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Anomalous segregations at the *Sr6* locus for stem rust resistance in wheat

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Abstract The action of the gene *Sr6* for stem rust resistance in wheat is affected by temperature, light, and the particular susceptible parent with which a line carrying *Sr6* has been crossed. Two experiments were carried out to determine whether the effect of the susceptible parents was due to modifier genes, the general genetic background, or interallelic interactions. The data indicated that the susceptible parents carried different *sr6* alleles that interacted with *Sr6*, possibly in a paramutation-like process. In the course of the study, a number of anomalous results were obtained that may be due to the action of transposable elements.

Keywords Stem rust · Wheat · Paramutation · Gene*Sr6* · Transposable elements

Introduction

The gene *Sr6*, which conditions resistance to stem rust (*Puccinia graminis* f.sp. *tritici*) in wheat (*Triticum aestivum*) is unusual. It is sensitive to temperature and, to some extent, light (Forsyth 1956; Luig and Rajaram 1972). For example, a line carrying *Sr6* is resistant to stem rust when grown at a continuous temperature of 18°C, but is susceptible at 24°C. The gene may be either dominant or recessive depending on the race of the pathogen used and the cross in which it is studied (Knott and Anderson 1956). For example, the near-isogenic line of Marquis, Kenya 58/10*Marquis, was produced by crossing and backcrossing Kenya 58 ten-times to Marquis. It carries *Sr6*, derived from Kenya 58, and is resistant to both race TMHT(15B-1) and race MCCD(56) – the new race designations of Roelfs and Martens (1988) are given first, followed by the old designations of Stakman et al. (1962). When Kenya 58/10*Marquis (abbreviated as Marquis-*Sr6*) was crossed to Marquis, the F_1 plants were susceptible to TMHT and resistant to MCCD when grown at 20°C. However, when it was crossed to Pusa 12 the F_1 plants were susceptible to both races, and when it was crossed to LMPG-1 the F_1 plants were resistant to both races (Knott 1981a). Pusa 12 had been used to broaden the range of the genetically different susceptible parents tested. LMPG-1 is a day length-insensitive, stem rust susceptible line developed by the author from crosses involving Little Club, Marquis, Prelude and Gabo. LMPG-1 was found to have resistance to stem rust races TMBG (15B-1L) and LCBN(111), and was replaced by a susceptible sib line, LMPG-6 (Knott 1990). However, in the present study it became apparent that LMPG-1 and LMPG-6 carry different susceptible alleles at the *Sr6* locus. This is not surprising since the four parents of the LMPG lines are susceptible to race TMHT and each could carry a different *sr6* allele.

The effects of the susceptible parents on the dominance of *Sr6* could be due to: (1) different *sr6* alleles, (2) different alleles at a major modifier locus, or (3) differences in their general genetic backgrounds that affect *Sr6*. Two experiments were carried out in an attempt to distinguish between these possibilities.

Materials and methods

Experiment 1

For experiment 1, two crosses, Marquis-*Sr6*/LMPG-l and Marquis-*Sr6*/Pusa 12, were made in an attempt to distinguish between possibilities (1) and (2) above. Pusa 12 is an old cultivar from India. Marquis-*Sr6* and LMPG-1 are described above. The F_1 and F_2 populations from both crosses were tested with races TMHT and MCCD.

Experiment 2

Three lines carrying the gene *Sr6* had been produced previously – Marquis–*Sr6* (described above), LMPG-1-*Sr6* (Kenya 58/10*Mar-

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quis//9*LMPG-1) and Pusa 12-*Sr6* (Kenya58/10*Marquis//5*Pusa 12). Pusa 12 is a late cultivar with very compact spikes. Pusa 12-*Sr6* is also late, although a few days earlier than Pusa 12. However, unlike Pusa 12, it has very long, lax spikes. The *Sr6* locus is known to be linked to the locus for compact vs lax spikes (McIntosh et al. 1998). Apparently, the linkage between *Sr6* and *c* (lax spikes) was not broken during the four backcrosses used to produce Pusa 12-*Sr6*.

The three near–isogenic lines carrying *Sr6* were each crossed (including reciprocals) to the three susceptible parents, Marquis, LMPG–6 and Pusa 12, making a total of 18 crosses. The objective was to study the interactions between the genes in the three susceptible parents and the *Sr6* gene in the three parents carrying it. The 18 F_1 and F_2 populations were tested with races TMHT and MCCD. After some unexpected results were obtained, the crosses were repeated and the new populations tested with races TMHT and MCCD.

