The Effect of Temperature and Genetic Background on Host Gene Expression and Interaction to Puccinia graminis tritici

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ABSTRACT

Infection types produced by *Puccinia graminis* f. sp. *tritici* on seedlings of near-isogenic lines of Marquis wheat, and on homozygous and heterozygous combinations between them, were studied in growth cabinets at six temperature levels. At the high temperatures, 27 and 30 C, seedlings heterozygous or homozygous for the genes *Sr5*, *Sr8*, and *Sr9b* became susceptible or semisusceptible.

When *Sr5* and *Sr9b* were transferred by successive backcrosses to the highly susceptible background of W2691 and W3498, the infection types became much higher, and heterozygous seedlings were semisusceptible at the intermediate temperature range.

In most instances, combinations of host genes resulted in higher levels of resistance. Higher temperatures and susceptible genetic backgrounds interacted in decreasing the resistance provided by all genes.

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While it is well known that high temperatures can alter infection types caused by wheat stem rust, *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., towards susceptibility in instances where temperature-sensitive genes for resistance are involved (3, 4, 5, 8, 10), low temperatures can have the same effect (14). Low light intensity and chemicals can also render certain genes for resistance ineffective (2, 3, 5, 7, 8).

Knott & Green (9) reported that the gene action of *Sr6*, *Sr8*, *Sr9a*, and *Sr9b* was not modified to any extent by other genes present in the cultivars which they used. However, when *Sr10* and *Sr7* were backcrossed to Marquis more than 3 and 6 times, respectively, a distinct loss of resistance occurred.

Watson & Luig (21) demonstrated that the genes *Sr6* and *Sr11* each were capable of interactions resulting in low infection types at three different levels of expression. Thus, in a given environment, avirulence of rust cultures could be further characterized according to the infection types induced by these two genes. Furthermore, it was found that the expression and the dominance relationship of *Sr6* were greatly altered by changes in temperature (10). The present study, utilizing *Sr6* and five genes previously considered temperature-insensitive, reports on (i) interactions between these genes when present in pairs either in a heterozygous or homozygous condition; (ii) influence of constant temperatures on infection type; and (iii) effects of the genetic background on gene expression.

MATERIALS AND METHODS.—Six near-isogenic lines in a Marquis background, originally provided by D. R. Knott, Saskatoon, Sask., Canada, namely, Kenya 58 X Mq^6 W2400 *(Sr6)*, Mq^6 X Red Egyptian W2401 *(Sr8)*, Mq^6 X Kenya 117A W2402 *(Sr9b)*, Thatcher X Mq^6 W2928 *(Sr5)*, Khapstein X Mq^6 W2931 *(Sr13)*, and Lee X Mq^{10} W3015 *(Sr11)*, were used to synthesize lines having different paired combinations for low reaction to stem rust. A diallele set of crosses between the six lines was made. Approximately 25 to 35 seedlings raised from 300 F_3 lines of each cross were inoculated with appropriate strains of *P. graminis tritici* virulent on one of the parents of each cross. Residual seed of homozygous-resistant lines were sown, and at least 20 seedlings were inoculated with strains virulent on the second parent. Again, lines homozygous-resistant were chosen, and these were further tested with strain 34-1,2,3,6,7 which is virulent on plants with *Sr5*, *Sr6*, *Sr8*, *Sr9b* and *Sr11*. Except for lines possessing the *Sr13* allele, the resulting susceptible infection types were additional evidence for the presence of the genes derived from the near-isogenic parents.

By the above procedures, the homozygous combinations (i) *Sr5*, *Sr9b* (21-1,2,3,7, 34-2,4,5); (ii) *Sr5*, *Sr13* (21-1,2,3,7, 126-1,6,7); (iii) *Sr6*, *Sr8* (126-6,7, 21-1,2,3,7); and (iv) *Sr8*, *Sr9b* (126-1,6,7 21-1,2,3,7), were obtained. The strains used for inoculation which have been described earlier (20) are indicated in parentheses.

In addition to the four homozygous combinations, four double heterozygous combinations (the F_1 from crossing two isogenic lines), five single heterozygotes (the F_1 from crossing an isogenic line with Marquis), and the six isogenic parents were tested. Marquis (as the susceptible check), Reliance *(Sr5)*, and Kanred *(Sr5)* were used for purposes of comparison.

Two very susceptible lines, W2691 and W3498, which have been described (12) were used as the recurrent parents in a program designed to obtain near-isogenic lines possessing major genes for resistance in

a susceptible background. Three lines (A-*Sr5*, B-*Sr9b*, C-*Sr11*) resulting from this program, as well as W2691 and W3498, were included in the study.

Strain 21-4,5, which resembles strain 21-2 in interactions with *Sr6* (21) and which is also avirulent on the other isogenic lines (20), was chosen. After inoculation, seedlings (10-20 in each line) were transferred to "Sherer" plant growth rooms where temperatures of 15, 18, 21, 24, 27, and 30 C with an average fluctuation of ± 1 C were maintained. Light intensity was ca. 3,000 ft-c. Infection types were recorded after 12 days. The system of Stakman et al. (18) was followed, excepting a "3+" denoting full susceptibility. Backcross lines involving W2691 and W3498 were tested mainly in the greenhouse at 15 to 24 C.

