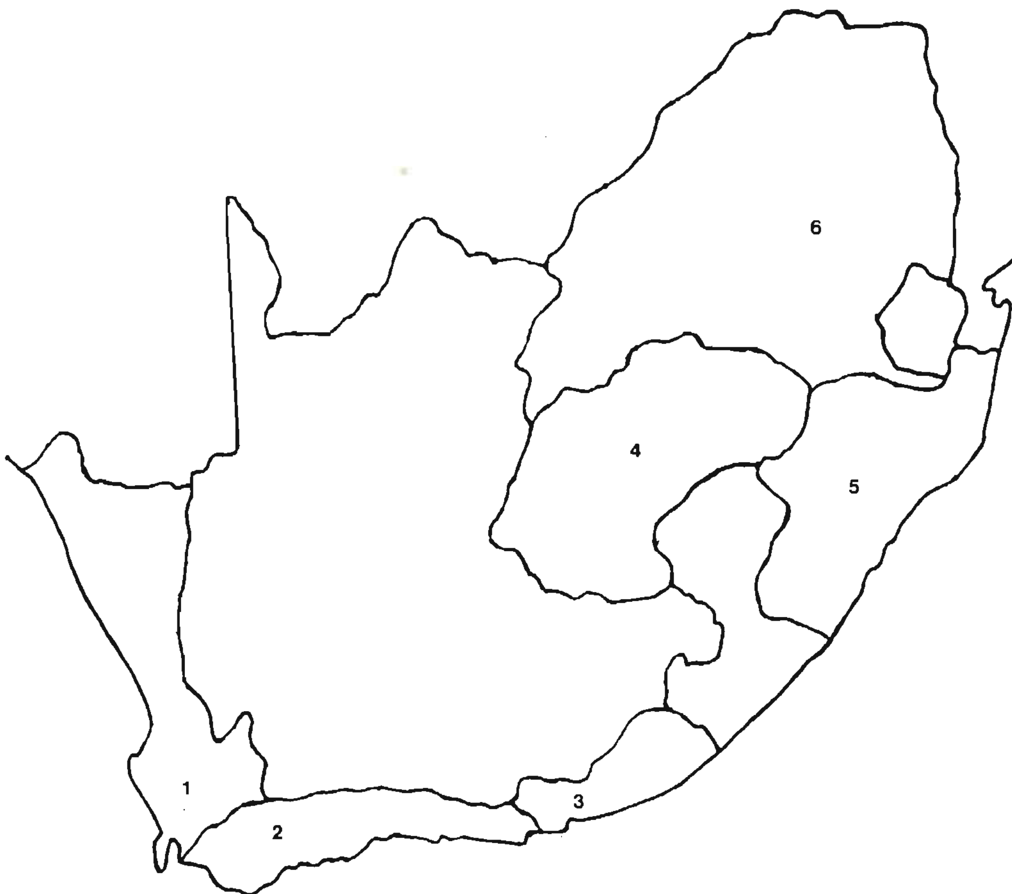

Fig.1.1. Ecological areas for wheat production in South Africa. Area 1: Western Cape Province, hard red spring wheats, winter rainfall region, dryland; Area 2: Southern Cape Province, hard red spring wheats, winter rainfall region with limited summer rains, dryland; Area 3: Eastern Cape Province, hard red spring wheats, winter rainfall region, dryland; Area 4: Orange Free State, hard red winter wheats, summer rainfall region, dryland; Area 5: Natal, hard red spring wheats, summer rainfall region, predominantly under irrigation; Area 6: Transvaal, hard red spring wheat, summer rainfall region, irrigated and dryland.

TABLE 1.1 Low seedling infection types produced on wheat genotypes carrying designated genes for resistance to *Puccinia graminis* f. sp. *tritici*

Gene Sr-	Low infection types[a]	Cultivar/Line[b]	C.I.[c]
5	0;, ;1=	ISr5Ra	14161
6	;, ;1	ISr6Ra	14163
7a	2+3c, 3c	MqSr7a	15083
7b	2	ISr7bRa	14165
8b	;1++n, x=cn	Barleta Benveneto	
9b	2-	W2691Sr9b	17386
9e	;cn, ;1=c	Vernal	
9g	;1cn, ;1++	Acme	
12	;1++, x+	BtSr12Tc	17783
13	;1, 12=	W2691Sr13	17387
15	;1+, x+	Norka	
17	;x+	Renown (Sr7b)	
21	11+	Einkorn	2433
22	1+2	*Triticum boeoticum* derv.	
23	3n	Exchange	
24	12-	BtSr24Ag	
25	2+	Agatha (=Tc + Sr25)	
26	o;, ;1=	Eagle	
27	0;, ;n+	WRT238-5	14141
29	1++2, 2	Etiole de Choisy	

TABLE 1.1 (Continued) Low seedling infection types produced o wheat genotypes carrying designated genes for resistance t Puccinia graminis f. sp. tritici

Gene *Sr*-	Low infection types[a]	Cultivar/Line[b]	C.I.[c]
30	1+, 2	Festiquay	P.I.330957
31	0;, ;12=c	Veery#3 + ?	
32	1+	GS Sr32	
35	;, ;1-	82.2692 (Univ. Sydney)	
36	0;, ;, ;1n	W2691SrTt1 (Line C sel.)	
37	;n, ;1=n	W2691 Sr37	
Gt	12-, 2++	BtSrGt	P.I.320230
dp-2	;1, 2	Medea Ap9d	3255
TAF_2d	1+	Agropyron intermedium	
?	1+	Enterlago de Montijo	
McN	3c	McNair 701	

a Comma seperates infection type variation due to differen cultures

b Name in front of Sr-gene refers to genetic background, nam at the end to the gene-source. No avirulence was observe for Sr-genes 7a, 8a, 9f, 10, 11, 14, 16, 18, 19, 20 and 28

c C.I. = U.S.A. Cereal Investigation Number, P.I. = U.S.A Plant Introduction number

TABLE 1.2 Avirulence/virulence combinations of Puccinia graminis f.sp. tritici identified in South Africa during 1984 and 1985[a]

S.A. Ac-cess.No.	Standard race	Avirulence/virulence formula (Sr-genes)	Year	Percentage of isolates for each area[b]						TOTAL
				W-Cape	S-Cape	E-Cape	O F S	Natal	T V L	
2SA100	222	9e, 9g, 30, 36/5, 6, 7b, 9b, 17, 24	1984	0	46,7	100	0	75,0	100	58,2
			1985	7,1	54,5	84,8	18,2	0	91,3	55,0
2SA4	321	9g, 24, 36/5, 6, 7b, 9b, 9e, 17, 30	1984	66,7	23,4	0	0	25,0	0	20,2
			1985	89,4	15,5	6,1	0	0	4,3	20,2
2SA10	98	6, 7b, 9b, 9e, 17, 24, 30, 36/5, 9g	1984	0	14,4	0	0	0	0	9,6
			1985	0	5,4	0	0	0	0	3,6
2SA101	34	9e, 30, 36/5, 6, 7b, 9b, 9g, 17, 24	1984	0	6,7	0	0	0	0	4,5
			1985	3,5	7,0	3,0	0	0	4,3	5,7
2SA32	326	5, 6, 7b, 9b, 9e, 9g, 17, 24, 30, 36/	1984	33,3	4,4	0	0	0	0	4,5
			1985	0	11,2	6,1	0	0	0	8,2
2SA2	21	5, 6, 9b, 9e, 17, 24, 30, 36/7b, 9g	1984	0	4,4	0	0	0	0	3,0
			1985	0	1,6	0	18,1	0	0	1,7
2SA6	34	9e, 24, 36/5, 6, 7b, 9b, 9g, 17, 30	1984	0	0	0	0	0	0	0
			1985	0	1,1	0	54,6	0	0	2,8
2SA43	40	24, 36/5, 6, 7b, 9b, 9e, 9g, 17, 30	1984	0	0	0	0	0	0	0
			1985	0	1,6	0	9,1	0	0	1,4
2SA48	222	9e, 9g, 24, 30, 36/5, 6, 7b, 9b, 17	1984	0	0	0	0	0	0	0
			1985	0	2,1	0	0	0	0	1,4

a No virulence was detected on resistance conferred by genes 8b, 13, 21, 22, 25, 26, 27, 29, 31, 32, 35, dp2, Tt3.
No avirulence was detected for 7a, 8, (9a, 9d, 9f), 10, 11, 14, 16, 18, 19, 20, 28.
Sr 15 was not included as a differential genotype.

b W-Cape - Western Cape; S-Cape - Southern Cape; E-Cape - Eastern Cape; OFS - Orange Free State; TVL - Transvaal

1.4.1 Year 1981

The incidence of stem rust on cultivated wheats was much less than in the previous two years, with no reports of serious yield losses. Data for the six ecological areas of South Africa are presented in Table 1.2.

On a basis of avirulence/virulence combinations seventeen pathotypes were identified from a total of 203 collections. The most frequent pathotypes, 2SA6 and 2SA45, differing only in respect of virulence for Sr9g, occurred in all the ecological areas except the western Cape Province and Natal, respectively. Explanation of designation; 2 identifies P. graminis f.sp. tritici, SA: South Africa, 6: record number for each distinctive pathotype. The third most frequent pathotype, 2SA48, similar to 2SA45 but avirulent for Sr30, was restricted to the spring wheat production areas of the western and southern Cape Province.

The incidence of virulence for the individual host resistance genes tested in 1981 is presented in Table 1.4. Virulence for Sr36 occurred in five pathotypes including 2SA36(8,5% of all isolates tested), 2SA49 (2,5%), 2SA33 (1%), 2SA20(0,5%) and 2SA53 (0,5%).The latter combined virulence for both Sr36 and Sr9e. Pathotypes virulent for Sr9e, viz. 2SA53, 2SA43 and 2SA4, isolated at a very low combined frequency(1%),

occurred only in the eastern Cape Province and Transvaal. A further characteristic of the 1981 stem rust survey was the importance of _Sr9g_ as a host differential gene. Forty percent of pathotypes were virulent for _Sr9g_.

No virulence was detected for stocks with Sr_8b_, _13_, _21_, _22_, _24_, _25_, _26_, _27_, _29_, _31_, _32_, _35_ and _dp2_. Resistance genes Sr_7a_, _8a_, _10_, _11_, _14_, _16_, _18_, _19_, _20_ and _28_ failed to condition resistance to any of the pathotypes evaluated.

1.4.2 Years 1982 and 1983

Environmental conditions were highly unfavorable for stem rust development during 1982 and 1983. The South African sub-continent suffered the worst drought in many years, with winter and summer grain production at a near record low. These conditions, combined with the cultivation of resistant cultivars, resulted in an absence of stem rust. Even susceptible trap plots of cultivars such as Morocco, distributed throughout the country, were not infected. Only six collections, all consisting of pathotype 2SA48, were processed over both seasons. Although growing conditions improved towards the end of the 1983 season, inoculum levels were too low for rust outbreaks to occur.

1.4.3 Years 1984 and 1985

In September 1984 during the course of the regular survey, a few scattered pustules were found on commercially irrigated hitherto resistant SST44 and Gamka plants near Worcester in the western Cape Province. The center of the outbreak was found 250 km further eastward in the mistbelt area of Albertinia in the southern Cape Province. Susceptible responses of 80S(Cobb scale) were recorded on SST44 and, almost simultaneously, on the cultivar Gamka. Limited yield losses, confined to the Albertinia region, were recorded. One week later, similar infection levels were observed in a breeder's crossing block(Condor/Agent derivatives) near Groblersdal. This restricted outbreak, situated in the summer rainfall region of the Transvaal was ±2000 km northeast of the first observation.During December, stem rust was identified on SST44 and Gamka in the eastern Cape Province.

A survey was conducted during March 1985 following abnormally high summer rains in the wheat production areas of the southern and eastern Cape Province. Stem rust survived on volunteer wheat plants and *Hordeum murinum* Huds., furnishing adequate inoculum levels for the following wheat season.

The incidence of stem rust during 1985 was moderate to severe compared to 1982, 1983 and 1984. The pathogen, which had been confined in occurrence to specific areas of the southern Cape Province, Natal and Transvaal during 1984, spread to adjacent areas of the western and eastern Cape Province and Orange Free State. However extensive yield losses, ranging between 17% and 75%, were confined to Albertinia.

The 1984 and 1985 surveys permitted the identification of six and nine pathotypes respectively(Table 1.3) in contrast to 17 identified during 1981 and one in 1982-1983. Two new pathotypes, 2SA100 and 2SA101, both unique in virulence for stocks having _Sr24_ were identified. Pathotype 2SA100 predominated in both years constituting 58% and 55% of isolates. In 1984 it was found in the Transvaal, Natal and eastern Cape Province only. During 1985 it spread to the western Cape Province and Orange Free State. The related pathotype, 2SA101, differing from 2SA100 in being virulent for _Sr9g_ and occurring in the western, southern and eastern Cape Province and Transvaal constituted in 4,5% of collections.

The second most frequent(20%) pathotype,2SA4, characterized by virulence for _Sr9e_, was exclusively isolated from the spring wheat cultivars SST33 and SST66 in all production areas except the Orange Free State. A rela-

TABLE 1.3 Avirulence/Virulence combinations of *Puccinia graminis* f.sp. *tritici* identified in South Africa during 1981[a]

S.A. Access. No.	Standard race	Avirulence/virulence formula (Sr-genes)	Percentage of isolates for each area[b] W-Cape	S-Cape	E-Cape	O F S	Natal	T V L	TOTAL
2SA6	34	9e, 24, 36/5, 6, 7b, 9b, 9g, 17, 30	33,3	11,7	15,4	57,1	0	45,7	31,6
2SA45	222	9e, 9g, 24, 36/5, 6, 7b, 9b, 17, 30	0	28,8	26,9	35,7	100	45,7	31,1
2SA48	222	9e, 9g, 24, 30, 36/5, 6, 7b, 9b, 17	42,4	28,8	0	0	0	0	14,3
2SA36	222	9e, 9g, 24, 30/5, 6, 7b, 9b, 17, 36	0	13,5	38,5	0	0	0	8,5
2SA49	222	9e, 9g, 24/5, 6, 7b, 9b, 17, 30, 36	0	0	0	7,2	0	5.2	2,5
2SA10	98	6, 7b, 9b, 9e, 17, 24, 30, 36/5, 9g	9,1	1,9	0	0	0	0	2
2SA39	21	5, 9b, 9e, 17, 24, 30, 30/6, 7b, 9g	0	5,6	0	0	0	0	1,5
2SA54	343	7b, 9e, 9g, 24, 36/5, 6, 9b, 17, 30	6,1	0	3,8	0	0	0	1,5
2SA18	98	7b, 9b, 9e, 24, 30, 36/5, 6, 9g, 17	0	3,9	0	0	0	0	1,0
2SA51	222	9b, 9e, 9g, 17, 24, 30, 36/5, 6, 7b	0	3,9	0	0	0	0	1,0
2SA52	34	9e, 24, 30, 36/5, 6, 7b, 9b, 9g, 17	6,1	0	0	0	0	0	1,0
2SA33	343	7b, 9e, 9g, 17, 24, 30/5, 6, 9b, 36	0	0	7,7	0	0	0	1,0
2SA2	21	5, 6, 9b, 9e, 17, 24, 30, 36/7b, 9g	3,0	0	0	0	0	1,7	1,0
2SA20	34	9e, 24, 30/5, 6, 7b, 9b, 9g, 17, 36	0	1,9	0	0	0	0	0,5
2SA53	40	24/5, 6, 7b, 9b, 9e, 9g, 17, 30, 36	0	0	3,8	0	0	0	0,5
2SA43	40	24, 36/5, 6, 7b, 9b, 9e, 9g, 17, 30	0	0	3,8	0	0	0	0,5
2SA4	321	9g, 24, 36/5, 6, 7b, 9b, 9e, 17, 30	0	0	0	0	0	1,7	0,5

a No virulence was detected on resistance conferred by genes 8b, 13, 21, 22, 24, 25, 26, 27, 29, 31, 32, 35, dp2, Tt3. No avirulence was detected for 7a, 8a, (9a, 9d, 9f), 10, 11, 14, 16, 18, 19, 20 and 28. A host tester with Sr15 was not included.

b W-Cape - Western Cape; S-Cape - Southern Cape; E-Cape - Eastern Cape; OFS - Orange Free State; TVL - Transvaal.