The *Sr6* gene involved in both experiments was derived from the same line, Kenya 58//10*Marquis, and should, therefore, have been identical in each cross. It should not have been a source of variation.

Rust tests

In some of the early tests, plants were grown in the greenhouse with 16 h of light at 20°C and 8 h of darkness at 15°C. Supplemental fluorescent lighting was used as needed to provide 16 h of light. During sunny days, the temperature at times rose above 20°C despite the use of coolers. However, the 8-h dark period at 15°C was normally sufficient to prevent any high-temperature breakdown of the resistance controlled by *Sr6*. Most of the later tests, particularly those in experiment 2, were run in growth chambers with 16 h of light at 20°C and 8 h of dark at 15°C, except for one test that was run at 19°C/14°C. With the exception of the latter test, the environmental conditions in the growth chamber tests were identical and resulted in normal development of the resistance conditioned by *Sr6*. Any breakdown of resistance was even less likely at 19°/14°C.

Depending on seed availability, particularly in the F_1, up to eight seeds were planted in a 15 cm-diameter, plastic pot. Seedlings at the two-leaf stage were sprayed with uredospores suspended in Soltrol, a light mineral oil. They were then kept in a chamber in the dark with a mist humidifier for approximately 16 h at 15°C. The lights were then turned on and the temperature raised to 20°C. The infection types (ITs) on the seedlings were recorded after 14 days, using the 0 to 4 scale described by Stakman et al. (1962). In this system, the scale is as follows: 0 – immune, 0;– hypersensitive flecks, 1 – minute uredia surrounded by yellow chlorosis, 2 – small uredia surrounded by light-green islands, 3 – medium-sized uredia with some chlorosis, 4 – large uredia, possibly with some light chlorosis, and X – mixed ITs. Pluses and minuses are used to indicate small deviations from the typical ITs. Infection types 0, 1, 2 and X are considered resistant and 3 and 4 are susceptible. If a line shows more than one IT, a range is shown. Often it was desirable to obtain the reactions of the same plants to both races. In these cases, when the pustules from the first race were just about to sporulate (usually after 7 or 8 days), the seedlings were inoculated with the second race using the same procedure. The reactions of the seedlings to the first race were recorded after 14 days and the infected leaves removed. Seven or eight days later the reactions to the second race were recorded. No evidence of cross-protection has been noted in such tests.

Results

Experiment 1

For the cross Marquis-*Sr6*/LMPG–l the results expected differ depending on whether the effect of the LMPG-l parent is due to the susceptible allele it carries at the *Sr6* locus (designated *sr6l*) or to a modifier gene (designated *Ml*) (Table 1). Marquis is assumed to carry a different modifier allele, *Mm*. Based on previous results (Knott 1981a), it was expected that the F_1 plants from the cross would be resistant to both races under either hypothesis, and they were (ITs 0;1⁻). However, with race TMHT the F_2 plants should segregate 3R:lS (R is resistant and S is susceptible) if the effect is due to the susceptible allele, but 10R:6S if the effect is due to a modifier (Table 1). The actual F_2 segregation was 61R:25S which fits both a 3:1 ratio (P=0.25–0.50) and a 10:6 ratio (P=0.10–0.25). The population size was too small to distinguish between

Table 1 Results expected for F_1 and F_2 seedlings from the cross Marquis-*Sr6*/LMPG, depending on whether the effect of the susceptible parent, LMPG-1, is due to the *sr6l* allele that it carries or to a modifier, *Ml*

Type	Effect due to *sr61*			Effect due to a modifier		
Cross – Genotypes –	Marquis-*Sr6*/LMPG-1 *Sr6Sr6*/*sr61sr61*			Marquis-*Sr6*/LMPG-l *Sr6Sr6MmMm*/*sr6sr6MlMl*		
		Reaction to			Reaction to	
		TMHT	MCCD		TMHT	MCCD
F_1 genotype	*Sr6sr6l*	R[a]	R	*Sr6sr6MlMm*	R	R
F_2 genotypes	1 *Sr6Sr6*	R	R	1 *Sr6Sr6MlMl*	R	R
	2 *Sr6sr6l*	R	R	2 *MlMm*	R	R
	1 *sr61sr6l*	S	S	1 *MmMm*	R	R
				2 *Sr6sr6MlMl*	R	R
				4 *MlMm*	R	R
				2 *MmMm*	S	R
				1 *sr6sr6MlMl*	S	S
				2 *MlMm*	S	S
				1 *MmMm*	S	S
Expected ratio		3:1	3:1		10:6	12:4

