The Residual and Interactive Expressions of "Defeated" Wheat Stem Rust Resistance Genes

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ABSTRACT

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Seven near-isogenic lines of Chinese Spring wheat and Red Egyptian (CI 14181) representing all combinations of the resistance alleles *Sr*6, *Sr*8, and *Sr*9a were studied for their reaction to an isolate of *Puccinia graminis* f. sp. *tritici* race RKQQ that possesses virulence alleles corresponding to *Sr*6, *Sr*8, and *Sr*9a. The seven lines with one or more of the resistance genes significantly reduced pustule size and sporulation compared to the Chinese Spring control line. Lines with two resistance genes were more effective in reducing pustule size and sporulation than were lines with single resistance genes. The line with three resistance genes was more effective in reducing pustule size and sporulation than lines with two genes. We conclude that each of the three resistance genes has a residual expression when confronted by matching virulence genes.

Additional key words: disease resistance.

We have been working with powdery mildew of winter wheat for several years as a model to evaluate certain genetic strategies designed to manage plant diseases at some acceptable threshold level. Much of our research has utilized six of the near-isogenic winter wheat lines developed by Briggle (2) in a Chancellor background, each containing a different race-specific powdery mildew resistance gene (*Pm*). Working with a single isolate of *Erysiphe graminis* DC. f. sp. *tritici* E. Marchal possessing all the virulence genes needed to overcome the six resistance genes, Nass et al (6) determined that some of the "defeated" resistance genes expressed significant residual effects in the form of reduced infection and disease efficiency and in sporulation capacity. Royer (11) subsequently demonstrated that each of the six resistance genes exhibited residual effects against certain isolates and that the ability to express residuality was dictated by the interaction of the host and pathogen genes.

It has been hypothesized (7,8) that nonspecific or rate-reducing resistance could be obtained by combining a number of "defeated" race-specific resistance genes. Put another way, specific and nonspecific resistance are expressions of different actions of the same genes in different genetic backgrounds. To test that hypothesis, we bred four two-gene lines and one four-gene line, each containing different sets of *Pm* genes. When both of the *Pm* genes expressed residual effects against the test isolate of *E. graminis*, the two-gene lines significantly reduced lesion number or sporulation capacity when compared to the corresponding one-gene lines (9). The four-gene line was more effective than the two-gene line in reducing lesion number and sporulation (9).

It seemed natural to ask whether the phenomena that we observed with powdery mildew of winter wheat may occur in other diseases. Consequently, we obtained seeds of eight wheat lines representing all combinations of stem rust resistance genes *Sr*6, *Sr*8, and *Sr*9a. Our research with the wheat stem rust system is reported herein.

MATERIALS AND METHODS

The pathogen. One isolate of race RKQQ of *Puccinia graminis* Pers. f. sp. *tritici*, obtained from A. P. Roelfs, USDA Cereal Rust Laboratory, University of Minnesota, St. Paul, was used throughout the study. This isolate is virulent toward *Sr* genes 5, 6,

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7b, 8, 9a, 9d, 9f, 9g, 12, 14, 15, 16, 18, 19, 20, 21, 23, 28, 36, McN, Kt '2,' and BB, and it is avirulent toward *Sr* genes 2, 9e, 10, 11, 13, 17, 22, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 37, Tt-3, Tmp, Gt, dp-2, x, Wst-2, Wld-1, H, and U (10).

Inoculum for the studies was obtained by inoculating 12-day-old wheat (cultivar Chancellor) seedlings grown in sterilized soil in clay pots. The plants were inoculated by rubbing the leaves with a cotton swab moistened with a sterile distilled water suspension of urediospores of *P. g.* f. sp. *tritici*. Inoculated plants were placed in dark dew chambers for 24 hr and then transferred to a growth chamber maintained at 20 ± 2 C for 10 days to obtain an ample quantity of urediospores for making subsequent inoculations.