TABLE 1.4 Percentage virulence[a] in *Puccinia graminis* f. sp. *tritici* pathotypes to single-gene differential lines used in 1985. Totals for 1985 are compared with those for 1981 and 1984

Gene	Production areas[b]					South Africa		
Sr-	W-Cape	S-Cape	E-Cape	O F S	TVL	1981	1984	1985
5	100	87	94	82	100	99	93	90
6	100	82	94	94	100	97	83	87
7b	100	83	94	82	100	95	83	88
9b	100	82	94	82	100	95	86	87
9g	7	17	3	82	4	39	17	15
9e	89	17	6	9	4	1	20	22
17	100	88	94	82	100	93	83	87
24	11	61	87	18	96	0	63	60
30	89	18	6	64	4	57	20	24
36	0	0	0	0	0	13	0	0

[a] No virulence was detected on resistance conferred by genes 8b, 13, 21, 22, 25, 26, 27, 29, 31, 32, 35, dp2 and Tt3. No avirulence was identified for Sr7a, 8, 10, 11, 14, 16, 18, 19, 20 and 28

[b] W-Cape - Western Cape; S-Cape - Southern Cape; E-Cape - Eastern Cape; OFS - Orange Free State; TVL - Transvaal

ted pathotype, 2SA43, virulent for Sr9e and Sr9g, made up 1,4% of isolates. Virulence for Sr9e was always associated with virulence for Sr30(Festiquay).

Pathotypes 2SA10 (Standard race 98), 2SA32(326) and 2SA2(21) were characteristically avirulent for Sr6, Sr9b, Sr17, Sr24 and Sr36. Pathotypes 2SA6 and 2SA45, which dominated the 1981 pathogen population, were absent in 1984 and 2SA6 was present at a very low frequency in 1985(Table 1.3).

The incidence of virulence in different years for various host genes are presented in Table 1.4. No virulence was detected for lines with Sr8b, 13, 21, 22, 25, 26, 27, 29, 31, 32, 35 and dp2. Sr7a, 8a, 10, 11, 14, 16, 18, 20 and 28 conditioned susceptibility to all pathotypes.

1.5 DISCUSSION

The pathogenic attributes of the South African P. graminis tritici populations,indicate a continuous process of adaptation. From surveys conducted over the period 1965 to 1981, Lombard(23), showed that most variation was toward the accumulation of virulence for the resistance genes Sr6, Sr9b, Sr11 and Sr17.

The present study contextualizes an earlier report of the incidence of two new pathotypes virulent on Sr24 (20, see also Chapter 2) occurring in all major wheat

TABLE 1.5 Production[a] of spring wheat cultivars SST44, SST66 and SST33 in the major wheat production areas of South Africa during the period 1980-1985

Year	Production areas				
	South Africa	Cape Province	Orange Free State	Transvaal	Natal
SST44 (Sr24)					
1980	0.1	0.7	-	-	-
1981	0.7	1.5	0.1	0.9	-
1982	8.6	16.9	1.9	12.1	-
1983	14.1	15.7	4.3	31.5	4.5
1984	7.1	11.7	2.1	12.7	3.1
1985	5.6	6.9	1.9	7.6	1.0
SST66 (Sr9e)					
1980	0.4	0.9	-	0.1	-
1981	9.7	11.7	-	0.3	-
1982	8.2	14.2	0.1	19.2	10.8
1983	12.3	20.9	0.2	11.6	36.4
1984	14.3	31.0	0.5	9.2	35.8
1985	29.3	43.0	4.6	21.9	35.4
SST33 (Sr9e)					
1980	0.4	1.0	-	-	-
1981	2.8	7.5	-	0.4	-
1982	4.1	8.5	0.2	5.3	1.1
1983	5.4	9.4	0.2	4.4	3.4
1984	4.3	8.6	0.2	6.0	9.1
1985	7.0	10.7	0.5	4.4	2.7

[a] Percentage of total production.

producing areas of South Africa in 1984 and 1985. These presumably single-gene mutants were selected due to the extensive cultivation(Table 1.5) of cultivars SST44 and Gamka possessing Sr24. Consequently pathotypes 2SA100 and 2SA101 now constitute a major threat to wheat production in Southern Africa and in other areas where this gene is extensively employed.

The second major shift in virulence frequencies since 1981, concerns Sr9e. Pathotypes virulent for Vernal (Sr9e) have occurred at very low frequencies in South Africa since 1929(23, 66). With the cultivation of the spring wheats SST33 and SST66(Table 1.5), there has been a corresponding and significant increase in the frequencies of pathotypes 2SA4 and 2SA43, virulent for Sr9e. Presently, SST33 and SST66 constitute 36% and 44% respectively of cultivated wheats in South Africa and the Cape Province.

The present study, as well as that of Lombard(23), also provides evidence that an increase in the cultivation of certain cultivars is not necessarily associated with a corresponding increase in the frequency of pathotypes virulent for those cultivars. Virulence for Sr36, first observed in South Africa in 1964(24), reached a peak of 13% during 1981, but was not detected in the 1984 and 1985 seasons(Table 1.4). This was despite the presence of several cultivars with Sr36, including Gouritz,

Dipka, Flamink, SST101 and Zaragoza, all susceptible in both the seedling and adult plant stage to Sr36-virulent pathotypes. One explanation for these results is that Sr36-virulent pathotypes such as 2SA36, 2SA49, 2SA33, 2SA20 and 2SA53, identified up to 1981 have not possessed, like 2SA100 and 2SA4, the ability to combine increased virulence with aggressiveness and good survival features. It is fortuitous that the mutant pathotype with virulence for Sr24 lacks virulence for Sr36. Again 2SA53 occurred only at very low frequencies and may not have survived the widespread drought conditions in 1982 and 1983.

Therefore, taking the avirulence/virulence behavior of the genes Sr6, 9b, 11 and 17(23),as well as Sr9e, Sr24 and Sr36 into consideration,there is considerable evidence that the virulence structure of P. graminis tritici is to a great extent,but not solely, determined by the resistance genes present in local cultivars. Similar reports for Sr6, Sr9b, Sr11, Sr17 and Sr36 have emanated from eastern Australia(29). However data from certain regions of North America(46) has shown that the cultivation of resistant cultivars has not resulted in major changes in resistance or virulence attributes of the pathogen over many years.

Wheat lines with 33 different genes were investigated in these studies. Of these, 10 lines reacted differentially, 10 were susceptible to all pathotypes and 13

were resistant to all pathotypes. The differentiating group, which included lines with genes Sr5, 6, 7b, 9b, 9g, 9e, 17, 24, 30 and 36 was selected as the basis of a future set of host differentials, whereas the uniformly resistant group viz., Sr8b, 13, 21, 22, 25, 26, 27, 29, 31, 32, 35, Tt3 and dp2, are being used as sources of resistance by wheat breeders.

Considering the identification of two Sr24-virulent pathotypes, as well as the significant increase in the frequencies of pathotypes virulent for Sr9e, combined with the decrease in virulence for Sr36, the present study demonstrated the importance of annual pathogenicity surveys. The significant changes in pathotype frequencies which occurred in two seasons, have had a paramount effect on future breeding strategies.

CHAPTER 2

PATHOTYPES OF PUCCINIA GRAMINIS f.sp. TRITICI WITH INCREASED VIRULENCE FOR SR24

2.1 ABSTRACT

Pathogenic studies of two pathotypes of *Puccinia graminis* f.sp.*tritici* first isolated during 1984 on the previously resistant cultivars SST44 and Gamka, demonstrated that both possessed increased virulence for *Sr24*. These pathotypes, designated 2SA100 and 2SA101, which appeared to be mutations of earlier types, became widespread throughout the South African wheat production areas within two seasons. Seedling and adult-plant response studies indicated genetic vulnerability in 60% of the cultivars. Nine of the 23 recommended cultivars possess *Sr24*, either alone, or in combination with other resistance factors. Susceptibility ratings, measured in terms of latent period and uredinium density, separated cultivars into three distinctive groups. Differences in the degree of susceptibility in lines/cultivars having *Sr24* as a major stem rust resistance gene were evident. Pathotypes 2SA100 and 2SA101 or races similar to these constitute a major threat to wheat production in Southern Africa and other regions where *Sr24* is deployed, since they combine increased virulence with aggressiveness and good survival ability.

2.2 INTRODUCTION

Stem rust, caused by Puccinia graminis f.sp. tritici Eriks. & Henn., is an important disease of wheat (Triticum aestivum L.)in the winter rainfall regions of South Africa.

The pathogen oversummers on volunteer wheat plants and certain grasses (Hordeum murinum Huds.) which are supported by periodic summer rains. Moreover, the likelihood of stem rust outbreaks is enhanced because wheat is grown throughout the year in different climatic regions. In order to reduce the frequency and extent of epidemics, resistant cultivars have been released since the mid 1960's.

In the process of the production of new resistant cultivars, wheat breeders and pathologists have exploited the available genetic sources. The resistance genes currently deployed in local cultivars include: Sr9e (SST33, SST66); Sr24 (SST44, SST102, Gamka); Sr31 (Gamtoos) and Sr36 in Zaragoza and SST107 (see Chapters 3 and 4). By the end of 1984, 54% and 61% of the wheat areas in the winter rainfall region and the Transvaal irrigation areas respectively were planted with resistant cultivars. The use of resistant cultivars in combination with a serious drought in the summer rainfall region where more susceptible cultivars are grown, resulted in a sharp decline in the

incidence and severity of stem rust in the period 1982 to 1983 (see Chapter 1).

SST44(C.I.13523/3*T4[Anza]), a high-yielding stem rust-resistant spring wheat, released during 1980, became the predominant cultivar in the winter rainfall and Transvaal areas. During 1983, SST44 reached a production peak of 15% and 31% respectively of wheat produced in both areas(see Table 1.5, Chapter 1).Although it possesses genes Sr5, Sr8a, Sr9b, Sr12 and Sr24(53), only the last conferred resistance to the predominant local strains (see Chapter 1).

Until recently, Sr24, a dominant gene present in a spontaneously translocated chromosome of *Thinopyrum elongatum*(=*A. elongatum* Host.Beauv.) to wheat chromosome 3D(9), conditioned resistance in both seedling and adult plants to stem rust worldwide(23,27,31,46). Only one virulent culture, a putative *P. graminis tritici*/*P. graminis secalis* hybrid has previously been reported by the University of Sydney Plant Breeding Institute(37). Artificial mutation studies suggested that the avirulence gene corresponding to Sr24 rarely mutates to virulence(25).

Because of the widespread effectiveness of Sr24 in controlling stem rust, it has been exploited quite extensively in e.g. Australia, where it occurs in Sun-

dor, Skua, Torres,Bass, Sunelg and Vasco(27), United States of America where it is present in Agent(37), Blueboy II, Cloud(26) and Siouxland(50), and South Africa occurring in SST44, Gamka and SST102 (see Chapters 3 and 4). Presently, Sr24 also serves as a universal resistant tester in pathogen variability surveys worldwide(23, 27, 31, 47).

This study circumscribes two stem rust pathotypes, 2SA100 and 2SA101, first found in South Africa during 1984(20). Both are characterized by increased virulence for Sr24 and are currently still the only pathotypes that have been found to be virulent on commercial cultivars possessing this gene.

2.3 MATERIALS AND METHODS

During the annual rust survey, stem rust samples collected from each of the various localities were inoculated onto 7-day old seedlings of wheat (cv. McNair 701), treated with maleic hydrazide to enhance spore production. After 10-12 days, urediniospores from two single uredinia were separately collected and increased. The subsequent cultures were inoculated onto the following differential testers: Reliance (with gene, Sr5), ISr7bRa(Sr7b), ISr8Ra(Sr8a), W2402 (Sr9b), Vernal(Sr9e), Acme(Sr9g), Festiquay(Sr30), W2691 + Sr36 (Sr36), Isr6Ra(Sr6), Renown(Sr7b + Sr17), Agent(Sr24),

SST44 and Gamka(local Sr24 testers), Yalta(Sr11), TAF2d (Agropyron intermedium - unnamed gene) and Enterlago de Montijo(unnamed gene). Inoculated seedlings were placed in an incubation chamber held at 100% relative humidity for a 14h dark period, followed by 3h of gradual drying at a light intensity of 10 000 lux. Temperature inside the chamber was maintained at 20 ± 2C. Plants were then moved to a greenhouse where they received natural daylight supplemented with illumination from cool-white fluorescent tubes for 14h each day. Temperature was controlled at 22±2C. Infection-type responses (IT) were recorded after 10-12 days according to the method of Stakman et al.(55).

Following the demonstration of two new pathotypes, seedlings of an extended differential host series (Table 2.1), including all previous designated genes were assessed.

2.3.1 Additional Sr24 testers

In a subsequent test, in addition to Agent(37), two independently produced translocation stocks, designated 3D/Ag 1 and 3D/Ag 3; three lines having Sr24 in a Baart background and seven lines with Sr24 as a possible resistance factor selected from nurseries distri-

buted by CIMMYT(Table 2.2) were tested as seedlings (CIMMYT = International Maize and Wheat Improvement Centre). Two P. graminis tritici cultures viz. 2SA48 and 2SA52, avirulent and virulent respectively on stocks with Sr9g, and avirulent for Sr24, were included to represent the pre-1984 stem rust spectrum with the two pathotypes virulent for Sr24.

2.3.2 Seedling Response in South African Cultivars

Since resistance derived from Agent has been used extensively in local wheat breeding programs to control stem rust, it was imperative that recommended South African winter and spring wheats be evaluated for reaction to the new pathotypes. Accordingly, 20 cultivars(Table 2.3) were inoculated with 2SA48,2SA52 and the two Sr24-virulent pathotyes.

2.3.3 Adult Plant Response in South African Cultivars

To determine the relationship between seedling and adult plant response, six South African cultivars (Table 2.3), shown on the basis of above tests to possess Sr24, were inoculated with pathotypes 2SA100 and 2SA48. Tillers at growth stage 60(60) of each test plant were quantitatively inoculated with 0.11 ml (3mg urediniospores/ml) suspension (in Soltrol 130®), by means of the Andres inoculator(2). The trial consisted

of 12 replications, each replication being one plant. Plants were arranged in a randomized block design within the same greenhouse cubicle. This procedure was repeated for each cultivar-pathotype combination.

Inoculated adult plants were exposed to the same incubation procedures as described for seedlings. Latent period (LP) was determined on the terminal two internodes of adult plants by counting sporulating uredinia daily after inoculation until no more erupted. The LP was calculated by linear regression as the time from inoculation until the stage at which 40% of uredinia had erupted. Uredinium density (UD) was determined as the number of uredinia/cm stem length when all had erupted. The significance of differences in LP and UD between cultivars and pathotypes was tested by analysis of variance ($P \leq 0,05$) and Tukey's procedure (56).

2.3.4 Prevalence of Sr24 virulence during 1985

Following abnormally high summer rains in the wheat production areas of the southern and eastern Cape, a rust survey to assess oversummering of inoculum, was conducted during the beginning of March 1985.

2.4 RESULTS

During March 1985, stem rust survived on volunteer wheat plants and *Hordeum murinum*, furnishing an adequate inoculum level for the following wheat season.