[a] R=resistant and S=susceptible

the alternative hypotheses. With race MCCD, the expected segregation is the same (3R:1S) under either hypothesis. However, if the effect is due to the particular susceptible allele present, all F_2 plants should give the same reaction to both races, either resistant to both or susceptible to both. If the effect is due to a modifier, the 2/16 of the F_2 plants (or 11 plants) that were *Sr6sr6MmMm* should have been susceptible to TMHT but resistant to MCCD. All of the 86 plants gave the same reaction to both races, although the resistance was greater to MCCD (ITs 0;1$^-$) than to TMHT (ITs 1$^-$X – mostly X$^-$X). Thus, the data suggest that the effect is due to the *sr6l* allele and provide no evidence for a major modifier. However, a modifier tightly linked to *sr6* would not have been detected.

For the cross Marquis-*Sr6*/Pusa 12 the expected results are somewhat similar to those for the cross Marquis-*Sr6*/LMPG-l. The F_1 plants are expected to give the same results under either hypothesis, susceptible ITs to both races. They were susceptible (ITs 4$^-$4), except in one test in which they gave an IT 4 with race TMHT but an IT XX$^+$ with race MCCD. The F_2 population should segregate in a lR:3 S ratio for both races if the effect is due to the susceptible allele, *sr6p*, but 1R:3S to race TMHT and 6R:10S to MCCD if the effect is due to a modifier. Again, plants of one F_2 genotype, *Sr6sr6MmMm*, were expected to be susceptible to race TMHT but resistant to race MCCD. The F_2 segregation with race MCCD of 15R:47S is a good fit to a 1:3 ratio (P=1.0), but does not quite fit a 6:10 ratio (P=0.02–0.05). One plant was susceptible to race TMHT (IT 3$^+$), but resistant to race MCCD (IT 1$^-$). If the modifier hypothesis is correct, 2/16 or 8 plants should have been of this type. A number of the F_2 plants gave X ITs with race TMHT and it is likely that the plant with an IT 3$^+$ had been misclassified and should have been an X or X$^+$. The data suggest that the effect of the susceptible parent, Pusa 12, is due to the susceptible allele, *sr6p*, that it carries and provide no evidence for a modifier gene unless it is tightly linked to *sr6p*.

Experiment 2

F_1 generation

The genotypes expected in the F_1 generation for the 18 crosses under each of the three hypotheses are given in Table 2 (the expectations are the same for reciprocal crosses). The hypothesized alleles for susceptibility are designated *sr6l* (from LMPG–6), *sr6m* (from Marquis) and *sr6p* (from Pusa 12). The hypothesized modifiers are designated *Ml*, *Mm* and *Mp*, i.e., alleles at one locus. However, the results expected would not change if they are at separate loci. Similarly, linkage to the *Sr6* locus would not change the results expected in the F_1 generation.

If the effect on *Sr6* is due to different *sr6* alleles, each set of three F_1 populations from the three crosses between one susceptible parent and the three *Sr6* parents should give the same reactions to a particular race since all three populations have the same genotype (Table 2). Under either of the other two hypotheses, in each set of three crosses involving a common susceptible parent the F_1 populations will have either different modifiers or different genetic backgrounds. In the sets of three crosses involving Marquis and Pusa 12, respectively, the F_1 plants from different crosses should give different rust reactions. The exception is the set of three crosses involving LMPG–6 for which the F_1 plants are expected to give the same results under either hypothesis.