RESULTS.—Infection types are listed in Table 1. Seedlings of W2928 *(Sr5 Sr5)* exhibited infection type "O-O;" at 15, 18, 21, and 24 C, "O; 1=" at 27 C, and "3cn" at 30 C, whereas F_1 plants of the cross W2928 X Marquis (*Sr5 sr5*) produced "O-O;" at the 4 lower temperatures, ";1=" at 27 C, and "3" at 30 C. These results indicated that *Sr5* in a Marquis background was completely dominant at the lower temperatures, partially dominant at 27 C, and recessive at 30 C. However, at the last temperature, *Sr5* when present in W2928 was largely ineffective, whereas seedlings of Kanred *(Sr5)* and Reliance *(Sr5)*, remained fully resistant ("O" and "O;" infection type).

The temperature sensitivity of *Sr5* at 30 C was confirmed in the tests involving homozygous and heterozygous combinations with the genes *Sr9b* and *Sr13* (Table 1). Since infection types on seedlings having the gene combinations were lower than those on seedlings possessing *Sr5* alone, the data suggest additive gene interaction. This was surprising in the case of the heterozygous combination *Sr5 sr5 Sr9b sr9b*, as the heterozygote *Sr9b sr9b* appeared to be fully susceptible at 30 C.

Lines of *Sr5* in a W2691-W3498 background were tested against strain 21-4,5 in the glasshouse at average temperatures of 21 C. Those homozygous and heterozygous for *Sr5*, and backcrossed to W2691 and W3498 five times, exhibited ";" and "X=" infection types, respectively. The same genotypes after seven backcrosses showed "X" and "3c"; and the infection types recorded after nine backcrosses were "X+3" and "3". Thus, by changing the genetic background, it was possible to achieve a partial breakdown of the *Sr5* resistance similar to that occurring at constant temperatures of 30 C. The infection types recorded in the growth chambers on a homozygous line synthesized after nine backcrosses are shown in Table 1.

As can be seen in the second part of Table 1, the gene *Sr6*, when homozygous in a Marquis background, was ineffective at 24 C and above, whereas the heterozygous F_1 plants were susceptible at all six temperatures. In the glasshouse, plants with *Sr6* were much more resistant to strain 21-4,5, especially at temperatures of 15 to 18 C when this gene is dominant (10). Better light conditions in the glasshouse were probably responsible for this difference. *Sr8* when heterozygous was ineffective at high temperatures. The homozygous and heterozygous combinations between *Sr6* and *Sr8* produced lower infection types than those with the two components singly, again indicating additive gene interaction.

Gene interaction was also observed in the homozygous and heterozygous combinations between *Sr8* and *Sr9b*. At 30 C, the two single heterozygotes were susceptible, whereas the double heterozygote exhibited "2+3−" infection types.

The expression of the gene *Sr11* when homozygous was less influenced by genetic background than that of *Sr9b*. *Sr11* was also less affected by high temperatures, and by the temperature-background interactions (Table 1).

DISCUSSION.—Host genes conditioning resistance to *P. graminis tritici* can be grouped according to the stability of their expression under different environmental conditions. Extreme cases of thermal sensitivity are genes *Sr6* (10) and *Sr15* (20), whereas five genes (*Sr5, Sr8, Sr9b, Sr11*, and *Sr13*) condition similar infection types at temperatures normally encountered in our glasshouses. However, at constant temperatures of 30 C, *Sr5* in W2928 (Thatcher X Marquis[6]) conditioned a semisusceptible reaction only. This is the first report of thermal sensitivity concerning this gene. Aamodt (1) found *Sr5* to be completely dominant.

At the same constant temperature, Kanred *(Sr5)* and Reliance *(Sr5)* exhibited their characteristic immunity ("O" and "O;" infection type). This shows that high temperatures are unable to influence the resistant reaction expressed by *Sr5* in the genetic background of these two cultivars.

Furthermore, it was shown that when *Sr5* was transferred by several backcrosses to the "susceptible" genetic background of W2691 and W3498, it became largely ineffective. This clearly demonstrates that susceptible wheat cultivars could influence different segregation patterns in crosses with resistant cultivars possessing one or more major genes for resistance. Even in a favorable environment, the expression of such genes would be determined by the genetic background.

W2691 and W3498 were selected for susceptibility to rye stem rust, *Puccinia graminis* f. sp. *secalis*, and Sanghi & Luig (15) have shown that the resistance to this pathogen in wheat cultivars susceptible to wheat stem rust is due to many previously unknown genes. Since W2691 and W3498 are not only susceptible to certain strains of *P. graminis secalis* but also provide a susceptible genetic background for resistance genes to *P. graminis tritici*, it is suggested that the genes for resistance to *P. graminis secalis* act as modifiers of genes for resistance to *P. graminis tritici*. The effect of the genes for resistance to rye stem rust on reducing the spread of wheat stem rust in the field on cultivars which possess no factor for resistance to this pathogen has not been determined; however, several independent observations suggest that these may constitute one kind of nonspecific resistance as defined by Watson (19) or van der Plank's horizontal resistance (13).