2.4.1 Pathotype identification

From the rust survey two new pathotypes were identified and were designated 2SA100 and 2SA101. The avirulence/virulence formulae were as follows: 2SA100: *Sr8b*,*9e*,*9g*,*13*,*15*,*21*,*22*,*25*,*26*,*27*,*29*,*30*,*31*,*32*,*35*,*36*,*37*, *Tt3*,*dp2*,TAF2d,E. de Montijo/*5*,*6*,*7a*,*7b*,*8a*,*9b*,*10*,*11*,*12*, *14*,*16*,*17*,*18*,*19*,*20*,*24*,*28*. 2SA101:*8b*,*9e*,*13*,*15*,*21*,*22*,*25*, *26*,*27*,*29*,*30*,*31*,*32*,*36*,*37*,*Tt3*,*dp2*,TAF2d,E. de Montijo/*5*, *6*,*7a*,*7b*,*8a*,*9b*,*9g*,*10*,*11*,*12*,*14*,*16*,*17*,*18*,*19*,*20*,*24*,*28*. Detailed IT responses for 70 differential lines, representing 46 single host genes, for resistance to *P. graminis tritici* pathotypes 2SA100,2SA101,2SA48 and 2SA52, are presented in Table 2.1. Data indicated that 2SA100, standard race 222 on the Stakman *et al*. key (55) and 2SA101, standard race 34(55),only differ in avirulence and virulence, respectively,for *Sr9g*(Acme). Comparing 2SA100 and 2SA101 with selected pre-1984 pathotypes, it was found that 2SA100 could not be distinguished from 2SA48 nor 2SA101 from 2SA52, except for their *Sr24* response. A further characteristic of

TABLE 2.1 Comparison between line/cultivar seedling responses to *P. graminis tritici* pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for *Sr24*

Gene *Sr-*	Line/Cultivar	Accession number	*P. g. tritici* pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
5	Reliance	C.I. 14159	4	4	4	4
	ISr5Ra	C.I. 14161	4	4	4	4
6	McMurachy		4	4	4	4
	ISr6Ra	C.I. 14163	3+	4	4	4
7a	Marquis + *Sr7a*		2+3	3	3	3
	Mq *Sr7a* EG101 sel.	C.I. 15083	2+3	3	3+	33+
7b	Marquis	C.I. 3641	3+	3	3+	4
	I*Sr7b*Ra	C.I. 14165	2++3	2++3	33+	2++3
	CSHope 4B		33+	33+	33+	3+
8a	Mentana		4	4	4	4
	I*Sr8*Ra	C.I. 14167	4	4	4	4
8b	Barleta Benvenuto		X=cn	X=cn	;1++n	;1++n

TABLE 2.1 (continued). Comparison between line/cultivar seedling responses to *P. graminis tritici* pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for *Sr24*

Gene *Sr-*	Line/Cultivar	Accession number	*P. g. tritici* pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
9b	Line AA	C.I. 17386	4	3+	3+	3+
9d	Arnautka	C.I. 1493	4	4	3+	4
	Mindum	C.I. 5296	4	4	4	4
	Spelmar	C.I. 6236	4	4	4	4
9e	Vernal	C.I. 3686	;cn	;cn	;1-n	;1-n
	P.I. 192334	C.I. 8155	;1-cn	;1=cn	;1-	;1-
9g	Acme	C.I. 5284	;cn	4	;cn	4
	Kubanka	C.I. 2094	;cn	4	;cn	4
10	Federation		4	4	4	4
	Line F	C.I. 17388	4	4	4	4
11	Yalta		4	4	4	4
	I*Sr11*Ra	C.I. 14171	3	3+	4	4

TABLE 2.1 (continued). Comparison between line/cultivar seedling responses to _P. graminis tritici_ pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for _Sr24_

Gene _Sr-_	Line/Cultivar	Accession number	_P. g. tritici_ pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
12	Line R		4	4	3+	3+
	Bt_Sr12_	C.I. 17783	4	4	3+	3+
13	Line S sel	C.I. 17387	12=	12=	;1	;1
	Marquis + _Sr13_	C.I. 15088	12=	12=	;1	;1
14	Line A		4	4	4	4
	Line A (new)		4	4	4	4
15	Norka		X-n	X-n	X	X
	W2691/+_Sr15_		X-n	X-n	X	X
16	Marquis + _Sr16_		4	4	4	4
17	Renown		4	4	4	4
18	Mq-A		4	4	4	4
19	Mq-B		4	4	4	4

TABLE 2.1 (continued). Comparison between line/cultivar seedling responses to *P. graminis tritici* pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for *Sr24*

Gene *Sr-*	Line/Cultivar	Accession number	*P. g. tritici* pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
20	RL-C		4	4	4	4
21	Einkorn	C.I. 2433	1+	1+	;1+c	;1+
	Triticum monococcum derv.		1++	1++	1++2	1++
22	SW*Sr22*TB 11-70-565-resel.		1++	2	2	2
23	Selkirk	C.I. 13100	3cn	X++cn	3cn	3cn
24	Agent	C.I. 13532	2++3-	2++3	12=	12=
25	Ars-3 sel	C.I. 17473	1+2	1-	1+	1
	Agatha		1-	1	;1-	;1-
26	Eagle	P.I. 365582	;1-	;c	1-	1-
	Avocet		;1-	;c	;1=	1
27	WRT 238-5	C.I. 14141	;n	;	;n	;n

TABLE 2.1 (continued). Comparison between line/cultivar seedling responses to *P. graminis tritici* pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for *Sr24*

Gene *Sr-*	Line/Cultivar	Accession number	*P. g. tritici* pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
28	Kota	C.I. 5878	3+	4	4	4
	Line AD		3+	3+	4	4
29	RL6046 *Sr29*		2+	2	2++	2+
	Tc6+/Etiole de Choisy	RL6045	2+	2-	1++	1
30	Festiquay	P.I. 330957	1+	1++	2++	2+
	Bt + *Sr30*		2	2	2+	2+
31	Veery#3 Gamtoos		;12=	;1=c	0;	0;
32	CS + *Sr32*		1	1+	1++	1++
	W3598		1+	1+	1	1
35	82.2692	Univ. Sydney	;1-	0;c	;	;
36	Line C	C.I. 17385	1n	;n	;	;0
	Mengavi		0;	0;	0;	;

TABLE 2.1 (continued). Comparison between line/cultivar seedling responses to *P. graminis tritici* pathotypes virulent (2SA100, 2SA101) and avirulent (2SA48, 2SA52) for *Sr24*

Gene *Sr-*	Line/Cultivar number	Accession	*P. g. tritici* pathotypes[a]			
			2SA100	2SA101	2SA48	2SA52
37	W2691 + *Sr37*	Univ. Minn.	;n	;	;1=n	;1=
Tt3	74-3452		2+3-	Xcn	1++c	1++
Gt	Bt + *SrGt*	P.I. 329230	2++3	2	3-	1++3-
	Gamut		2++	12-	3	1++3-
dp-2	Medea Ap9d	3255	2	2	;1-	;1=
	Golden Ball derv.	W 3504	2	2	;	;c
	Agropyron intermedium		1+	12-	1+	1+
	Enterlago de Montijo	192847	1++	1+	1	1
	Rosner Triticale	15013	;n	;n	;n	;n
	Morocco	W 1107	4	4	4	4
McN	McNair 701		3+c	3+c	3+c	3+c

a South African designation for *P. graminis tritici* cultures. For avirulence/virulence formulae, see text.

the 2SA100 and 2SA101 pathotypes was their moderate virulence to host lines with Sr7b(Marquis). Variable IT scores, 2+3, 3c, 33+ and 3+c led Le Roux(20) to conclude that 2SA100 was standard race 343(55).However the present study showed that both 2SA100 and 2SA101 were relatively virulent on Marquis, ISr7bRa and CS Hope4b(Table 2.1) when compared with pathotype 2SA10 (Standard race 98), which produced IT 2- on the stocks.

Pathotypes 2SA100 and 2SA101 constituted 58% and 5% respectively, of survey cultures during 1984. A similar situation prevailed during 1985 when the corresponding frequencies were 55% and 6% (see Chapter 1).

The distribution of 2SA100 and 2SA101 for 1984 and 1985 is indicated in Fig. 2.1. Pathotype 2SA100 was confined in occurrence to the wheat production areas of the southern Cape, Natal, Transvaal and eastern Cape during 1984, but spread to the adjacent areas of the south western Cape, eastern Cape and Orange Free State in the following season. Yield losses of up to 75% were experienced in the Albertinia region of the southern Cape. In contrast to 2SA100, pathotype 2SA101 occurred only in the southern Cape in 1984, whence it spread to the eastern Cape region and Transvaal in 1985.

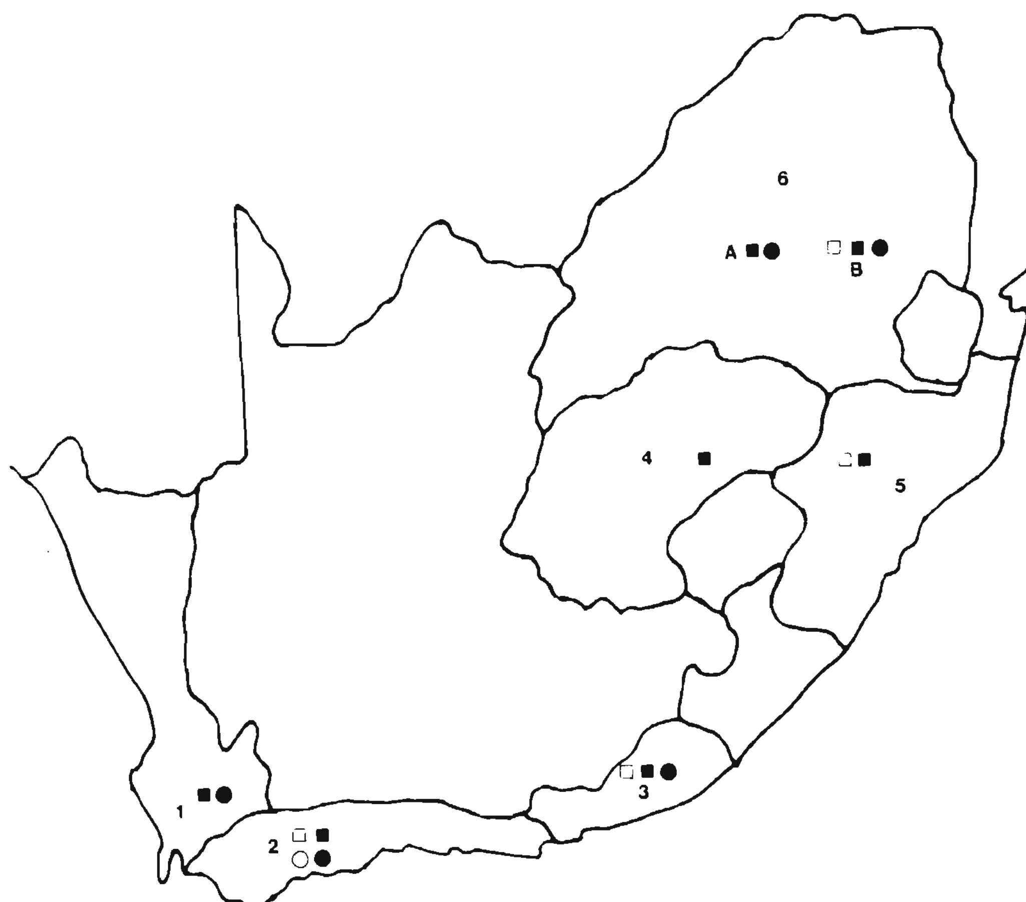

Fig. 2.1. Ecological areas for wheat production and the occurrence of *Puccinia graminis tritici* pathotypes 2SA100 and 2SA101 in South Africa: Area 1: Western Cape Province, hard red spring wheats, winter rainfall region, dryland; Area 2: Southern Cape Province, hard red spring wheats, winter rainfall region with limited summer rains, dryland; Area 3: Eastern Cape Province, hard red spring wheats, winter rainfall region, dryland; Area 4: Orange Free State, hard red winter wheats, summer rainfall region, dryland; Area 5: Natal, hard red spring wheats, summer rainfall region, predominantly under irrigation; Area 6: Transvaal, hard red spring wheats, summer rainfall region : a) Springbokflats - dryland, b) Groblersdal - irrigated.
Legend □ 2SA100, 1984; ■ 2SA100, 1985; ○ 2SA101, 1984; ● 2SA101, 1985.

2.4.2 Response of Agent and related genotypes to pathotypes virulent for Sr24

The seedling responses of Agent and 12 other lines with Sr24, to pathotypes 2SA100 and 2SA101 and those from which they in all probability were derived, 2SA48 and 2SA52, are given in Table 2.2. Increased virulence for Sr24 in 2SA100 and 2SA101 was clearly indicated by the responses of 12 lines representing six different genetic backgrounds. Two response groups were evident. Lines BtSr24Ag, 3Ag#1, 3Ag#3, Agent/Inia66*6, Agent/Ska*7, BtSr24Agent and Agent/Yecora70*4, responded with fully susceptible reactions,clearly confirming the full virulence of the pathotypes for Sr24 and also the presence of Sr24 in these lines. The second response group included Agent(C.I.13532), Agent/Yecora70*5 and Agent/Yecora70*2, and gave distinctly less susceptible responses with 2SA100 and 2SA101. All lines carrying Sr24 gave typically low responses for Sr24 when inoculated with the avirulent pathotypes (Table 2.2).

2.4.3 Response of South African wheat cultivars to pathotypes virulent for Sr24

The seedling reactions of 20 South African commercial cultivars to pathotypes 2SA100 and 2SA101 and the pathotypes from which they were in all probability

TABLE 2.2 Seedling infection types produced by Agent and related genotypes inoculated with four _P. graminis tritici_ pathotypes

Line/Cultivar	Source of seed[b]	_P. graminis tritici_ pathotype[a]				
		2SA100	2SA101	2SA48	2SA52	Sr24[c]
Bt_Sr24_Ag(Baart Ag-2)	Univ. Minn.	4	3+	2-	2-	+
Bt_Sr24_Ag(resel)	Univ. Minn.	4	33+	1+	1+	+
3AG#1	Univ. Sydney	4	3+	12-	12-	+
3AG#3	Univ. Sydney	4	3+	12-	12-	+
Agent/6*Inia66	1984LRRM/124	33+	3+	12=	12=	+
Agent/6*Inia66	1984LRRM/125	3+	3+	12=	12=	+
Agent/7*Ska	1984LRRM/144	3+	3+	;1=	1-	+
Agent/7*Ska	1984LRRM/145	3+	3+	12=	12=	+
Baart _Sr24_ Agent	1984ISWRN/13	4	4	12-	12-	+
Agent/4*Yecora70	1984LRRM/80	3+	4	;12=	12=	+

TABLE 2.2 Seedling infection types produced by Agent and related genotypes inoculated with four *P. graminis tritici* pathotypes

Line/Cultivar	Source of seed[b]	*P. graminis tritici* pathotype[a]				
		2SA100	2SA101	2SA48	2SA52	Sr24[c]
Agent (C.I. 13532)	Univ. Sydney	2++3-	2++3	12=	12=	+
Agent/5*Yecora70	1984LRRM/79	3	1++3	;12=	;12=	+
Agent/2*Yecora70	1984LRRM/81	3	1++3	12=	12=	?
Morocco		4	4	4	4	-

a South African designation for *P. graminis tritici* pathotypes. For avirulence/virulence formulae see text.

b LRRM - Leaf rust resistant material nursery, CIMMYT.
ISWRN - International Spring Wheat Rust Nursery.

c + present; - absent

derived, are presented in Table 2.3. Significantly increased virulence of 2SA100 and 2SA101 compared with that of 2SA48 and 2SA52 provided evidence of the presence of Sr24 in Gamka, SST44, Kinko, Palmiet, SST25, SST102 and possibly Wilge and Karee (see Chapters 3 and 4).