Table 2 The genotypes expected in the F_1 generation of the 18 crosses under the three hypotheses when the three near-isogenic *Sr6* lines are each crossed with the three susceptible parents (reciprocal crosses are assumed to give the same results)

Cross		Hypothesis								
		Susceptible alleles			Modifier alleles			Background genotype		
		F_1 genotypes	Expected reactions[a]		F_1 genotypes	Expected reactions[b]		F_1 genotypes[c]	Expected reactions	
			TMHT	MCCD		TMHT	MCCD		TMHT	MCCD
LMPG-6	/LMPG-1-*Sr6*	*Sr6 sr6l*	R	R	*Sr6 sr6 Ml Ml*	R	R	*Sr6 sr6* LMPG LMPG	R	R
	/Marquis-*Sr6*	*Sr6 sr6l*	R	R	*Sr6 sr6 MlMm*	R	R	*Sr6 sr6* LMPG Marquis	R	R
	/Pusa 12-*Sr6*	*Sr6 sr6l*	R	R	*Sr6 sr6 MlMp*	R	R	*Sr6 sr6* LMPG Pusa	R	R
Marquis	/LMPG-1-*Sr6*	*Sr6 sr6m*	S	R	*Sr6 sr6 MmMl*	R	R	*Sr6 sr6* Marquis LMPG	R	R
	/Marquis-*Sr6*	*Sr6 sr6m*	S	R	*Sr6 sr6 MmMm*	S	R	*Sr6 sr6* Marquis Marquis	S	R
	/Pusa-12-*Sr6*	*Sr6 sr6m*	S	R	*Sr6 sr6 MmMp*	S	S	*Sr6 sr6* Marquis Pusa	S	S
Pusa 12	/LMPG-1-*Sr6*	*Sr6 sr6p*	S	S	*Sr6 sr6 MpMl*	R	R	*Sr6 sr6* Pusa 12 LMPG	R	R
	/Marquis-*Sr6*	*Sr6 sr6p*	S	S	*Sr6 sr6 MpMm*	S	R	*Sr6 sr6* Pusa 12 Marquis	S	S(?)
	/Pusa-12-*Sr6*	*Sr6 sr6p*	S	S	*Sr6 sr6 MpMp*	S	S	*Sr6 sr6* Pusa 12 Pusa 12	S	S

[a] Based on previous data (Knott 1981a)

[b] The expected reactions are based on the assumption that the dominance is *Ml*>*Mm*>*Mp*

[c] The cultivar names are used to represent the two background genotypes combined in the F_1

Table 3 The infection types for F_1 seedlings of 18 crosses and their parents when tested with races TMHT and MCCD (reciprocal crosses gave the same results and have been combined)

Cross or parent		Infection type with	
		Race TMHT	Race MCCD
LMPG-6	/LMPG-1-*Sr6*	34	0;1⁻
	/Marquis-*Sr6*	34	0;X⁻
	/Pusa 12-*Sr6*	4⁻4	0;1⁻
Marquis	/LMPG-1-*Sr6*	4⁻4, X⁻X[a]	0;1⁻
	/Marquis-*Sr6*	4⁻4	0;1⁼
	/Pusa 12-*Sr6*	4⁻4	0;1⁼
Pusa 12	/LMPG-1-*Sr6*	0;1X⁼	0;1⁻
	/Marquis-*Sr6*	1⁺2X[b]	0;1⁼
	/Pusa 12-*Sr6*	1⁺2X[b]	0;1⁼
LMPG-1-*Sr6*		0;	0;
Marquis-*Sr6*		0;1⁻	0;
Pusa 12-*Sr6*		1⁼1⁻	0;
LMPG-1		4⁻4	4⁻4
LMPG-6		4	4⁻4
Marquis		4	4
Pusa 12		4	4

[a] In growth chamber tests with race TMHT, the seedlings usually gave ITs 4⁻4
[b] In one growth chamber test, some 4⁻4 ITs were obtained

The F_1 results were clear (Table 3). With race MCCD, all 18 crosses gave similar, resistant ITs. Unexpectedly, the three crosses with Pusa 12 gave resistant F_1 plants. Based on earlier results (Knott 1981a), it was expected that these F_1 plants would all be susceptible. A check was made in our seed store and two packages labelled Pusa 12 were found. When they were grown out, they had very different head types. Both types were crossed with LMPG-1-*Sr6*. The F_1 plants were tested with race TMHT and gave different results. The plants from one cross were susceptible and from the other resistant. Evidently, the first line was used in the original crosses and the second line in the crosses for the present experiment.