The host. Eight lines representing all combinations of stem rust resistance genes *Sr*6, *Sr*8, and *Sr*9a were used in this study. The line possessing all three *Sr* genes was a line of Red Egyptian (CI 14181) as shown by Sears et al (13). The relevant Chinese Spring (Red Egyptian) substitution lines were used by Loegering and Harmon (4) to develop lines homozygous for combinations of two genes for resistance. With the exception of Red Egyptian, all lines were in a cultivar Chinese Spring background. To quote from Loegering and Harmon (4), "In tests made with these materials it appeared that all behaved as expected; however, they cannot be considered isogenic." The main potential for the presence of different, undetected background genes would be on each of the three chromosomes in the substitution lines.

Inoculation. Seeds of the various wheat lines were planted in 12 × 16.5 × 6-cm plastic trays containing a mixture of soil, fertilizer, and peat moss (1:1:1, v/v), one line per tray. Ten seeds were planted in a single row parallel to the longest side of the tray. Primary leaves of 12-day-old seedlings were secured with masking tape over an 8 × 5 × 10-cm wire grid so that they would lie horizontally across it, exposing 8 cm of the adaxial surface of each leaf. Leaves were held in position by securing the tip of each leaf with a paper clip.

The trays were then placed in a simplified version of the Melching (5) settling tower. The inoculum consisted of a suspension of 3 mg of urediospores per milliliter of oil (Soltrol 170, Phillips Chemical Company, Borger, TX). This quantity of deposited inoculum is equivalent to ~150 urediospores per square centimeter of leaf. A DeVilbiss No. 15 nozzle attached to a vacuum pump (maximum 68.9 kP) was used to spray the inoculum into the top of the settling tower. The trays were rotated on a turntable at 4.5 revolutions per minute to permit uniform deposition of inoculum. The inoculated plants were incubated in a moist atmosphere in a darkened dew chamber at 20 C for 24 hr, then placed in a growth chamber at 20 ± 2 C, 50–60% relative humidity (RH), with a 12-hr photoperiod at a light intensity of approximately 14,000 lux. Ten plants per replicate of each of the eight lines were simultaneously inoculated in the settling tower. Data for pustule size and sporulation were recorded from four random leaves for each line per replicate. Each of the three experiments was designed as a randomized complete block with three replications.

Pustule size. Pustule size was measured 10 days after inoculation by a technique devised by D. R. MacKenzie (*unpublished*) and utilized by Castano (3). Pustule size was measured by using an Apple II Plus Computer (Apple Computer Inc., Cupertino, CA) fitted with a video camera and an analog-to-digital converter for calculation of irregular areas. This computer has as components a digisector board (DS-65 Digisector; The Micro Works, Del Mar, CA), a video camera, two video monitors, and a printer. Each pustule was measured with the computer system by using the following procedure: The pustule-bearing leaf was placed under the video camera and its image was transmitted to a video monitor via the computer and digisector board. The digisector digitized the image by assigning addressed pixels a numerical value within the range from 0 to 60. Thresholds were then set to differentiate the pustule from the "healthy" leaf background. The scan area (including the pustule) was chosen, and the computer scanned the area and compiled the frequency of all the shades of gray in the scan area and converted the shades of gray within the threshold to square millimeters.

Sporulation. Ten days after inoculation, sporulation data were obtained every 2 days for 8 days (four harvests) by removing the urediospores by vacuum suction from all pustules on the 8-cm section of adaxial leaf surface. Each experiment had three replicates of each line, with each replicate containing four leaves and 8–10 pustules per leaf. A bent glass tube, functioning as a nozzle, was inserted through a rubber stopper into the collection tube. Another glass tube, functioning as an exhaust outlet, was inserted through the rubber stopper and connected to a vacuum pump with a rubber hose. The collection tube contained 5 ml of 1.0% NaCl solution containing 0.1% Tween 80. The resulting spore suspensions were adjusted to a volume of 26 ml with a 1.0% NaCl solution, and 0.5-ml aliquots were counted with a model ZB Coulter counter (Coulter Electronics Industrial Division, Franklin Park, IL). The number of urediospores per 0.5-ml aliquot was converted to the mean number of urediospores per pustule per collection. Cumulative sporulation was obtained by summing the data from the four collection dates.