Although Palmiet, SST25 and SST102 responded with a significantly increased seedling IT, these cultivars were only moderately susceptible(IT 20MS) at the mature plant stage. In contrast, the adult plant response of Gamka and SST44, when compared to seedling IT, increased from 40MS to 70S. Yield losses in these two cultivars ranged between 17% and 75%. The response of the other South African cultivars listed in Table 2.3 clearly differed from those mentioned above. The responses of Inia66, Scheepers69, Belinda(Sr5, see Chapter 4) and SNK108 to the pre-1984 pathotypes were too high to be considered as typical of Sr24. The remaining cultivars gave similar low responses to all four pathotypes. If any of these possess Sr24, they must carry additional genes as well. Moreover the presence of Sr24 cannot be predicted from the pedigrees. SST66 and SST33 are known to carry Sr9e(23, 52, see Chapter 3). In an earlier study with two *P. graminis tritici* pathotypes 2SA48 and 2SA36, differing only in virulence for Sr36, IT produced by Zaragoza and SST107 suggested the presence of Sr36(Le Roux,

TABLE 2.3 Seedling infection types of South African wheat cultivars inoculated with P. graminis tritici pathotypes virulent and avirulent for Sr24

	P. graminis tritici pathotypes				
Cultivars	2SA100	2SA101	2SA48	2SA52	Sr24[a]
Gamka	4	4	12-	12-	+
SST44	33+	2++3	;1=	;1=	+
SST25	3	2++3	12=	12=	+
Palmiet	33+	33+	;1+	;1+	+
Kinko	33+	3+	1+2	1+2	+
SST102	33+	2++3	12-	12=	+
Wilge	1++3	2++	2-	2-	?
Karee	1++3-	1++3-	1-	1-	?
Inia	3+	4	4	4	-
SST33	;1-	;1-	;1-	;1-	-
SST66	12-	;12-	;12-	12-	-
Gamtoos	;1=	;	1	1	-
Harts	;n	;n	;n	;n	-
SST107	;c	;n	;	;	-
Zaragoza	;n	0;	0;	;n	-
Scheepers 69	3+c	2++3	4	4	-
Betta	2++	2+	2+	2++	-
Belinda	4	4	4	4	-
Flamink	1+cn	1+cn	1+cn	1+cn	-
SNK 108	4	4	4	4	-
Morocco (check)	4	4	4	4	-

[a] + present; - absent

unpublished data). Flamink probably possesses Sr36(23, 52) and Gamtoos and Harts undoubtedly carry Sr31(52). The resistant IT displayed by Betta was not only too high, but this cultivar does not give adequate adult plant resistance to 2SA48 and 2SA52 under epidemic conditions, to be typical of Sr24.

2.4.4 Latent period and uredinium density response

Virulence enhancement, as indicated by the seedling data, was confirmed during the adult plant stage by calculating the corresponding effect on the LP(Table 2.4) and UD(Table 2.5) of Agent, SST44 and Gamka by pathotypes 2SA100 and 2SA48. Since Kinko, Palmiet and SST25 did not support sporulation when inoculated with the Sr24-avirulent pathotype(2SA48),their LP and UD responses to pathotype 2SA100 with those of Morocco, Gamka, SST44 and Agent are presented separately (Table 2.6).

When compared with 2SA48, pathotype 2SA100 reduced the LP of Agent, SST44 and Gamka by 38%, 33% and 37% respectively ($P \leq 0,05$). SST44 and Gamka responded with LP's similar to that of Morocco, the susceptible check. Although susceptible in terms of lesion size, Agent reacted with a LP significantly longer than that determined for SST44 and Gamka. Considering the pathotype means, 2SA100 significantly increased the UD

TABLE 2.4 Mean latent period for various host : pathogen combinations resulting from inoculation of adult plants with *Puccinia graminis* f. sp. *tritici* pathotypes virulent (2SA100) and avirulent (2SA48) for *Sr24*

	Latent period[a] (hours)				
Pathotype	Morocco[b]	Agent	SST44	Gamka	Pathotype mean
2SA48	153.23 a	343.94 d	241.05 bc	253.36 c	247.89 h
2SA100	138.56 a	213.49 b	160.76 a	158.38 a	167.80 i
Cultivar mean	145.89 e	278.72 g	200.90 f	205.87 f	

[a] Latent period are expressed as number of hours from inoculation to 40% uredinia formed.

[b] Values are means of 12 replications. Means followed by the same letter are not significantly different (P=0.05) according to Tukey's procedure.

TABLE 2.5 Mean uredinium density values for various host : pathogen combinations resulting from inoculations of adult plants with *Puccinia graminis* f. sp. *tritici* pathotype virulent (2SA100) and avirulent (2SA48) for *Sr24*

Pathotype	Uredinium density[a]				
	Morocco	Agent	SST44	Gamka	Pathotype mean
2SA48	9.83 a	7.90 b	5.67 d	6.99 bc	7.60 f
2SA100	9.61 a	6.25 cd	8.35 b	9.72 a	8.48 e
Cultivar mean	9.72 g	7.07 i	7.01 i	8.36 h	

[a] Uredinium density values are expressed as the number of uredinia/1 cm stem length. Values are means of 12 replications. Means followed by the same letter are not significantly different (P=0.05) according to Tukey's procedure.

TABLE 2.6 Latent period (LP) and mean uredinium density (UD) response of South African wheat cultivars inoculated with a *P. graminis tritici* culture (2SA100) virulent for *Sr24*

Cultivars	Latent period[a] (hours)	Uredinium density[b]
Morocco(check)	138.56 a	9.61 a
Gamka	158.38 a	9.72 a
SST44	160.26 a	8.35 ab
Agent	213.49 b	6.25 cd
Kinko	226.19 b	7.48 bc
Palmiet	234.47 b	7.03 c
SST25	267.16 c	5.64 d

[a] Latent period is expressed as number of hours from inoculation to 40% uredinia formed.

[b] Uredinium density values are expressed as number of uredinium/cm stem length. Values are means of 12 replications. Means followed by the same letter are not significantly different (P=0.05) according to Tukey's procedure.

(Table 2.5). The UD of SST44 and Gamka was increased by 47% and 39% respectively. In contrast to LP, the UD for Agent decreased after inoculation with pathotype 2SA100. This may serve to indicate that background germplasm can independently modify the expression of these two resistance components.

Cultivars Kinko and Palmiet exhibited similar LP and UD values to those of Agent, with SST25 showing the longest LP and lowest UD of all cultivars tested.

2.5 DISCUSSION

Comparative seedling and adult plant evaluations with two P. graminis tritici pathotypes, 2SA100 and 2SA101, isolated during 1984 in South Africa from the cultivars SST44 and Gamka, clearly showed that the pathogen had acquired virulence for Sr24. The frequent incidence of pathotype 2SA100, 58% of all isolates in 1984 and 55% in 1985, suggests that it may have been prevalent in the population some time prior to detection, but gone undetected as a result of the preceding drought. Similar important changes in the rust flora, which remained undetected for a period of at least a year, have also occurred elsewhere(30,38).

Comparative infection type studies between pre- and post-1984 pathotypes suggest that 2SA100 and 2SA101 were single-locus mutations from 2SA48 and 2SA52 re-

spectively. Pathotype 2SA48 predominated in the South African virulence surveys until 1981, with 2SA52 constituting 6% of isolations from the western Cape during 1981(see Chapter 1). Alternatively, there may have been a mutation with respect to Sr24, with a second event affecting the gene corresponding to Sr9g, considering the high mutation rate documented for this pathogen gene(25, 26 see also Chapter 1). Since the first alternative involves only a single step mutation it is the favored explanation.

P. graminis tritici pathotypes 2SA100 and 2SA101 constituted 64% of all survey collections for 1985(see Chapter 1). 2SA100 has been isolated from every major wheat-producing area in South Africa, whereas the incidence of 2SA101 has been restricted to the south western Cape and Transvaal. The wide virulence range of both pathotypes, with respect to both current and older cultivars, could explain the rapid increase in prevalence especially during 1984. Moreover, pathotypes 2SA100 and 2SA101 appear to combine their increased virulences with aggressiveness and good survival abilities.

Presently, P. graminis tritici pathotypes with virulence for Sr24 constitute a threat to wheat production in the winter rainfall and irrigation areas of South Africa, because three of the seven recommended culti-

vars for these areas are susceptible to both pathotypes. Nine of the 23 cultivars currently recommended in South Africa possess Sr24 either alone or in combination with other resistance factors.

Susceptibility of cultivated wheats in terms of LP and UD, separated cultivars into three distinct response classes, viz. fully compatible: SST44 and Gamka, moderately susceptible: Kinko, Palmiet and moderately resistant: SST25.

When the presence of Sr24-virulent pathotypes was demonstrated during 1984, SST44 and Gamka were removed from the list of recommended cultivars in high risk areas. However rapid replacement of a prominent cultivar such as SST44 presents major logistical problems. Meanwhile, SST44 and other susceptible cultivars continue to be cultivated on alarmingly large areas.

Differences in susceptibility among lines having Sr24 were evident in both seedling and adult plant stages. BtSr24Ag, 3Ag#1 and 3Ag#3 were fully susceptible to pathotypes 2SA100 and 2SA101, whereas Agent was moderately susceptible. The lower seedling response of Agent to these pathotypes was obvious when compared with the fully susceptible SST44 and Gamka. These differences were further supported by the significantly longer LP and lower UD values obtained with adult

plants. Nevertheless, Agent must be regarded as susceptible in terms of size and type of pustules as well as LP and UD. In inheritance studies of Agent, Gough and Merkle(9) and McIntosh *et al*.(37) demonstrated the presence of additional genes for resistance to certain pathotypes. The presence of such additional resistance factors, as well as differences in genetic background, may explain the lower seedling and adult plant reactions of Agent compared with those of SST44 and Gamka.

In the development of commercial wheat cultivars, resistant to the prevailing pathotypes of *P. graminis tritici* in Southern Africa, wheat breeders have exploited a number of resistance genes. These genes, including *Sr9e*, *Sr36*, *Sr24* and *Sr31*,mostly derived from species related to *T. aestivum*, were used on an individual basis to broaden the genetic diversity between cultivars rather than in the enhancement of resistance reserves within the same cultivar. The present study, as well as that of Lombard(23), clearly shows that the incorporation of single resistance genes has placed the rust resistance of South African cultivars on a very narrow genetic base. Furthermore, there is no evidence that resistance transferred from species such as tetraploid (*T. timopheevi*-*Sr36*; *T. turgidum*-*Sr9e*, *Th. elongatum* - *Sr24*) or diploid (*T. monococcum*-*Sr21*) species will prove durable when used individually. Therefore, care must be taken to accumulate, within

the same cultivar, newly found resistance to the complete virulence spectrum of stem rust in proven combinations.

To expedite this, carefully selected pathotypes, such as the Sr24-virulent pathotypes identified in this study, must be used. In addition, international co-operative testing, making use of critical pathogen genotypes available in other countries, will need to be conducted on a much broader basis than is the case at present.

CHAPTER 3

INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS f. sp. TRITICI IN SOUTH AFRICAN WHEATS. I. SPRING CULTIVARS

3.1 ABSTRACT

The inheritance of resistance to five pathotypes of Puccinia graminis tritici was investigated in eight South African wheat cultivars. Resistance was conferred by Sr9e in SST33 and SST66,and by Sr24 in Gamka. Gamtoos carried one partially dominant gene, postulated to be Sr31. Gouritz carried Sr36 and an unidentified gene, whereas SST23 carried Sr24 and an unidentified gene. Resistance in Palmiet was conferred by one dominant(Sr24) and one unknown recessive gene. Palala carried three partially dominant genes, one of which conferred an infection type ;c at low temperatures but proved to be temperature sensitive. These observations are discussed in relation to genetic diversity of resistance in South African wheats.

3.2 INTRODUCTION

Spring wheat production is traditionally confined to the winter rainfall regions of the southern Cape Province and to the irrigation areas of Natal, Transvaal and Orange Free State. Stem rust caused by Puccinia graminis f.sp. tritici Eriks. and Henn., is a major limiting factor in the production of spring wheats in the Republic

of South Africa(16,22,23,24,40). Periodic epidemics are enhanced by the cultivation of wheat throughout the year (23) in southern Africa and by the oversummering of the pathogen on volunteer wheat plants and grasses such as Hordeum murinum Huds.(66).

The potential danger of stem rust was again apparent during the 1984 and 1985 wheat-growing seasons, when extended yield losses ranging between 17% and 75% were recorded in certain areas of the southern Cape Province (see Chapter 2). These outbreaks firstly demonstrated the deficiencies of chemical control which proved inadequate under the prevailing conditions. Secondly, they highlighted the lack of genetic variability in stem rust resistance of local wheat cultivars.

In order to reduce the vulnerability of wheat to future stem rust outbreaks worldwide, resistance breeders are encouraged to place special emphasis on genetic diversity(36,64). However, good gene management requires a thorough knowledge of the pathogenic capabilities of the pathogen population and of the diversity and effectiveness of the available resistance(10).

Resistance breeding in South Africa has proceeded without adequate information concerning the available resistance sources. Genetic analyses of resistant genotypes and current cultivars are few(23). From 1935 incomplete

data(15,16,18,19,22,39) were generated by means of host response studies using selected pathotypes permitting postulations of certain resistance factors.In addition, while several South African cultivars were clearly resistant, details on the genetic basis of their resistance to stem rust have not been determined/published.

The present study was undertaken to determine the genetic basis for resistance in eight important South African spring wheats.

3.3 MATERIALS AND METHODS

3.3.1 Host cultivars

Eight hard red spring wheat(*Triticum aestivum* L.) cultivars(Table 3.1) were used as male parents in crosses with Morocco(University of Sydney, accession number, W1107), which is highly susceptible to all South African pathotypes. The origin and pedigrees of the cultivars are included in Table 3.1. Palala and Gamtoos were selections from nuseries distributed by CIMMYT(International Maize and Wheat Improvement Centre), whereas the remaining six cultivars were bred and selected in South Africa.

The F_1 and F_2 generations were grown in pots in a commercial plastic-covered tunnel. A water soluble-fertilizer, Chemicult ® was applied weekly at a rate of 0,5g/pot from growth stage 5(60) to growth stage 87.

Seedlings for rust testing were raised in 10-cm pots (12 pots/tray). Trays were kept in a rust-free environment at 25C for six days. Seeding rate was 30-45 per pot for all populations, including the parents. Separate plantings were made for each selected stem rust pathotype. A minimum of 335 F_2 seedlings were evaluated for each pathotype used, while more than 60 seedlings were tested for each F_3 family.

3.3.2 Pathotypes

Single-pustule-derived cultures of *P. graminis tritici* pathotypes 2SA2, 2SA32 and 2SA10 were chosen as representative of the avirulent population for use throughout the study. In addition, pathotypes 2SA4, 2SA36 and 2SA100 virulent respectively, for *Sr9e*, *Sr36* and *Sr24*, were used in supporting studies to inoculate hybrid and parental materials. Avirulence/virulence formula for the six pathotypes are presented in Table 3.2.

3.3.3 Inoculation and incubation

Seedling populations at the first leaf stage, were inoculated with _P. graminis tritici_ urediniospores suspended in light-weight mineral oil(Soltrol 130®). Inoculated seedlings were kept in a high humidity chamber (temp. 20±2C) for a 14h dark period followed by a 3h gradual drying period at a light intensity of 10 000 lux. Seedlings were then placed in an airconditioned greenhouse, receiving natural daylight supplemented with illumination of 10 000 lux from cool-white fluorescent tubes, providing a 14h photoperiod. Temperatures were controlled at 25±1C and 15±1C, respectively, for warm and cold incubation treatments.

Seedling infection types(IT) of individual F_2 seedlings and F_3 families were recorded 12 days after inoculation. These were based on the scale described by Stakman _et al_.(55). The Chi-square test for goodness of fit(56) was used in analysing the F_2 and F_3 data.

3.4 RESULTS

3.4.1 Gamka

Gamka responded with IT ;c to pathotypes 2SA32 and 2SA10 at 15C. An IT 2 was recorded with all other pathotypes at both high(25C) and low(15C) temperatures except with 2SA100 to which Gamka was fully susceptible(Table 3.3).