With race TMHT, in each case the F_1 plants from the three crosses involving the same susceptible parent gave similar ITs. The one minor exception was the cross, Marquis/LMPG-1-*Sr6*. In two greenhouse tests, the F_1 plants from this cross looked healthy when they were rusted, but died from unknown causes before the ITs could be classified. All of the other crosses grown and rusted at the same time were normal. However, in four tests run in growth chambers the plants from the cross remained healthy. The ITs on them were usually 4⁻4, but occasionally X⁻X. Again, unexpectedly, the F_1 seedlings from the crosses involving Pusa 12 were resistant. In addition, the F_1 seedlings from crosses involving LMPG-6 were susceptible, the reverse of previous results (Knott 1981a). However, the earlier crosses had involved LMPG-1, not LMPG-6. The crosses with LMPG-1 gave results very similar to those from crosses with Little Club. LMPG-1 undoubtedly obtained its *sr6* allele from its Little Club parent. LMPG-6, on the other hand, must have received its *sr6* allele from one of the other three parents in its pedigree. Nevertheless, since all three crosses involving one susceptible parent gave similar ITs in each case, the F_1 data fit the hypothesis that different alleles for susceptibility have different effects on the resistance controlled by *Sr6*. They do not fit either of the other two hypotheses, including the possibility that a major modifier locus is tightly linked to *sr6*. The F_2 genotypes expected are the same regardless of whether the postulated modifiers are linked to or independent of *Sr6*.

F_2 generation

In the F_2 populations, the separation between resistant and susceptible plants was clear. Nevertheless, the F_2 generation gave a number of unexpected results.

With race TMHT, the F_1 plants of the 12 crosses involving LMPG-6 and Marquis gave susceptible ITs, indicating that resistance was recessive. Therefore, the F_2 populations were expected to segregate 1R:3S. However, in three of the crosses some of the F_2 families segregated 1R:3S, but others segregated 3R:1S (identified with an A in column 3 of Table 4). In other words there was a reversal of the dominance of *Sr6* in the progeny of some F_1 plants, but not in others, even though all of the F_1 plants had been susceptible. Apparently, the change occurred only in the germ line of the F_1 plants. Of the three groups of families in which a reversal of dominance had occurred, in two the F_2 segregation to race MCCD fits the 3R:1S ratio expected since the F_1 plants were resistant. In the third group, the cross Marquis/LMPG-1-*Sr6*, the F_2 segregation did not quite fit a 3R: 1S ratio (P=0.025–0.050). Possibly this deviation occurred by chance. There are 20 χ^2 values in Table 4 and, on average, 1 in 20 should give a probability value below 0.05 just by chance.

In the cross, LMPG-6/LMPG-1-*Sr6*, the seven F_2 families that showed a reversal of dominance segregated for atypical, resistant ITs with both races. Instead of the typical 0;X ITs, they gave 1⁺2 ITs. Furthermore, three plants were resistant to race TMHT but susceptible to MCCD and two were the reverse. Since resistance was dominant to both races, all plants should have given the same reaction to both as expected with *Sr6*.

In the cross, Marquis/LMPG-l-*Sr6*, normal ITs for *Sr6* were obtained, but, again, in the families that showed a reversal of dominance with race TMHT and segregated 3R:1S to both races, many plants did not give the same reaction to both races. In one group of four sib families, 9 of 61 seedlings were susceptible to race TMHT and resistant to MCCD whereas 11 were the reverse. In another group of four sib families, 9 of 46 seedlings were susceptible to race TMHT and resistant to MCCD, but none was the reverse. Three F_3 families were grown from plants of one F_2 family. Two segregated in a 3R:1S ratio to both races. Of 20 plants tested, three were susceptible to race TMHT but resistant to MCCD while six were the

Table 4 The F_2 segregation with races TMHT and MCCD in the 18 crosses (including reciprocals) between three susceptible parents and three parents carrying *Sr6*. + or – these families had an excess or deficiency of resistant plants, respectively. A – based on the F_1 data, these families should have segregated 1R:3S but actually segregated 3R:1S. B – Based on the F_1 data these families should have segregated 3R:1S but actually segregated 1R:3S