The data were analyzed and mean differences were determined by using Duncan's multiple range test, $P = 0.05$.

RESULTS

Pustule size. The infection type for all the lines was 3+, but pustules on the wheat line with *Sr*8 were significantly smaller than pustules on lines with *Sr*6 or *Sr*9a. The contribution of any combined two genes to pustule size was not statistically significant. The line with all three genes had significantly smaller pustules than lines with either single genes or pairs of genes. Moreover, pustules on the susceptible check were significantly longer than on all other lines.

The net quantitative contribution of gene effect on pustule size was calculated by subtracting the observed pustule size value of any of the single combinations from the size of those of the gene-deficient check (*sr*6, *sr*8, or *sr*9a) and then dividing by the susceptible check pustule size value. The net expected contribution of gene combination was calculated by summing the contributions of the values of single genes.

The expected net gene contribution of the three single genes in combination (*Sr*6 + *Sr*8 + *Sr*9a) to pustule size is less than the observed net contribution. It is interesting that the gene contribution of *Sr*6 and *Sr*9a in combination with *Sr*8 is close to additive (42.23 versus 38.01 for *Sr*6 + *Sr*8, and 44.34 versus 37.33 for *Sr*8 + *Sr*9a), whereas the combination of *Sr*6 + *Sr*9a was not additive (45.25 versus 26.92) as expected. The nonadditive gene contribution of either *Sr*6 and/or *Sr*9a is further expressed in the observed contribution of the three genes to pustule size. The observed net contribution of these three genes to pustule size is much higher (pustule size much smaller) than expected (65.16 versus 51.13).

Sporulation. The three genes were significantly different in their ability to suppress sporulation. The difference between the contribution of the combined two genes, *Sr*6 + *Sr*8, and *Sr*9a to sporulation was not statistically significant. The combination of the three genes produced significantly fewer spores per pustule from either single genes or any paired gene combination. The net quantitative contribution of gene effect on sporulation was calculated by subtracting the observed number of spores produced per pustule of any of the single combinations from the number of spores produced per pustule of the gene-deficient check (*sr*6, *sr*8, or *sr*9a) and then dividing by the number of spores produced per pustule on the susceptible check.

The expected net gene contribution to sporulation of the three single genes in any combination is higher than the observed net contribution. It is clearly expressed in the observed net combined contribution of these three genes to sporulation. The observed net contribution in this case is much lower than expected (76.76 versus 91.14).

DISCUSSION

The effect of genes for resistance in the host when matched by genes for virulence in the pathogen is expressed in quantitative

parameters such as the number of sporulating colonies and spore production. Nass et al (6) have shown that where a gene for matching virulence in *Erysiphe graminis* was present, the number of sporulating colonies and the spore production per lesion were significantly lower than expected. The lower-than-expected quantitative expression of the virulence-resistance interaction was termed residual effect, namely, a higher level of resistance expressed in quantitative parameters than expected. Residuality was considered to be the expression of partial resistance by a near-isogenic line with a particular *Pm*x gene, after having been overcome ("defeated") by a virulent isolate, compared to the recurrent parent with no known *Pm*x genes (6). The stability of the interaction was further verified by the repeated ranking of partial resistance in the lines tested when evaluated with different isolates than isolate 144 of *E. graminis*.

Residual effects on pustule size and spore production were observed in the present study where near-isogenic lines possessing various combinations of *Sr*6, *Sr*8, and *Sr*9a were inoculated with race RKQQ of *P. g.* f. sp. *tritici*.

Thus, specific resistance genes, which function in a qualitative manner by resisting some races but not others (though not tested in this study), also appear to function in a quantitative fashion to restrict disease increase.