TABLE 3.1. Origin and pedigrees of spring wheat cultivars used in crosses with Morocco

Cultivar	Origin	Pedigree
Gamka	South Africa	*T. timopheevi* derv.P 6297/Agent
Palmiet	South Africa	SST3*3//Scout *5/Agent
SST23	South Africa	Inia 66*5//T4*3/ (11-53-541^{2}x C.I. 13523^{2}x 11-53-546 11 60 219)
SST66	South Africa	SST16*3/3/Flameks*3/LD398*2//LD357/Stewart 464
SST33	South Africa	SST3*3/3/Flameks*3/LD398*2//LD357/Stewart 464
Palala	U S A	ND487/Waldron
Gouritz	South Africa	Flameks//*4 Minturki/*Triticum timopheevi*
Gamtoos	CIMMYT	Veery #3

TABLE 3.2. Avirulence/virulence formulae[a] of *Puccinia graminis* f.sp. *tritici* pathotypes used to inoculate appropriate F_2 and F_3 generation seedlings

Formula number	Standard race[b]	Avirulence/virulence formula (Sr-gene)
2SA2	21	5, 6, 9b, 9e, 17, 24, 30, 36/7b, 9g
2SA32	326	5, 6, 7b, 9b, 9e, 9g, 17, 24, 30, 36/
2SA10	98	6, 7b, 9b, 9e, 17, 24, 30, 36/5, 9g
2SA100	222	9e, 9g, 30, 36/5, 6, 7b, 9b, 17, 24
2SA4	321	9g, 24, 36/5, 6, 7b, 9b, 9e, 17, 30
2SA36	222	9e, 9g, 24, 30/5, 6, 7b, 9b, 17, 36

a Pathotypes were virulent for *Sr* genes *7a*, *8a*, *9a*, *9d*, *9f*, *10*, *11*, *14*, *16*, *18*, *19*, *20*, *28* and avirulent for *Sr8b*, *13*, *21*, *22*, *25*, *26*, *27*, *29*, *31*, *32*, *35*, dp2 and Tt3

b As described by Stakman *et al*. (55)

TABLE 3.3 Seedling infection types produced by eight spring wheats when tested with six *P. graminis tritici* pathotypes at two different temperature regimes

Cultivar	Temperature (C)	Pathotype and seedling infection type response					
		2SA2	2SA32	2SA10	2SA100	2SA4	2SA36
Gamka	15	2+	;c	;c	4	2	2
	25	2	12=	2	4	12-	2
Palmiet	15	1	1	1	3	1	1-
	25	1+	1	1+	3cn	1	1-
SST23	15	;1+	1-	;1n	3	1	;1=c
	25	;1+	;1+	;1c	3c	;1c	;1=c
SST66	15	1+	;1=	;1	;1-	4	;1=
	25	1+	;1=	;1	;1-	4	;1=
SST33	15	;1	1-	;1n	;1-	4	;1=
	25	1+	1-	;1	;1-	4	;1=

TABLE 3.3. (continued) Seedling infection types produced by eight spring wheats when tested with six *P. graminis tritici* pathotypes at two different temperature regimes

Cultivar	Temperature (C)	Pathotype and seedling infection type response					
		2SA2	2SA32	2SA10	2SA100	2SA4	2SA36
Palala	15	2	;	;n	2	12-	2
	25	2	1+	1+	2	12-	2
Gouritz	15	;1-	0;	;	;1	;1-	4
	25	;1-	0;	;1	0;	;1-	4
Gamtoos	15	1	1-	1c	;1=c	;1=	1=
	25	1+2	1-	1+2	;1-	;1=	1=
Morocco	15	4	4	4	4	4	4
	25	4	4	4	4	4	4

The F_2 data(Table 3.4) from the cross Morocco/Gamka, tested with pathotypes 2SA32 and 2SA10 at 25C indicated segregation of one partially dominant gene. The seedling IT of resistant F_2 plants ranged between 1+2 and 2 for both pathotypes. No resistant F_2 segregate was observed following inoculation with pathotype 2SA100, which is virulent for Sr24 (Table 3.4). The hypothesis of the presence of one partially dominant gene was confirmed when the F_3 populations were inoculated with pathotype 2SA32 at 25C. Thirty three families were homozygous resistant, 69 segregating and 32 homozygous susceptible (Table 3.5).

On the basis of both specificity and genetic segregation it was evident that Gamka carried Sr24, a partially dominant gene to pathotype 2SA32.

3.4.2 Palmiet

Palmiet responded with IT 1 to 1+ with pathotypes 2SA2, 2SA32, 2SA10, 2SA4 and 2SA36 at both high and low temperatures(Table 3.3). In contrast a marked reduction in resistance(IT 3,3cn) was noted for the Palmiet: 2SA100 interaction.

The F_2 segregation ratios(Table 3.4) involving a total of 619 seedlings, and the distribution of 126 F_3 families (Table 3.5) conformed with the hypothesis of

TABLE 3.4. Segregation of F_2 seedlings from crosses of Gamka, Palmiet, SST23, SST66, SST33, Palala, Gouritz and Gamtoos with Morocco when tested with selected pathotypes

Cross	Temperature (C)	Pathotype	Number of F_2 seedlings Resistant	Susceptible	Expected ratio	P-value
Morocco x Gamka	25	2SA32	374	111	3:1	0.50-0.25
Morocco x Gamka	25	2SA10	249	86	3:1	0.90-0.75
Morocco x Gamka	25	2SA100	0	474	-	-
Morocco x Palmiet	25	2SA2	472	147	3:1	0.50-0.25
Morocco x Palmiet	25	2SA32	384	92	13:3	0.75-0.50
Morocco x SST23	15	2SA2	685	42	15:1	0.90-0.75
Morocco x SST23	25	2SA2	480	34	15:1	0.75-0.50
Morocco x SST23	25	2SA10	643	45	15:1	0.90-0.75

TABLE 3.4. (continued) Segregation of F_2 seedlings from crosses of Gamka, Palmiet, SST23, SST66, SST33, Palala, Gouritz and Gamtoos with Morocco when tested with selected pathotypes

	Temperature		Number of F_2 seedlings		Expected	
Cross	(C)	Pathotype	Resistant	Susceptible	ratio	P-value
Morocco x SST66	25	2SA32	460	103	13:3	0.90-0.75
					3:1	<.005
Morocco x SST66	25	2SA10	563	112	13:3	0.25-0.10
					3:1	<.005
Morocco x SST66	25	2SA2	558	124	13:1	0.75-0.50
					3:1	<.005
Morocco x SST66	25	2SA4	-	710	-	-
Morocco x SST33	25	2SA32	577	181	3:1	0.50-0.25
Morocco x SST33	25	2SA2	521	177	3:1	0.90-0.75
Morocco x SST33	25	2SA10	634	236	3:1	0.25-0.10
Morocco x SST33	25	2SA4	-	640	-	-

TABLE 3.4. (continued) Segregation of F_2 seedlings from crosses of Gamka, Palmiet, SST23, SST66, SST33, Palala, Gouritz and Gamtoos with Morocco when tested with selected pathotypes

Cross	Temperature (C)	Pathotype	Number of F_2 seedlings Resistant	Susceptible	Expected ratio	P-value
Morocco x Palala	25	2SA32	812	64	15:1	0.25-0.10
Morocco x Palala	25	2SA2	1102	74	15:1	0.975-0.950
Morocco x Palala	15	2SA2	616	10	63:1	0.95-0.90
Morocco x Gouritz	25	2SA2	650	42	15:1	0.90-0.75
Morocco x Gouritz	25	2SA32	688	50	15:1	0.75-0.50
Morocco x Gamtoos	25	2SA10	750	250	3:1	0.995
Morocco x Gamtoos	25	2SA4	648	233	3:1	0.90-0.75

TABLE 3.5. Frequencies of F_3 families in response classes from crosses of Gamka, Palmiet, SST23, SST66, SST33, Palala, Gouritz and Gamtoos with Morocco when tested with selected P. graminis tritici pathotypes

Cross	Temperature (C)	Pathotype	Number of families			Expected ratio	P-value
			Resistant	Segregating	Susceptible		
Morocco x Gamka	25	2SA32	33	69	32	1:2:1	0.950-0.90
Morocco x Palmiet	25	2SA2	23	72	31	1:2:1	0.25 -0.10
Morocco x SST23	25	2SA2	40	50	6	7:8:1	0.950-0.90
Morocco x SST66	25	2SA32	29	65	25	1:2:1	0.75 -0.50
Morocco x SST33	25	2SA2	28	65	28	1:2:1	0.75 -0.50
Morocco x Palala	15	2SA2	90	62	5	37:26:1	0.50 -0.25
Morocco x Gouritz	25	2SA2	78	88	12	7:8:1	0.975-0.950
Morocco x Gamtoos	25	2SA10	23	44	21	1:2:1	0.975-0.950

segregation for a single partially dominant gene, when tested with pathotype 2SA2. The F_2 and F_3 populations segregated for IT ; , ;1 and 2-. However when 476 F_2 seedlings from the same cross were tested with pathotype 2SA32, a segregation ratio of 13 resistant: 3 susceptible seedlings was observed. This indicated the presence of one dominant and one recessive gene for resistance. Corresponding IT suggested the operation of the same dominant gene to both pathotypes used.

3.4.3 SST23

SST23 proved to be highly resistant(IT ; , ;1+) to all pathotypes at both temperatures(Table 3.3), except 2SA100 (IT 3 and 3cn). The F_2 data from Morocco/SST23 inoculated with pathotypes 2SA2 and 2SA10, suggested segregation for two partially dominant genes(Table 3.4). These results were confirmed in the F_3 tests based on 96 families(Table 3.5). The resistant F_2 seedlings responded with IT 0, ;c, ;1, 12= and 3c to both pathotypes. Of the 23 resistant F_3 lines identified, six responded with IT 0 and 0;, nine lines with ; and ;1 and eight lines with IT 1 and 1++2.

3.4.4 SST66

SST66 was developed by means of a backcrossing program which incorporated resistance into a local cultivar. At both temperature regimes, SST66 responded with an IT;1

to all pathotypes(Table 3.3), except 2SA4 which is known to be virulent for Sr9e.

When tested with pathotypes 2SA32, 2SA10 and 2SA2(avirulent for Sr9e), the F_2 population segregated in ratios consistent with an expected ratio of 13 resistant: 3 susceptible seedlings, indicating the presence of one dominant(IT ;1+) and one recessive(IT 3) gene in SST66 (Table 3.4). Inoculation with the Sr9e-virulent 2SA4, resulted in the absence of resistant F_2 segregates. This suggested that if SST66 possessed two genes for resistance to 2SA32, 2SA10 and 2SA2, then pathotype 2SA4 must be virulent for both of these genes.

When F_3 families were tested with pathotype 2SA32, a segregation ratio of 1:2:1 was observed, indicating the presence of only one dominant(IT ;1+) gene. The inability to identify the presence of the recessive gene (IT 3) may be due to difficulty in classifying these intermediate infection types. However Luig and Watson(28) reported that F_2 segregation in crosses between Vernstein(Sr9e) and W2691 did not follow a typical single gene pattern. Similar results were reported by McIntosh and Baker(34) for F_3 lines segregating for Vernstein and C.I.12632 resistances, who ascribed the aberrant segregation to differential transmission.

3.4.5 SST33

SST33 and its sister line SST66 responded similarly to all pathotypes used(Table 3.3). When inoculated with pathotypes 2SA32 and 2SA2, the F_2 ratios(Table 3.4) conformed to those expected for segregation of one dominant gene(IT ;1+). Again no resistant F_2 segregate was observed when the test was conducted with pathotype 2SA4, virulent for Sr9e. The F_2 results were confirmed in F_3 tests, where the family ratio fitted a 1:2:1 ratio (Table 3.5). Both SST33 and SST66 were susceptible as adult plants when infected with pathotype 2SA4 (Le Roux, unpublished).

3.4.6 Palala

Palala responded with IT 2 when tested with pathotypes 2SA2, 2SA100, 2SA4(IT 12-) and 2SA36. Pathotypes 2SA32 and 2SA10 produced more resistant reactions (IT ;n), especially at lower temperatures(Table 3.3). The F_2 segregation ratios indicated the presence of three genes for resistance, one of which is temperature sensitive (Table 3.4).

The second and third genes conditioned IT ;1++n and ;3cn, respectively, with pathotypes 2SA2 and 2SA32. The presence of the first gene(IT ;c) together with the second and the third was confirmed in the F_3generation

when an expected segregation ratio of 37 homozygous resistant: 26 segregating: 1 homozygous susceptible family was indicated at 15C(Table 3.5). Fifty-five of the 90 homozygous resistant F_3 lines responded with IT ;1++n, 26 lines exhibited a ;1=n and ;3cn reaction and 9 lines a 0; and ;1=n infection type.

3.4.7 Gouritz

Gouritz was highly resistant to all pathotypes except 2SA36, which has virulence for Sr36. The Gouritz: 2SA32 interaction gave the highest level of resistance(IT 0;) at both temperatures of all combinations(Table 3.3).

The F_2 generation from Morocco/Gouritz(Table 3.4) inoculated with pathotypes 2SA32(IT 0;) and 2SA2(IT ;1), segregated for two partially dominant genes as indicated by ratios of 15 resistant: 1 susceptible seedling(Table 3.4). F_2 seedling hybrids segregated clearly for two phenotypic groupings viz. IT ;, ;1- and IT 3cnx+, 3-cn. The behavior of the F_3 families agreed with the expected ratio of 7 homozygous resistant :8 segregating :1 homozygous susceptible(Table 3.5).

3.4.8 Gamtoos

Gamtoos, a Veery#3(=Genaro F81) derivative was released for its seedling and adult plant resistance to all South African P. graminis f.sp.tritici and Puccinia recondita

pathotypes. Infection types, produced by Gamtoos with the various pathotypes, ranged between IT ;1=c and 1+2. It was the only cultivar of the group chosen with a high level of resistance to all pathotypes(Table 3.3).

With pathotypes 2SA10 and 2SA4, the F_2 ratios of resistant and susceptible seedling conformed to monogenic segregation(Table 3.4). Pathotype 2SA4, was selected for its virulence with respect to most of the known Sr genes. The segregation ratio for F_3 families confirmed the expected presence of a single gene viz., 1 homozygous resistant :2 segregating :1 homozygous susceptible individuals(Table 3.5). Resistant F_2 seedlings of the Gamtoos :2SA4 interaction showed variation of IT ;c, ;1- and 1++2, whereas with Gamtoos :2SA10, infection type variation was limited to reactions 0; and ;1+. Homozygous F_3 families were characterised by IT ;c and 1++2.

3.5 DISCUSSION

The present study determined that inheritance of resistance in eight South African cultivars to selected pathotypes of P. graminis tritici was relatively simple.

Both SST33 and SST66 possessed Sr9e on the basis of specificity and genetic segregation. Since 22% of the pathotypes identified during the 1985 annual rust survey were virulent for Sr9e(see Chapter 1), the future value of both cultivars appears to be in question.

Gamka which responded with an IT 2 to *Sr24*-avirulent pathotypes, was fully susceptible with 2SA100, which is virulent for *Sr24*. This indicates the presence of only *Sr24* in Gamka. Further confirmation comes from the fact that no resistant F_2 segregate was recorded when Morocco/Gamka was tested with pathotype 2SA100. During 1985, *Sr24*-virulent pathotypes represented about 60% of the stem rust samples evaluated in the South African pathotype survey(see Chapters 1 and 2), indicating that the future of Gamka is also in the balance.

Gamtoos, a third cultivar having a single gene for resistance, exhibited resistance to all pathotypes used in this study. Because Gamtoos was selected from Veery#3, and since the dominant gene, *Sr31*, is one of the few genes charaterized by IT ;1= to 1+2 with all South African *P. graminis tritici* pathotypes(23), the single segregating gene(F_2 and F_3 population) in Morocco/ Gamtoos is postulated to be *Sr31*. Thus far there appears to be no virulence for this gene in South Africa and the rest of the world.

The second group of cultivars, including SST23, Palmiet and Gouritz, conditioned resistance to selected pathotypes by at least two genes. The significant reduction in resistance(IT 3,3c) when SST23 and Palmiet were tested with the *Sr24*-virulent pathotype(2SA100) suggests the presence of *Sr24* which probably originated from Agent, a

known carrier of this gene(37). The second partially dominant gene present in SST23(IT 0; and ;c) which appeared to be temperature stable, was not identified. The recessive gene shown to be operative in Palmiet, and characterized by a moderately susceptible response(IT 3c) was more strongly expressed in the adult plant stage, IT-moderately resistant(see Chapter 5). This gene was also not identified.