Cross	Number of F_2 families	Race TMHT	*P* (3:1 or 1:3) [a]	Race MCCD	*P* (3:1)
LMPG-6/LMPG-1-*Sr6*	7	121:35 [b]A	0.50–0.75	111:28 [b]	0.10–0.25
	3	7:23	0.90–0.95	29:2+	0.025–0.05*
Reciprocal	8	48:145	1.0	173:38+	0.01–0.025*
LMPG-6/Marquis-*Sr6*	3	16:55	0.50–0.75	43:16	0.75–0.90
Reciprocal	7	31.89	0.90–0.95	76:19	Het.[d]
LMPG/Pusa 12-*Sr6*	4	9:18	0.25–0.50	22:8	1.0
Reciprocal	6	32:128	0.10–0.25	81:44–	0.01–0.025*
Marquis/LMPG-1-*Sr6* [c]	8	83:20A	0.10–0.25	90:17+	0.025–0.050*
	18	50:149	1.0	152:25+	<0.001**
Reciprocal [c]	6	73:27A	0.50–0.75	48:10	0.10–0.25
	5	37:89	0.25–0.50	114:13	<0.001**
Marquis/Marquis-*Sr6*	5	24:81	0.50–0.75	71:22	0.75–0.90
Reciprocal	6	53:113	0.025–0.050*	128:29	0.05–0.10
Marquis/Pusa 12 -*Sr6*	5	9:44	0.10–0.25	43:11	0.25–0.50
Reciprocal [c]	9	29:95	0.50–0.75	71:27	0.75–0.90
Pusa 12/LMPG-1-*Sr6*	9	67:22	1.0	86:28	1.0
Reciprocal [c]	2	23:5	0.50–0.75	31:7	0.25–0.50
	6	11:29B	0.75–0.90	–	
Pusa 12/Marquis-*Sr6* [c]	10	72:27	0.50–0.75	72:26	1.0
	4	13:22B	0.10–0.25	6:1	0.50–0.75
Reciprocal [c]	2	30:13	0.50–0.75	30:12	0.50–0.75
	1	3:7B	1.0	–	
Pusa 12/Pusa 12-*Sr6*	5	31:6	0.25–0.50	33:6	0.10–0.25
Reciprocal [c]	9	88:41	0.05–0.10	75:33	0.10–0.25
	2	2:9B	0.75–0.90	–	

[a] The segregations were fitted to either a 3:1 or a 1:3 ratio, whichever was appropriate

[b] These data came from families that segregated for an atypical IT with race TMHT and race MCCD ($1^{-}2^{-}$ instead of 0;X) and gave a 3R:1S ratio instead of a 1R:3S

[c] The families fell into two groups for reaction to race TMHT, some segregating 3R:1S and some 1:3

[d] Het. means the families were heterogeneous

reverse. The third F_3 family segregated lR:3S to race TMHT but 3R:1S to MCCD. Two of ten plants in this family were resistant to race TMHT and, if they were homozygous for a normal *Sr6* allele as expected, should have also been resistant to race MCCD. However, one of the two was susceptible.

Of the 12 groups of families from the LMPG-6 and Marquis crosses that gave the expected F_2 segregation of 1R:3S with race TMHT, seven also gave the expected F_2 segregation of 3R:1S with race MCCD, although there was often a tendency for an excess of resistant plants. However, the remaining five deviated significantly from the expected 3R:1S ratio, four having an excess of resistant plants and one an excess of susceptible plants (marked with a + or –, respectively, in column 5 of Table 4).

For the six crosses involving Pusa 12, the F_1 plants were generally resistant to both races, although the ITs tended to be somewhat variable with race TMHT (Table 3). Occasionally, in tests with race TMHT a few seedlings gave 4–4 ITs, for some unknown reason. The results were confirmed in the tests on the F_2 populations. In four of the six crosses, some of the families segregated 1R:3S to race TMHT (marked with a B in column 3 of Table 4) instead of the expected 3R:1S. Again a reversal of dominance had occurred in the progeny of some F_1 plants, presumably those that had been susceptible to race TMHT, but not in others. Unfortunately, tests with race MCCD were obtained on only one small family in the four groups of families that showed the reversal of dominance. It segregated 3R:1S as expected since the F_1 plants were resistant.

Some of the crosses in experiment 2 provided additional data to distinguish between the hypotheses that the observed effects on *Sr6* are due either to different *sr6* alleles or to modifiers, as in experiment 1. In fact, it was originally thought that the same two crosses had been repeated. However, in the cross, Marquis-*Sr6*/LMPG-1, LMPG-1 had been replaced with LMPG-6, and in the cross, Marquis-*Sr6*/Pusa 12, a different ”Pusa 12” had been used.

The crosses LMPG-6/Marquis-*Sr6*, Marquis/Marquis-*Sr6* and Pusa 12/LMPG-1-*Sr6* (and reciprocals) were of no use since the expectations were the same under either hypothesis.