In many host-pathogen interactions that showed temperature sensitivity, mesothetic infection types

TABLE 1. Infection types on homozygous and heterozygous lines and three cultivars of wheat when tested with strain 21-4,5 of *Puccinia graminis tritici* at six constant temperatures under artificial light

Genotypes	Temperature ± 1 C					
	15	18	21	24	27	30
Marquis	3+c[a]	3+c	3+c	3+c	3+	3+
Kanred (*Sr5 Sr5*)	0	0	0	0	0	0
Reliance (*Sr5 Sr5*)	0	0	0	0;	0;	0;
Sr5 Sr5 (W2928)	0-0;	0	0-0;	0-0;	0;1=	3cn
Sr5 sr5	0-0;	0	0	0;	;1=	3
Sr9b Sr9b (W2402)	23c	2-3c	2-3c	3-c	2+3-c	2+3c
Sr9b sr9b	2+3-	2+3+c	2+3+c	3+c	3+c	3+
Sr5 Sr5 Sr9b Sr9b	0	0	0	0	0	;2=
Sr5 sr5 Sr9b sr9b	0	0	0;	0;	;1=	X=
Sr13 Sr13 (W2931)	2	2-	2=2	2=2	2-	2
Sr5 Sr5 Sr13 Sr13	0	0	0	0	0;	;1-
Sr5 sr5 Sr13 sr13	0	0	0;	0		
Sr6 Sr6 (W2400)	;	1+3-cn	3cn	3+	3+	3+
Sr6 sr6	33+	33+	3+	3+	3+	3+
Sr8 Sr8 (W2401)	2-2	2-2	2-2	2-2	2-2	2
Sr8 sr8		22+	2+3+	2+3+	3+	3+
Sr6 Sr6 Sr8 Sr8	0;	;1-2-	2-	2=2–	2-	
Sr6 sr6 Sr8 sr8	2-2	2+3c	2+3c	2+3c	2+3c	3c3+
Sr8 Sr8 Sr9b Sr9b	2-	2-	2=2–	2=2–	2=2–	2-
Sr8 sr8 Sr9b sr9b	22+	22+	22+	2+3=	2+3-	2+3-
Sr11 Sr11 (W3015)	2≡2=	2≡2=	2≡2=	2≡2=	2≡2=	2=2–
Sr11 sr11	2=2-	2≡2=	2≡2=	2≡2=	2=2–	
W2691	3+	3+	3+	3+	3+	3+
W3498	3+	3+	3+	3+	3+	3+
Line A *Sr5 Sr5*	X+3-	X+3-	X+3	X+3	X+3	3
Line B *Sr9b Sr9b*	2+	2+	2+	2+3-	2+3-	3-
Line C *Sr11 Sr11*	2≡2=	2=	2=	2=	2=	2-

[a]Infection types are according to Stakman et al. (18). 0 = immune; 0; = nearly immune; 1 = very resistant; 2 = moderately resistant; 3 = susceptible; X = heterogeneous; ≡ and = indicate lower limits of type; + and – indicate variation within a given infection type; c = chlorosis; b = browning.

have been exhibited (6, 10). In this regard, *Sr5* resembles *Sr6* and *Sr15*. Although, at the average normal temperature range experienced in our glasshouse (21-24 C), the infection types of the three genes when tested with strain 21-4,5 are quite different ("O",";1++", and "X+3"), it now seems that these different phenotypes are due to different temperature requirements of the three genes. Thus, a labile mesothetic "X" infection type can be produced by all three genes; namely, by *Sr5* at constant 27-30 C, by *Sr6* at 18 C, and by *Sr15* at 15 C. Slight variations in temperature greatly influence the amount of necrosis produced in the three host-pathogen combinations, and this in turn changes the infection types. On the other hand, our studies suggest that the chlorotic "2" infection type conditioned by *Sr8, Sr9b, Sr11,* and *Sr13* is less influenced by high temperature. However, plants heterozygous for certain of these were rendered susceptible by the highest temperatures.

Additive gene interaction was found in a number of instances, especially when *Sr6* was involved. Such nonallelic interaction was described earlier (16). Since Marquis did not provide a fully susceptible background (Table 1), it was difficult to identify the effects of individual genes and to study interactions between them. To minimize the interference of the genetic background, near-isogenic lines in a susceptible background will have to be developed and employed in a diallel crossing program. At present, many genes have been transferred by backcrossing to W3498, and these lines should prove valuable for future studies on gene interaction.

The findings reported here might be applied in combining two resistance genes when the three appropriate cultures of the pathogen are unavailable. High temperatures could be used to inactivate one while selection is practized for the other. Simons (17) distinguished genetically different sources of oat crown rust resistance using environmental modifications such as high temperature and high nitrogen fertilization. Green & Johnson (4) separated foundation stock lines of Selkirk wheat employing the temperature sensitivity of *Sr6*. Under field conditions, cultivars having two or more genes conditioning moderate resistance normally exhibit a higher level of resistance to avirulent strains than is shown by those having the same genes present singly (11).

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