Of the two partially dominant genes operating in Gouritz, it was postulated that the resistance conferred by the first, producing IT ; and ;cn with 2SA2 and IT ; and ;1 with 2SA32,was due to *Sr36*. This gene probably originated from *T. timopheevi*. The second partially dominant gene conditioned IT 3=c with pathotypes avirulent for *Sr36*. Virulence for *Sr36* in the South African stem rust population was last detected during the 1981 season when 13% of the pathotypes evaluated in the survey were virulent (see Chapter 1).

In the third group of cultivars represented by Palala, stem rust resistance was conditioned by three partially dominant genes. The first gene, characterized by IT;n, was temperature-sensitive and only operated at 15C. The second and the third genes, conditioning IT ;1++n and ;3cn respectively, could not be identified by comparing the pattern of reaction to *P. graminis* f.sp.*tritici* pathotypes used in this study. Unless all patterns of

virulence and avirulence occur in the pathotypes, the identity of such genes are rather difficult to determine(45).

The genetical analysis of currently grown cultivars indicated a low level of genetic diversity. Sr24 featured in three(Gamka, SST23 and Palmiet) of the eight cultivars, and Sr9e confers resistance in SST33 and SST66. It is clear that backcrossing programs, aimed at incorporating stem rust resistance into adapted,high-yielding cultivars should be planned to exploit a broader spectrum of effective gene combinations, rather than the use of single gene resistances.

Furthermore, the present study emphasised the importance of progeny testing as part of a breeding program so as to ensure that the required resistance factors are in fact incorporated. For example, Gamka was bred from the cross T. timopheevi derivative-Sr36(35)/Agent-Sr24 (37), in an effort to obtain the combination Sr36 + Sr24. The present study indicated that only Sr24 was successfully introduced. This cultivar suffered extensive yield losses three years after its release when Sr24-virulent pathotypes appeared in South Africa(see Chapter 2). Since pathotypes virulent for Sr24 were avirulent for Sr36, this cultivar would not have succumbed to rust at the same time as others, had the original objectives of the cross been achieved.

As stated previously(36), single genes, as identified in this study, may ensure successful disease control if adequate genetic diversity is maintained by means of effective combinations in more resistant backgrounds.

CHAPTER 4

INHERITANCE OF RESISTANCE TO *PUCCINIA GRAMINIS* f.sp. *TRITICI* IN SOUTH AFRICAN WHEATS. II. WINTER CULTIVARS

4.1 ABSTRACT

The genetic basis of resistance in seven winter wheat cultivars to six *P. graminis* f.sp. *tritici* pathotypes was studied. Resistance was conferred by one dominant gene in each of Belinda(*Sr5*), SST102(*Sr24*), Karee and Betta. Two partially dominant genes were identified in Wilge, whereas one recessive and one dominant gene operated in Molen. Tugela carried three partially dominant genes for resistance, one of which was positively identified as *Sr5*. The studies proved that the stem rust resistance of South African winter wheats has a relatively simple genetic basis.

4.2 INTRODUCTION

Hard red winter wheats constitute 45% of the annual wheat production in South Africa. Confined to the dry-land areas of the summer rainfall and highveld regions, which are less prone to stem rust, these wheats usually mature before damaging levels of infection develop. However, extended winter wheat sowings combined with early spring rains may result in moderate infection levels.

In view of the known potential danger of stem rust caused by _Puccinia graminis_ f.sp._tritici_ Eriks. & Henn., increased emphasis has been placed on breeding rust resistant cultivars for both winter and spring wheat areas. In the development of these cultivars, breeders have exploited various genes for resistance, including _Sr9e_, _Sr24_, _Sr31_ and _Sr36_. With the registration and subsequent widespread cultivation of SST66 and SST44, in particular, there has been a corresponding significant increase in the frequencies of pathotypes virulent for _Sr9e_ and _Sr24_(see Chapter 1). Luig and Watson(29) and Lombard(23) reported a similar evolutionary relationship between host and pathogen following the release of cultivars with _Sr6_, _Sr8a_, _Sr9b_, _Sr11_, _Sr15_, _Sr17_, _Sr30_ and _Sr36_. Because of the lack of durability in resistance, breeders and farmers have been encouraged to use wheats with a diversity of resistance genes so that pathogenic changes can affect only part of the crop.

To ensure genetic diversity, a detailed knowledge of the resistance genes present in both commercial cultivars and potential breeding parents is imperative. Knowledge of the genes present in commercial cultivars permits potentially significant changes in the pathogen to be anticipated, whereas knowledge of the genes in breeding parents enhances the breeders' ability to synthesise effective resistances for the future.

The present study was carried out to determine the genetic basis for resistance to *P. graminis tritici* in seven recommended South African winter wheat cultivars. Conventional genetic analysis of segregating populations were supplemented by consideration of the individual infection type responses with a range of pathotypes.

4.3 MATERIALS AND METHODS

4.3.1 Host cultivars

Seven hard red winter wheat cultivars viz., Betta, Belinda, Karee, Molen, SST102, Wilge and Tugela, resistant to at least some pathotypes, were used as male parents in crosses with the susceptible cultivar Morocco, originally obtained from the Plant Breeding Institute, University of Sydney. The origin and pedigrees of the seven cultivars are presented in Table 4.1. F_1 and F_2 populations were grown in pots in a plastic-covered tunnel. Seedlings of parents and F_2 and F_3 populations for rust testing were planted at the rate of 30-45 seedlings per 10-cm pot.

4.3.2 Pathotypes

Single-pustule-derived cultures of pathotypes 2SA2, 2SA32, 2SA39 and 2SA10 were chosen to inoculate hybrid materials. In certain instances they were supplemented

by single-pustule derivatives of pathotypes 2SA100 and 2SA48, respectively virulent and avirulent for Sr24(see Chapter 1). The avirulence/virulence formula for each pathotype is presented in Table 4.2.

Urediniospores of *P. graminis* f.sp.*tritici* were applied as atomized suspensions in Soltrol 130® mineral oil by the method of Browder(5). Inoculated seedlings were placed in an incubation chamber held at 100% relative humidity and 20±2C for a 14h dark period, followed by a 3h gradual drying period at a light intensity of 10 000 lux. Plants were then moved to a greenhouse where they received natural daylight supplemented with illumination from cool-white fluorescent tubes(10 000 lux) for 14h each day. The temperature was controlled at 22±2C except where stated otherwise. Individual F_2 seedlings and F_3 populations were scored on the basis of the infection type scale described by Stakman *et al.* (55).

The Chi-square test for goodness of fit(56), was used to test comparisons of the observed ratios with those expected on the basis of Mendelian segregation.

4.4 RESULTS

4.4.1 Belinda

Belinda gave a highly resistant reaction(IT 0,0;) with pathotypes 2SA2, 2SA32 and 2SA39 avirulent for Sr5 (Table 4.3). When inoculated with Sr5-virulent pathotypes (2SA10, 2SA48 and 2SA100), Belinda was fully susceptible(IT 4). Incubation at either high(25C) or low (15C) temperatures had no significant effect on the expression of resistance(Table 4.3).

The segregation of F_2 seedlings of Morocco/Belinda when inoculated with pathotypes 2SA2 and 2SA32(both avirulent for Sr5) fitted 3:1 ratios, indicating that Belinda has a single gene for resistance to each pathotype(Table 4.4). Resistant segregates displayed a range of infection types viz., 0, ;, ;1- and 1+2 suggesting that the gene was partially dominant. When inoculated with pathotype 2SA10 which has virulence for Sr5, only susceptible segregates were identified in the F_2 population. The F_3 family ratio(Table 4.5) agreed with the expected 1 homozygous resistant :2 segregating :1 homozygous susceptible when tested with pathotype 2SA2. All 17 homozygous resistant F_3 families responded with IT 0, confirming the dominant behavior of the gene.

TABLE 4.1. Origin and pedigrees of seven winter wheat cultivars used in crosses with Morocco

Cultivar	Origin	Pedigree
Belinda	U S A	Ottowa/2*Cheyenne
Betta	Argentina	Klein Impacto
Karee	South Africa	Betta//Triumph/Agent
Molen	South Africa	Betta/3/Yaktana//Norin 10 Brevor/Mazoe
SST102	South Africa	Agent/*2 Betta
Wilge	South Africa	Bella/Reward/C.I. 12632
Tugela	South Africa	Kavkaz/Jaral 'S'

TABLE 4.2. Avirulence/virulence formulae[a] for *Puccinia graminis* f.sp. *tritici* pathotypes used to inoculate test populations

Formula number	Standard race[b]	Avirulence/virulence formula (Sr-genes)
2SA2	21	5, 6, 9b, 9e, 17, 24, 30, 36/7b, 9g
2SA32	326	5, 6, 7b, 9b, 9e, 9g, 17, 24, 30, 36/
2SA39	21	5, 9b, 9e, 17, 24, 30, 36/6, 7b, 9g
2SA10	98	6, 7b, 9b, 9e, 17, 24, 30, 36/5, 9g
2SA100	222	9e, 9g, 30, 36/5, 6, 7b, 9b, 17, 24
2SA48	222	9e, 9g, 24, 30, 36/5, 6, 7b, 9b, 17

[a] All pathotypes were virulent for *Sr7a*, *8a*, *9a*, *9d*, *9f*, *10*, *11*, *14*, *16*, *18*, *19*, *20*, *28* and avirulent for *Sr8b*, *13*, *21*, *22*, *25*, *26*, *27*, *29*, *31*, *32*, *35*, dp2 and Tt3.

[b] As described by Stakman *et al*. (55).

TABLE 4.3. Seedling infection types[a] resulting from inoculations of seven parental cultivars of wheat and Morocco (check) with six pathotypes of Puccinia graminis tritici at two different temperatures

Cultivar	Temp. (C)	Pathotype 2SA2	2SA32	2SA39	2SA10	2SA100	2SA48
Belinda	15	0;	0	0	4	4	4
	25	0;	0;	0	4	4	4
Betta	15	2+c	2+3	;3-	;1++c	2++	3c
	25	2+c	2+3	;3	;1+	2++	3c
Karee	15	1	0;n	;	;n	22+	1
	25	;	0;	1	0;	33+c	1+2
Molen	15	2+	2	2	;n	3+	2
	25	2	12-	2	2	3+c	2

TABLE 4.3. (continued) Seedling infection types[a] resulting from inoculations of seven parental cultivars of wheat and Morocco (check) with six pathotypes of *Puccinia graminis tritici* at two different temperatures

Cultivar	Temp. (C)	Pathotype 2SA2	2SA32	2SA39	2SA10	2SA100	2SA48
SST 102	15	2	2	2c	2	33+	1++2
	25	2	2	2	2	2++3	2
Wilge	15	2	2-	2	2	2+	2
	25	2	12-	2	2	3c	1+2
Tugela	15	0	0	0	0	0;	0
	25	0	0	0	0;	0;	1
Morocco	15	4	4	4	4	4	4
	25	4	4	4	4	4	4

a Infection types as described by Stakman *et al*. (55).

TABLE 4.4. Segregation of F_2 seedlings of crosses of Belinda, Betta, Karee, Molen, SST102, Wilge and Tugela with Morocco when tested with appropriate Puccinia graminis f.sp. tritici pathotypes

Cross	Pathotype	Number of F_2 seedlings		Expected	P-value
		Resistant	Susceptible	ratio	
Morocco x Belinda	2SA2	537	167	3:1	0.50-0.25
Morocco x Belinda	2SA32	504	143	3:1	0.10-0.05
Morocco x Belinda	2SA10	0	710	-	-
Morocco x Betta	2SA10	307	108	3:1	0.75-0.50
Morocco x Betta	2SA2	270	101	3:1	0.50-0.25
Morocco x Betta	2SA32	281	93	3:1	0.975-0.950
Morocco x Karee	2SA10	530	185	3:1	0.75-0.50
Morocco x Karee	2SA32	520	165	3:1	0.75-0.50
Morocco x Karee	2SA2	564	166	3:1	0.25-0.10
Morocco x Karee	2SA48	491	157	3:1	0.75-0.50

TABLE 4.4. (continued) Segregation of F_2 seedlings of crosses of Belinda, Betta, Karee, Molen, SST102, Wilge and Tugela with Morocco when tested with appropriate <u>Puccinia graminis</u> f.sp. <u>tritici</u> pathotypes

Cross	Pathotype	Number of F_2 seedlings		Expected	
		Resistant	Susceptible	ratio	P-value
Morocco x Molen	2SA2	336	108	3:1	0.75-0.50
Morocco x Molen	2SA10	375	96	13:3	0.50-0.25
				3:1	0.025-0.010
Morocco x SST102	2SA10	503	158	3:1	0.75-0.50
Morocco x SST102	2SA32	522	161	3:1	0.50-0.25
Morocco x SST102	2SA100	0	684	-	-
Morocco x Wilge	2SA2	457	31	15:1	0.95-0.90
Morocco x Wilge	2SA10	479	160	3:1	0.99-0.98
Morocco x Wilge	2SA48	554	170	3:1	0.50-0.25

TABLE 4.4. (continued) Segregation of F_2 seedlings of crosses of Belinda, Betta, Karee, Molen, SST102, Wilge and Tugela with Morocco when tested with appropriate *Puccinia graminis* f.sp. *tritici* pathotypes

Cross	Pathotype	Number of F_2 seedlings		Expected	P-value
		Resistant	Susceptible	ratio	
Morocco x Tugela	2SA39	660	15	63:1	0.25-0.10
Morocco x Tugela	2SA2	826	10	63:1	0.50-0.25
Morocco x Tugela	2SA10	729	48	15:1	0.95-0.90

TABLE 4.5. Classification of F_3 families from crosses of Belinda, Betta, Karee, Molen, SST102, Wilge and Tugela with Morocco when tested with appropriate *Puccinia graminis* f.sp. *tritici* pathotypes

Cross	Pathotype	Number of F_3 families			Expected	P-value
		Resistant	Segregating	Susceptible	ratio	
Morocco x Belinda	2SA2	17	55	19	1:2:1	0.25-0.10
Morocco x Betta	2SA10	12	29	8	1:2:1	0.50-0.25
Morocco x Karee	2SA32	14	24	10	1:2:1	0.75-0.50
Morocco x Molen	2SA2	7	21	9	1:2:1	0.75-0.50
Morocco x Molen	2SA10	19	23	2	7:8:1	0.975-0.950
Morocco x SST102	2SA10	19	49	20	1:2:1	0.75-0.50
Morocco x Wilge	2SA2	46	49	7	7:8:1	0.95-0.90
Morocco x Tugela	2SA2	59	34	2	37:26:1	0.75-0.50

Thus on the basis of both specificity and on genetic segregation Belinda carried a single dominant gene. This gene was clearly Sr5.

4.4.2 Betta

Betta responded with seedling infection types(Table 4.3) ranging from resistant(IT ;1++c) when inoculated with pathotype 2SA10, to moderately susceptible(IT 3c) when tested with pathotype 2SA48. To pathotypes 2SA2, 2SA10 and 2SA32 the F_2 seedling progenies segregated in ratios of 3 resistant :1 susceptible(Table 4.4). To these pathotypes there was segregation for a range of infection types viz., 0;,1+, 2,2+3,3c and 3+. When an F_2 population of 213 adult plants were inoculated with pathotype 2SA10, 109 plants were resistant, 52 moderately resistant and 52 fully susceptible, indicating the presence of one dominant gene(P-value 0,90 - 0,75). The response of F_3 families tested with 2SA10 agreed with the ratio 1 homozygous resistant(IT 1+2) :2 segregating :1 homozygous susceptible (Table 4.5), confirming the F_2 data.

Therefore the single dominant gene identified in Betta was operating in both the seedling and adult plant stage (Table 4.6) without being temperature sensitive (Table 4.3).