For the remaining six pairs of reciprocal crosses, the expectations under the two hypotheses differ in either one or two ways. Either the expected F_2 segregation ratios differ for one of the two races or the frequency of F_2 plants susceptible to race TMHT but resistant to race MCCD (S/R) differs under the two hypotheses. In some crosses, the expected F_2 ratios and the frequency of S/R plants cannot be determined unless assumptions are made about the dominance of the modifiers. For example, the expected reaction of *Sr6sr6MlMm* plants to each race depends on which allele is dominant. For the purposes of the hypothesis, the dominance was assumed to be *Ml>Mm>Mp*, which conforms to the results obtained earlier (Knott 1981a). Even if the hypothesis is wrong, maximum possible frequencies of S/R plants can often be determined.

In four of the six pairs of reciprocal crosses, half of the plants should have been S/R if the effect was due to the *sr6* allele, but at most 6/16 if the effect was due to modifier alleles. The data from the four pairs of reciprocal crosses were combined for the families in which no reversal of dominance had occurred. The actual frequencies for the four pairs of crosses were 90/158, 51/107, 108/177 and 68/123. In all four cases, the frequency is significantly above 6/16, confirming that the effect on *Sr6* is due to the different *sr6* alleles. In the third case, the frequency is significantly above one-half. This is because, in this cross, the frequency of plants resistant to race MCCD was significantly higher than expected and, as a result, the frequency of S/R plants was significantly above one-half. In the remaining two crosses, no plants were expected to be S/R under either hypothesis, and none were. In these latter two crosses, the expected F_2 ratio with race TMHT is 3R:1S under the susceptible-alleles hypothesis and a maximum of 10R:6 S under the modifier-gene hypothesis. If the families that unexpectedly segregated lR:3S are ignored, then neither segregation fits a 10:6 ratio. The results from these two crosses also confirm the susceptible-alleles hypothesis.

Discussion

The purpose of experiment 1 was primarily to test for the presence of a major, independent modifier of the resistance controlled by *Sr6*. No evidence for a modifier was found. The evidence suggested that the effect of the the susceptible parents is due either to the *sr6* alleles for susceptibility that they carry or to a tightly linked modifier.

The F_1 generation of the crosses in experiment 2 also provided no evidence that major modifiers were present, including a modifier tightly linked to *sr6*, or that the general genetic background of the susceptible parents had any effect on *Sr6*. The F_2 generation of some relevant crosses in experiment 2 confirmed experiment 1. Thus, all of the data suggest that the effect of the susceptible parents on *Sr6* is due to the *sr6* alleles that they carry.

In the F_2 generation of the crosses in experiment 2, a number of unexpected results were obtained. These can be classified into four types:

(1) Reversals of the dominance of *Sr6* from recessive to dominant in the progeny of some F_1 plants of three crosses and from dominant to recessive in the progeny of some F_1 plants of four crosses.
(2) Deviations from a 3R:lS ratio with race MCCD because of an excess of resistant plants in some or all of the F_2 families from five crosses and a deficiency in one cross.
(3) An IT atypical of *Sr6* to both races in some F_2 families of the cross LMPG-6/LMPG-1-*Sr6*, but not in the reciprocal cross. In the families segregating for the atypical IT, unexpectedly, three plants were resistant to race TMHT but susceptible to MCCD, and two were the reverse.
(4) A similar unexpected occurrence of plants resistant to one race but not to the other in the F_2 families of the cross Marquis/LMPG-1-*Sr6* that segregated in a ratio of 3R:1S to both crosses.