TABEL 4.6. Adult plant infection types[a] resulting from inoculations of seven parental cultivars of wheat and Morocco (check) with two pathotypes of _Puccinia graminis tritici_

	Pathotype	
Cultivar	2SA10	2SA100
Belinda	S	S
Betta	MR	MR-MS
Karee	MR	MS-S
Molen	MR	MS-S
SST102	MR	S
Wilge	R-MR	MS-S
Tugela	HR	HR
Morocco	S	S

a S: Susceptible; MS: Moderately susceptible;
MR: Moderately resistant; R: Resistant:
HR: Highly resistant.

4.4.3 Karee

Karee derived from Betta//Triumph/Agent responded with a characteristic very low infection type with pathotypes 2SA2,2SA32,2SA39,2SA10 and 2SA48. Infection types varied between ;, 0;, 1 and 1+2. The Karee :2SA100 interaction produced intermediate to high responses (IT 22+, 33+c) depending on temperature(Table 4.3).

The F_2 data from Morocco/Karee cross, tested with pathotypes 2SA10, 2SA32, 2SA2 and 2SA48, was explained on the basis of segregation for one dominant gene for resistance to each pathotype(Table 4.4). The resistant F_2 segregates responded similarly with IT 1+2, ;1 and ; to all four pathotypes used. The postulated single gene was confirmed in an F_3 study involving 48 lines of the same cross tested with pathotype 2SA32(Table 4.5). Infection types ;1=c and 1+2 were equally distributed between the 14 homozygous F_3 resistant lines. As a result of insufficient F_3 seed the presence of the dominant gene operating against 2SA32 could not be identified with pathotypes 2SA2, 2SA10 and 2SA48.

4.4.4 Molen

The seedling reaction of Molen is presented in Table 4.3. To pathotypes 2SA2,2SA32 and 2SA39 Molen responded with a moderately resistant reaction(IT 2) at both high

(25C) and low(15C) temperatures. The Molen:2SA10 interaction resulted in a highly resistant reaction(IT ;n) at 15C and a moderately resistant response(IT 2) at 25C. The Molen: 2SA100 interaction was fully compatible at both the seedling(Table 4.3) and adult plant stage (Table 4.6).

When tested with pathotype 2SA2, the F_2 population of Morocco/Molen segregated in a 3 resistant :1 susceptible ratio, indicating that Molen possesses a single gene for resistance. Resistant F_2 segregates displayed a range of infection types viz., 0, ;, ;1= and 1++c suggesting that the gene is partially dominant. The F_3 family ratio (Table 4.5) agreed with the expected 1 homozygous resistant :2 segregating :1 homozygous susceptible when tested with pathotype 2SA2. Lines responding with IT ;1=n and those with IT 1++2 were equally distributed between the seven homozygous resistant F_3 families tested. The F_3 segregating population responded with 11 families segregating for IT ;1= and IT 4 and 10 families segregating for IT ;1+2 and IT 4.

However, when inoculated with pathotype 2SA10, the F_2 population(Table 4.4) segregated for a ratio of 13 resistant :3 susceptible, suggesting the presence of one dominant and one recessive gene. Reaction classes of the F_2 seedlings when tested with pathotype 2SA10,

were equally distributed between IT ;1= and 1++2. This segregation pattern was confirmed in the F_3 (Table 4.5) where segregation conformed with the expected ratio of 7 homozygous resistant :8 segregating :1 homozygous susceptible family.

In a supportive study with two _Puccinia_ _recondita_ pathotypes, differing only in virulence for _Lr24_ infection types produced by Molen suggested the presence of _Lr24_(Pretorius, _personal_ _communication_).

4.4.5 SST102

When incubated at temperatures of 15C and 25C, SST102 was moderately resistant(IT 1++2,2c and 2) to five pathotypes(Table 4.3). However, with 2SA100, infection types of 2++3(15C) and 33+(25C), respectively were obtained. The SST102:2SA100 interaction was fully compatible in the adult plant stage(Table 4.6).

With pathotypes 2SA10 and 2SA32, F_2 segregation was compared with that expected on the basis of segregation at a single locus(Table 4.4). Single gene segregation was confirmed in F_3 tests using pathotype 2SA10(Table 4.5). Seedling infection types of plants scored as resistant within the homozygous resistant F_3 populations ranged between ;, 11+, 1+2 and 2 indicating partial dominance.

No resistant F_2 segregates were detected in Morocco/SST102 when inoculated with pathotype 2SA100, which is virulent for Sr24. Thus the single gene in SST102 is apparently Sr24. SST102 failed to inherit the resistance from its Betta parent.

4.4.6 Wilge

Wilge, released for its outstanding quality and high level of resistance to stem rust, responded similarly to SST102 and Molen when inoculated with the six test pathotypes. Seedling infection types ranged between 1+2 and 2 for all pathotypes except 2SA100(Table 4.3). As with Molen and SST102, there was increased susceptibility(IT 2+ and 3c) to 2SA100 which is virulent for Sr24. Adult plants of Wilge were moderately susceptible when tested with the same pathotype(Table 4.6).

F_2 segregation(Table 4.4) of Morocco/Wilge, tested with pathotypes 2SA10 and 2SA48, suggested the presence of a partially dominant gene. Seedling infection types varying between ;1++ and 22+ suggested that the same gene operated against both pathotypes. When inoculated with pathotype 2SA2 a second dominant gene was identified(IT 1++3-). This was confirmed in the F_3 where segregation fitted a 7:8:1 ratio (Table 4.5).

4.4.7 Tugela

The infection types of Tugela(Kavkaz/Jaral"S") when inoculated with six pathotypes of P. graminis f.sp.tritici and incubated at a low(15C) and high(25C) temperatures, are presented in Table 4.3. The behavior of Tugela to five of these pathotypes, 2SA2,2SA39,2SA32,2SA10 and 2SA100, was found to be highly resistant(IT 0, 0;) and similar at both temperatures. The Tugela :2SA48 interaction at 25C resulted in a slightly higher reaction (IT 1), with some sporulation.

The F_2 progenies from Morocco/Tugela segregated in expected Mendelian ratios of 63 resistant :1 susceptible, when inoculated with pathotypes 2SA2 and 2SA39, both avirulent for Sr5 (Table 4.4). Seedling infection types of plants scored as resistant within the segregating F_2 population varied between 0, ;c, ;1+, 2 and 3-, indicating partial dominance. The distribution of F_3 families tested with 2SA2 agreed with the expected ratio of 37 homozygous resistant :26 segregating :1 homozygous susceptible, suggesting the presence of three dominant genes for resistance(Table 4.5). Fourty-four of the 59 homozygous resistant F_3 lines responded with IT 0;, seven lines exhibited a 12 IT and eight a ;c+ reaction.

However when an F_2 population of the same cross was inoculated with pathotype 2SA10, differing from 2SA2 and 2SA39 in being virulent for Sr5 and Sr7b, the segregation ratio of 15 resistant :1 susceptible indicated the presence of two dominant genes for resistance(Table 4.4). Because of insufficient seed this result was not confirmed in F_3.

4.5 DISCUSSION

Resistance in the cultivars Betta, Belinda, Karee and SST102 to one or more of the six P. graminis f.sp.tritici pathotypes utilized in this study, was conditioned by a single gene, in Molen and Wilge by two genes and in Tugela by three genes.

Lombard(23) identified Sr8a in four derivatives of Betta when tested with 22 P. graminis tritici pathotypes. In addition, he postulated the presence of Sr2 which conditioned the adult plant resistance of certain derivatives. In the present study all six pathotypes were virulent for Sr8a, but another gene was identified in Betta. Comparing the seedling infection types it may be reasoned that the gene operating against pathotypes 2SA2 (IT 2+c) and 2SA32(IT 2+3), was different to the one effective to pathotype 2SA10(IT ;1+, ;1++c). The resistance of the latter gene(to pathotype 2SA10) was also more pronounced in the adult plant stage(Table 4.6).

The single gene resistance of Belinda(Ottowa/2*Cheyenne) is probably derived from Cheyenne which is a known carrier of Sr5(26). The segregation for one dominant gene(IT 0,0;) when inoculated with a pathotype avirulent for Sr5(2SA2) and compared with homozygous susceptibility to pathotype 2SA10, supports postulation of the presence of Sr5. Pathotypes 2SA2 and 2SA10 differ only in reactions to Sr5 and Sr7b. The fact that the low infection types were 0,0; rather than 22+ supports the conclusion that the gene involved was Sr5.

SST102, a Betta derivative(Agent/*2 Betta) carried one gene for resistance to pathotypes 2SA10 and 2SA32. When inoculated with pathotype 2SA100, which is virulent for Sr24, no resistant segregates were identified. This, as well as the fact that SST102 was completely susceptible to 2SA100 in the adult plant stage(Table 4.6) indicated that the resistance gene was probably Sr24. The resistance gene identified in Betta(IT 2+3) was not detected in SST102.

Karee was derived from the cross Betta/Triumph/Agent. The infection type with pathotype 2SA100 was 33+ at 25C, whereas with the other five pathotypes low infection types(IT ;, 0; and 1+2) were encountered. From the infection type and genetic data, it was concluded that Karee carries gene Sr24. Again the resistance from Betta was not present in Karee.

Molen possesses two genes, one dominant and one recessive, for resistance to pathotype 2SA10, but only one dominant gene for resistance to 2SA2. On the basis of seedling infection type data and specificity, the dominant gene appeared to be _Sr24_. The presence of _Sr24_ was supported by the finding that _Lr24_ was also present in Molen(Pretorius, _personal communication_). _Sr24_ and _Lr24_ are usually completely linked in wheat(37).The presence of _Sr24_/_Lr24_ in Molen is of particular interest because these genes are not present in the presumed parents.

The recessive gene in Molen, detected with pathotype 2SA10 and conferring infection type ;n, appeared to be temperature-sensitive. At 25C it conferred IT 2 as opposed to IT ;n at 15C.

Wilge was derived from the cross Bella/Reward/C.I. 12632. Earlier genetic studies(23) indicated the presence of _Sr24_ in Bella and _Sr36_ in C.I.12632. However, the present study showed that Wilge possesses two partially dominant genes against pathotype 2SA2 and one dominant gene for resistance to 2SA10 and 2SA48. From pathological considerations it may be concluded that the major gene(IT 2) was similar in expression to _Sr24_, since the combination Wilge :2SA100 resulted in a significant reduction in resistance. The second dominant gene, identified with pathotype 2SA2(IT 2+3c), appeared not to be _Sr36_ which is known to be present in

the C.I.12632 parent. The fact that the infection type was moderately susceptible, 2+3c, rather than ; or ;1=n supports the conclusion that Sr36 was not involved. This unidentified gene produced moderate susceptibility in the adult plant stage when inoculated with pathotype 2SA100(Table 4.6), which is virulent for Sr24.

Tugela, originating from Kavkaz/Jaral"S", exhibited the highest level of seedling and adult plant resistance of all cultivars examined in this study. It possesses three dominant genes for resistance to pathotype 2SA2 and 2SA39. One of these genes is almost certainly Sr5. It is assumed that one of the two genes identified with pathotype 2SA10(IT ;1) was Sr31, derived from the Kavkaz parent. This gene in combination with the third unidentified dominant gene conferred extremely high levels of resistance in both the seedling(IT 0,0;,;1) and adult plant stages(IT, trace resistance).

The present genetic study indicated that the inheritance of resistance to wheat stem rust in each of the seven winter wheat cultivars was relatively simple. One to three genes conferred resistance to each selected *P. graminis tritici* pathotype. Each gene was normally effective against certain pathotypes. However, Sr31 was effective against all selected pathotypes. Moreover, there appeared to be little effective variability for stem rust resistance since four cultivars, viz. SST102,

Karee, Wilge and Molen possesses _Sr24_. Virulence for _Sr24_ is now widespread in South Africa(see Chapters 1 and 2).

Cultivar Belinda which possesses only _Sr5_ is susceptible to most of the current South African pathotypes. In addition, Betta has inadequate adult plant resistance. However, two of the three genes present in Tugela appeared to be effective against all South African pathotypes.

The information obtained in the present study should be of value to plant breeders. Knowledge of the resistance genotypes should enable breeders and pathologists to predict and assemble more resistances that are effective against all local pathotypes. Secondly such studies should facilitate the recognition of genetic homogeneity and hence vulnerability following pathogenic changes.

Knowledge of resistant genotypes combined with knowledge of the avirulence/virulence pattern should enhance the possibility of constructing multiple gene combinations. Genetic diversity within and between cultivars appears to be the only long-term solution for combating the everchanging wheat stem rust pathogen.

CHAPTER 5

QUANTITATIVE ASPECTS OF SPECIFIC RESISTANCE TO PUCCINIA GRAMINIS f. sp. TRITICI IN WHEAT CULTIVARS

5.1 ABSTRACT

Three spring wheat cultivars(SST44, Palmiet and Gamtoos), with specific resistance to stem rust, were evaluated for differences in latent period(LP) and uredinium density(UD) after inoculation at the adult plant stage with four isolates of *Puccinia graminis* f.sp.*tritici*. Pathotype variation was apparent at both the seedling and adult plant stages. Averaged over the three cultivars, latent period was extended by 57% and uredinium density reduced by 29%, relative to the susceptible check. The resistance of SST44 and Palmiet was more effective in increasing LP than that of Gamtoos, but was less effective with respect to UD. Both resistance components appeared to be under the same genetic control in Palmiet and SST44. Latent period appeared to be a race-nonspecific resistance component of all three cultivars. Although the pathogen isolates showed significant variation, the variability attributable to the host exceeded that due to isolates. This study indicated that the variation in UD associated with specific resistance factors was similar to that reported by other workers for compatible host-pathogen interactions.

5.2 INTRODUCTION

Plant breeders and plant pathologists traditionally have often sought resistance characterized by hypersensitive reactions. A frequent consequence of this has been the subsequent failure of resistance due to the appearance of pathogen variants capable of negating these types of resistance. Advantages of such resistances are their ease of manipulation and their high degree of effectiveness while the pathogen population remains avirulent. In this manner a diversity of genes has been employed to provide, often temporarily,control of wheat stem rust in North America and Australia over the last three decades(44,45).

In South Africa, wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* Eriks. & Henn., has been decreasing in incidence and severity during the last ten years. This decline, highlighted by the absence of stem rust outbreaks during the 1982 and 1983 seasons, may be explained in part by the use of identified resistance genes, in combination with some that remain to be characterized, in released cultivars. The resistance genes that have been deployed include *Sr9e*(in cultivars SST33 and SST66), *Sr24*(SST44, Gamka, SST102, Wilge, Palmiet and SST25); *Sr36*(Zaragoza, SST107 and Gouritz) and *Sr31*(Gamtoos), see Chapters 3 and 4.

Despite the apparent success of the South African and other breeding programs it is clear that resistance mediated by specific genes may be short-lived(26,48,61, 68). Rapid loss of resistance has encouraged plant breeders to seek forms of resistance that are more capable of remaining effective during "prolonged and widespread use in environments favourable to disease" (11). Increased durability of resistance has been sought in mechanisms such as "slow-rusting"(6,51), horizontal resistance(61) and partial resistance(43) each being characterized by a reduction in the apparent infection rate (61). Such resistance is often assumed to be the cumulative result of several genetically determined independent host : pathogen interaction components, such as decreased uredinium density, spore production and infectious period as well as an increased latent period(42).

Vanderplank(61,62,63) assumed that rate-reducing resistance is race-nonspecific, stable and under quantitative genetic control. More recently(64) he suggested that race-specific resistance may also be partial with rate-reducing attributes. Nelson(41) stated that most, but not all, rate-reducing resistances are polygenically controlled. Conversely Parlevliet(42) was of the opinion that various resistance genes in small grain cereals that confer resistance to the rusts and powdery mildews could be cited as examples of monogenic rate-reducing resistances.

The purpose of this investigation was to examine the responses of three moderately resistant wheat cultivars, each with a single gene for resistance(see Chapter 3),in terms of latent period and uredinium density, when infected with several pathotypes of P. graminis f. sp. tritici. Other objectives were to identify possible host-isolate interactions and to establish the rate-reducing attributes of cultivars with effective monogenic resistance.