Knott and Anderson (1956) and Knott (1981a) showed that, in a specific cross, *Sr6* could act as a dominant gene to race MCCD and as a recessive to TMHT. This raised the question as to whether *Sr6* was a compound locus, one component giving resistance to MCCD and another to TMHT. Additional evidence on this point came from the work of Knott (1981b) who produced five translocations between a chromosome 2D carrying *Sr6* and A- or B-genome chromosomes in the durum cultivar Kubanka. When the translocations carrying *Sr6* were transferred to a hexaploid, one of the derived lines had resistance to race MCCD, but not to race TMHT. When *Sr6* was returned to a normal chromosome 2D by crossing-over, it again conditioned resistance to race TMHT (Knott, unpublished). Apparently, one component of *Sr6* had been silenced when a segment of chromosome 2D carrying it was translocated to a new chromosome (7B), but was reactivated when it was returned to chromosome 2D. This provides further evidence that *Sr6* is a compound locus with two components that act separately. If *Sr6* is a compound locus, it is easier to explain some of the anomalies observed in this work. However, there is no evidence that the *Sr6* locus is similar to either the *Rp1* locus which gives resistance to *Puccinia sorghi* in maize (reviewed by Hulbert 1997) or the *M* locus which gives resistance to *Melanspora lini* in flax (*Linum usitatissimum*) (reviewed by Ellis et al. 1997). Both are clusters of tightly linked genes that have been shown to recombine at low frequencies by crossing-over. The *Sr6* locus may be more similar to the *L* locus for resistance to flax rust which has 17 alleles. The alleles differ by tandem duplications. In heterozygotes, unequal crossing-over can result in susceptible progeny at a very low frequency. As yet only one allele for resistance has been identified at the *Sr6* locus, unless the translocation described above is considered to be a second allele. However, the present work suggests that there may be several alleles for susceptibility.

If the affect of the susceptible parents in crosses with genotypes carrying *Sr6* is due to the *sr6* alleles that they carry, as the data indicate, then the key question is how do different alleles at the *Sr6* locus interact? Brink's (1956) study in maize (*Zea mays*) provides a possible model. He coined the term paramutation to describe a process in which an R^r allele (coloured plant and aleurone) that had come from an R^rR^{st} (stippled aleurone) plant crossed to an r^rr^r (colourless plant, coloured aleurone) pistillate parent resulted in reduced pigmentation in the progeny. All R^r alleles were effected. This phenomenon has since been found to occur in other plant species (reviewed by Dooner et al. 1991; Hollick et al. 1997). The examples with *Sr6* reported by Knott and Anderson (1956) and Knott (1981a), in which *Sr6* showed a reversal of dominance in all of the F_1 progeny of certain crosses, has some similarity to paramutation. It is assumed that *Sr6* is normally dominant, like most other *Sr* genes. In the F_1 plants from a cross involving Marquis, the dominance of resistance was reversed only to race TMHT, whereas in a cross involving Pusa 12 dominance was reversed to both TMHT and MCCD. Since the *Sr6* allele in all of the F_1 plants in a cross was affected, there are similarities to paramutation in which all R^r alleles from R^rR^{st} hybrids are changed.

In the present work a new phenomenon was found; reversals of dominance occurred in both directions, but only in the F_2 progeny of some F_1 plants from a cross. In one case the F_1 plants were not affected and the reversal of dominance appeared in their F_2 progeny. This reversal may have occurred only in the germ line of the affected F_1 plants. In the second case, the F_1 plants were more variable in IT in different tests and the reversal may have affected the F_1 plants as well as their F_2 progeny.

The fact that the progeny of only some F_1 plants were affected in the latter cases suggests that a phenomenon other than paramutation is involved. McClintock (1965) reviewed a number of examples in maize in which a wild-type gene was suppressed by the insertion of a particular genetic element at that locus. The genetic elements were transposable and when they were excised from the locus, normal function returned. A system of transposable elements could account for the excess of resistant plants that occurred in a number of crosses in the present study. If some of the *sr6* alleles are really *Sr6* suppressed by an inserted transposable element, then excision of the element in some F_2 plants would result in extra resistant plants. To explain the one cross in which there was an excess of susceptible plants, it would be necessary to assume that a transposable element, perhaps present at an *sr6* locus, was occasionally transposed to an *Sr6* allele and suppressed it, resulting in a susceptible F_2 plant. This seems less likely than the excision of a transposable element, but a change in only a few F_2 plants would result in a significant excess of susceptible plants. Even the occurrence of an atypical IT in one group of families could result from the insertion of a transposable element that only partially suppressed resistance. Since *Sr6* is almost certainly a compound locus, the occurrence in some crosses of plants that were resistant to one race but not the other could result from the silencing of one component of the locus but not the other. That this can occur was shown when a segment of chromosome 2D carrying *Sr6* was translocated to another chromosome (Knott 1981b), as mentioned above.

The present study adds greatly to the known complexity of the *Sr6* locus in wheat. The evidence strongly suggests that *Sr6* is a compound locus and that its action is changed by the effect of *sr6* alleles present with it in heterozygotes. Something akin to paramutation may be occurring in some crosses. Although it is only conjecture at this point, other anomalies are most easily explained by the presence of transposable elements.

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