5.3 MATERIALS AND METHODS

Three South African spring wheat(Triticum aestivum L.) cultivars, with specific resistance to stem rust, and a susceptible control, were selected for the study. These and the genes they possess included:-

- **SST44** (Sr5, Sr8, Sr9b, Sr12, Sr24)(53) =C.I.13523/ 3*T4; T4=Anza=WW15; C.I.13523=Agent.
- **Palmiet**(Sr24)=SST3*3//Scout*5/Agent. One major gene for resistance to South African pathotypes, possibly Sr24(see Chapter 3).
- **Gamtoos** = Veery#3. Possess one major gene, Sr31, to South African stem rust pathotypes(see Chapter 3).
- **Morocco,** was used as a susceptible check cultivar (Univ. of Sydney, accession W1107).

Four P. graminis f. sp. tritici accessions, viz. 2SA13, 2SA19, 2SA21 and 2SA36, pathotypes avirulent to SST44, Palmiet and Gamtoos and commonly found in South Africa,

were used as test cultures. The avirulence/virulence formulae, as initially identified on an internationally representative set of single-gene host differentials (47,68) are presented in Table 5.1. Single pustule isolates from each pathotype were increased in isolation on seven-day-old McNair 701 seedlings, which had been treated with maleic hydrazide to retard growth of secondary leaves and to enhance spore production. Urediniospores were collected in gelatin capsules with a cyclone spore collector(5) and suspended in a light weight mineral oil(Soltrol 130®) at a concentration of 3mg/ml.

5.3.1 Seedling inoculation

Primary leaves were inoculated by atomizing spore suspensions onto seven-day-old plants placed on a turntable, rotating at 36 r.p.m.

Inoculated seedlings were placed in an incubation chamber held at 100% relative humidity for a 14h dark period, followed by a 3h gradual drying period at a light intensity of 10 000 lux. The temperature inside the chamber was maintained at 20±2C. Plants were then moved to a greenhouse where they received natural daylight supplemented with illumination from cool-white fluorescent tubes(Gro-lux) for 14h each day. Temperature was controlled at 22±2C. Seedling infection types(IT) were based on the description of Stakman et al.(55).

5.3.2 Adult plant inoculation

Single plants were grown in 15cm diameter pots in a plastic tunnel-style greenhouse. A water-soluble fertilizer(6,5 N ; 2,79 P ; 13,0 K ; 7,0 Ca ; 2,2 Mg ; 7,5 S) was applied weekly at a rate of 0,5g/pot. Plants were thinned to three tillers one day before inoculation.

Tillers at growth stage 60(60) of each test plant were quantitatively inoculated with 0.11 ml urediniospore suspension(3mg urediniospores/ml), by means of an Andres inoculator(2). This procedure was repeated for each cultivar-pathotype combination. Inoculated adult plants were exposed to the same incubation procedures as described for inoculated seedlings.

Plants were arranged in a randomized block design within the same greenhouse cubicle. The trial consisted of 12 replications, each replication being one plant.

Latent period(LP) determinations were based on the penultimate and terminal internodes of adult plants. Sporulating uredinia were counted daily until no more erupted. The LP was calculated by linear regression as the time from inoculation to when 40% of the uredinia had become erumpent(1). Uredinium density(UD) was cal-

culated as the number of uredinia/cm stem length when all had erupted. Adult plant IT were recorded on the last internode of each test cultivar.

The arc sin square root function was used to normalize the variance of the UD data. The significance of differences in LP and UD between cultivars and pathotypes were tested by analysis of variance($P \leq 0,01$) and Tukey's procedure(56).

5.4 RESULTS

Seedling and adult plant responses for all cultivar: isolate combinations are presented in Table 5.2. Compared with the susceptible check, seedling IT varied from very resistant(IT;) to moderately resistant(IT 12=). Gamtoos was more resistant at the seedling stage than SST44 and Palmiet to all pathotypes except 2SA36, for which it showed restricted sporulation. Apart from 2SA21, all pathotypes differentiated Gamtoos from SST44 and Palmiet.

Taking the Palmiet and SST44 interactions into consideration, two distinct reaction classes were evident in the seedling stage viz. IT ;1= and ;12=. Although all four pathotypes were virulent for Sr5,Sr8,Sr9b and Sr12 of SST44, the low reaction(IT ;) with 2SA21 may be the result of an interaction of one or more of these single

TABLE 5.1 Avirulence/virulence[a] combinations of *Puccinia graminis* f. sp. *tritici* pathotypes

Pathotype	Race No.[b]	Avirulence/virulence formula (*Sr*-gene)
2SA13	321	7b, 9g, 36, E. de M.[d]/9e, 30, TAF_2[c]
2SA19	343	7b, 9e, 9g, 30, 36, TAF_2/E. de M.
2SA21	34	9e, 30, TAF_2/7b, 9g, 36, E. de M.
2SA36	222	9e, 9g, 30, TAF_2/7b, 36, E. de M.

[a] All pathotypes were avirulent for *Sr8b*, *13*, *21*, *22*, *24*, *25*, *26*, *27*, *29*, *31*, *35*, *dp2*, *Tt3* and virulent for *Sr7a*, *8a*, *10*, *11*, *14*, *16*, *18*, *19*, *20* and *28*.

[b] Race according to Stakman *et al*. (55).

[c] Alien chromosome substitution line with a resistance gene from *Agropyron intermedium*.

[d] Enterlago de Montijo.

TABLE 5.2 Seedling[a] infection types and adult plant reactions of test cultivars inoculated with four P. graminis tritici pathotypes

Pathotypes	Growth Stage	Cultivars			
		Palmiet	SST44	Gamtoos	Morocco(Check)
2SA13	Seedling	1=	;1=	;	4
	Adult	30MR-MS[b]	40MR-MS	10MR-MS	80S
2SA36	Seedling	1=	;1=	;12=	4
	Adult	5MR-MS	30R-MR	5MR-MS	80S
2SA19	Seedling	12=	12=	;	4
	Adult	20MR-MS	40MR-MS	TR	70S
2SA21	Seedling	;	;	;	4
	Adult	TMR	TR	50MR-MS	80S

a According to Stakman et al. (55).

b S - susceptible, MS - moderately susceptible, MR - moderately resistant, R - resistant.

resistance genes with <u>Sr24</u>. It may also be occasioned by the presence of an unidentified gene or a greater degree of avirulence in the pathogen(69).

The corresponding adult plants showed greater variation in response to the different pathotypes and reinforced the differences between cultivars. As was the case with seedling responses, Palmiet and SST44 reacted similarly in the adult plant stage to all pathotypes used.

As with seedling resistance, Gamtoos responded with a higher level of resistance in the adult plant stage than SST44 and Palmiet, except in its resistance to pathotype 2SA21. In the latter interaction moderate sporulation was restricted to the areas immediately above the internodes as is characteristic for <u>Sr2</u> which, according to McIntosh(<u>personal communication</u>), might be derived from Veery. Gamtoos usually responds with an adult plant reaction of trMR-MS (Le Roux, <u>personal observation</u>).

5.4.1 Latent period

In the analysis of variance for LP, all treatment effects, except the pathotype-cultivar interaction were significant (Table 5.3). The LP for all pathotypes on Morocco differed significantly from the other pathotype-cultivar combinations(Table 5.4). The LP's for

TABLE 5.3 Analysis of variance for latent period (LP) and uredinium density (UD) involving several host:pathogen combinations[a]

		Resistance component[b]			
		LP		UD	
Source	df	SS	F	SS	F
Total	191	669492.48		353.09	
Experiment	11	5503.65	1.50	3.28	0.77
Treatment	15	609066.68	121.99**	285.85	49.16**
Cultivar	3	572093.54	572.90**	189.37	162.82**
Isolate	3	28481.72	28.52**	20.49	17.62**
Cultivar x Isolate	9	8491.41	2.83	75.89	21.78**
Error	165				
CV		5.90		10.66	

a Morocco (susceptible check), SST44, Palmiet and Gamtoos. Pathotypes 2SA13, 2SA36, 2SA21 and 2SA19.

b LP = latent period in hours, UD = number of sporulating uredinia/cm stem length analysed as arc sine (square root) function.

** P=0.01.

TABLE 5.4 Mean latent periods for various host:pathogen combinations resulting from inoculation of adult plants with <u>Puccinia graminis</u> f. sp. <u>tritici</u> pathotypes

	Latent period[a]				
Pathotype	Morocco	Palmiet	SST44	Gamtoos	Pathotype means
2SA13	210.23 f	321.63 cd	336.80 bcd	294.79 e	290.86 j
2SA19	220.20 f	344.49 abc	339.44 bcd	316.70 de	305.23 k
2SA21	219.99 f	368.04 a	356.47 ab	331.73 bcd	319.06 l
2SA36	217.67 f	367.25 a	365.47 a	334.46 bcd	321.21 l
Cultivar means	217.04 g	350.35 i	349.54 i	319.42 h	

[a] Latent periods are expressed as number of hours from inoculation to 40% uredinia erupted. Values are means of 12 replications. Means followed by the same letter are not significantly different (P = 0.05) according to Tukey's procedure (56).

SST44, Palmiet and Gamtoos were 61%, 61% and 47%, respectively, longer than that of Morocco. There were no significant differences between Palmiet and SST44, but both differed from Gamtoos. Pathotypes 2SA21 and 2SA36 behaved similarly, but differed significantly from 2SA13 and 2SA19, which also differed from each other.

5.4.2 Uredinium density

The mean UD values are presented in Table 5.5. All sources of variation were significant in the analysis of variance(Table 5.3). The mean UD for Morocco was significantly different from that of all other cultivars except combinations 2SA13: Palmiet, 2SA19: Palmiet and 2SA21: Gamtoos. The UD's of Palmiet, SST44 and Gamtoos were 22%, 31% and 33%, respectively, lower than that of Morocco. Gamtoos and SST44 responded with the lowest UD, both differing significantly from Palmiet.

Considering the UD pathotype means, 2SA13 differed significantly from the other pathotypes. The significant cultivar-pathotype interaction could be explained by 2SA19 being high on SST44 and Palmiet and low on Gamtoos, as well as by 2SA21 differentiating SST44 and Gamtoos. In the seedling stage, 2SA19 rendered a low IT value on Gamtoos and an intermediate value on SST44. This pathotype, correspondingly, induced a lower UD on

TABLE 5.5 Mean uredinium density for various host:pathogen combinations resulting from inoculation of adult wheat plants with four *Puccinia graminis* f. sp. *tritici* pathotypes

	Uredinium denstity[a]				
Pathotypes	Morocco	Palmiet	SST44	Gamtoos	Pathotype means
2SA13	8.70 a	6.29 cdef	5.85 ef	4.74 hi	6.39 m
2SA36	7.22 b	5.62 efg	4.76 ghi	4.70 hi	5.58 o
2SA19	7.07 bc	5.99 def	5.66 ef	3.80 j	5.63 o
2SA21	6.85 bcd	5.43 fgh	4.25 bcde	6.49 bcde	5.76 o
Cultivar means	7.46 k	5.83 l	5.13 m	4.93 m	

[a] Uredinium densities are expressed as number of sporulating uredinia/cm tiller length. Values are means of 12 replications transformed with an Arc sin transformation. Means followed by the same letter are not significantly different (P=0.05) according to Tukey's procedure.

Gamtoos than on SST44. However, no differences could be detected between seedlings and adult plants of the two cultivars when inoculated with 2SA21. This may indicate that the gene conferring resistance in Gamtoos was more effective to certain pathotypes when UD was measured in the adult plant stage.

5.4.3 Correlation between latent period and uredinium density

The relationships between LP and UD were examined by determining coefficients of correlation for each paired combination of resistance components. A significant negative correlation($r= -0,72$; $P \leq 0,01$) existed between LP and UD.Individually, Morocco($r= -0,98$), SST44($r= -0,85$) and Palmiet($r= -0,97$) showed a close negative correlation between LP and UD. The poor correlation obtained for Gamtoos($r= 0,38$) was reflected by the inconsistent response to 2SA19 and 2SA21 when LP and UD were compared.

5.5 DISCUSSION

Differences and interactions between resistance components of wheat lines have been used extensively to study the rate-reducing attributes of quantitative resistance(3,4,12,17,43,49). These studies, restricted to compatible host-pathogen interactions, were aimed at

the evaluation of partial resistance and the slow-disease development phenomenon, in the hope of avoiding monogenic resistance and thus obtaining durability of resistance. In general, resistance characterized by the hypersensitive response has proved to be monogenic,or at most oligogenic, unstable and qualitative in type (62).

The stem rust resistances that were present in the cultivars(SST44 and Palmiet - Sr24, and Gamtoos - Sr31) used in this study may be described as specific (see chapter 3). They are controlled by single genes or simple gene combinations in different genetic backgrounds. Despite the operation of these major resistance factors, the cultivars expressed the same resistance components as those reported for compatible interactions.

These observations support the interpretation(58) that when inoculated with avirulent pathotypes, specific resistances appear to restrain disease development similarly to that expected of quantitative resistances, such as slow-rusting. According to Vanderplank(64) this type of resistance, where the pathogen conditions no symptoms or small uredinia with limited sporulation, should be referred to as vertical partial resistance and should not to be confused with "Horizontal" resistance.

Royer *et al.*(49) working with known powdery mildew resistance genes, concluded that the variability due to the host usually exceeded that due to pathotypes when LP and colony density were evaluated as components of partial resistance. The present study confirm these findings for an incompatible interaction. Nevertheless, it also demonstrated that LP and UD may be significantly influenced by different pathotype genotypes. Inoculation with 2SA21 and 2SA36 resulted in significantly longer LP's when compared with those induced by 2SA13 and 2SA19. Uredinium density reacted accordingly, with 2SA13 usually producing the highest value, a possible indication of a higher level of aggressiveness (64).

Uredinium density was decreased by an average of 29% when compared with the susceptible check. The significant difference in UD between SST44 and Palmiet when inoculated with 2SA21, emphasises the importance of genetic background, whereas the value of the specific resistance gene in the expression of resistance(41,49) is underscored by the difference in UD between SST44 and Gamtoos.

The significant correlations calculated between LP and UD for SST44(r= -0,85) and Palmiet(r= -0,97) indicate that both resistance components are possibly under the same genetic control in these cultivars. Similar re-

sults were obtained in a compatible interaction with powdery mildew of oats(13) and barley(3), suggesting that, sometimes, the selection for a longer LP will simultaneously lead to a reduction in UD. However, the poor correlation(r=0,38) between LP and UD for Gamtoos indicated that this relationship is not inevitable.

Significant cultivar-pathotype interactions for UD, showed that similar variation exists in both incompatible and compatible interactions, suggesting that quantitative and qualitative resistances may be essentially similar(except in magnitude) in expression. In this connection it is interesting to note that reductions in infection frequencies due to failure in production of elongating hyphae have been reported for cultivars exhibiting partial resistance to powdery mildew of barley(3) as well as cultivars possessing "major" resistance genes(21,32,33). Asher and Thomas(3) questioned whether on this basis there were any fundamental differences in the mechanisms of partial and "major" gene resistance expression.

Results reported here, confirm earlier reports(41,42) that the inheritance of rate-reducing resistance can be controlled by single genes or combinations of genes and correspond with Vanderplank's proposals(64) concerning partial vertical resistance. Vertical resistance, which allows low to moderate sporulation in the seedling and/

or adult plant stage has been well documented for powdery mildew of barley(14), rice blast(59), wheat leaf rust(58), downy mildew of sugar beet(54) and stem rust of wheat(64).

I conclude that at least some specific resistance, although not always easily detected, has quantitative attributes. Therefore, gradation in levels of resistance occurs in both compatible and incompatible host-pathogen interactions. Whether the resistance is partial or absolute, or characterized by hypersensitive or non-hypersensitive response, its usefulness will be determined by the level of disease control, its durability, and the ease with which it can be manipulated in breeding programs.

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