The three host genotypes containing two *Sr* genes had significantly smaller pustules and produced lower numbers of spores per pustule than host genotypes with one *Sr* gene. Yet, the quantitative expression of each *Sr* gene on either of the measured components varied. The line possessing *Sr*8 singly was statistically different in pustule size from those with *Sr*6 and *Sr*9a, which did not differ from one another (Table 1). The sporulation abilities of the three single gene lines (*Sr*6, *Sr*8, and *Sr*9a) significantly differed from each other (Table 2).

Moreover, the combination of any two genes (*Sr*6 and/or *Sr*9a) in the presence of *Sr*8 resulted in nonsignificant pustule size differences but differed statistically in spore number. The greater residual effect attributed to *Sr*8 was not expressed in two-gene combinations as expected. Furthermore, the observed net gene contribution resulted in greater quantitative residual effect than expected, indicating a significant interacting effect between the three genes.

The three "defeated" *Sr* genes, either combined in pairs or all three combined, expressed higher residual resistance than expected for pustule size but not for spore production.

The question of interpreting the quantitative contribution of each "defeated" major gene to gene combination remained open. It is possible that the greater residual effect of combined "defeated" genes may indicate an interaction between the three *Sr* genes of unequal magnitude when single or indicate an interaction with additional undetected genes with the same isolate.

Anderson (1) suggested that residual effects in quantitative components may result from differences in the produced near-isogenic lines that "were not as nearly isogenic as intended." It is possible that quantitative resistance genes may have been transferred from the resistant parents to these lines along with the qualitative resistance genes. Anderson proposed that if such unidentified genes were transferred along with the genes for resistance, then the results reported in this publication may be due to such quantitative genes rather than to residual effects of *Sr*6, *Sr*8, and *Sr*9a, or to *Pm*3c, *Pm*4, and MA as reported by Nass et al (6). If the near-isogenic lines were not isogenic with respect to a certain quantitative parameter, then differences in partial resistance ("residual effect") may be expected due to differences in background (unidentified quantitative genes), as well as differences in isolate virulence. Royer et al (12) have shown that the near-isogenic line CI 14118 (*Pm*2 from Ulka) did not perform the same as CI 14119 (*Pm*2 from CI 12632). Their interpretation was that the differences in components of partial resistance between the two near-isogenic lines possessing the same resistance genes within a different background thus emphasize the importance of a gene's source in the expression of resistance as previously stated by Nelson (7), or due to linked modifiers, or unidentified multiple alleles of the gene in question (1).

TABLE 1. Mean pustule size produced by an isolate of *Puccinia graminis* f. sp. *tritici* race RKQQ, on eight wheat lines representing all combinations of stem rust resistance genes *Sr*6, *Sr*8, and *Sr*9a

Gene(s)	Pustule size[w,x] (mm^2)	Net gene contribution Expected[y]	Observed[z]
*sr*6 + *sr*8 + *sr*9a	4.42 a	...	...
*Sr*6	3.81 b	...	13.80
*Sr*8	3.35 c	...	24.21
*Sr*9a	3.84 b	...	13.20
*Sr*6 + *Sr*8	2.55 d	38.01	42.23
Sr6 + *Sr*9a	2.42 d	26.92	45.25
*Sr*8 + *Sr*9a	2.46 d	37.33	44.34
*Sr*6 + *Sr*8 + *Sr*9a	1.54 e	51.13	65.16

[w] Data are averages of nine replicates, each replicate containing four leaves and five pustules per leaf.

[x] Numbers followed by a common letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

[y] Net expected contribution was calculated by summing contributions of the values for single genes.

[z] Net observed contribution = $100\ (Ps - Pr)/Ps$ in which Ps = pustule size on the line with no resistance gene and Pr = pustule size on the line with the indicated resistance gene(s).

TABLE 2. Cumulative sporulation per pustule produced by an isolate of *Puccinia graminis* f. sp. *tritici* race RKQQ on eight wheat lines representing all possible combinations of stem rust resistance genes *Sr*6, *Sr*8, and *Sr*9a

Gene(s)	Cumulative number of spores produced per pustule[w,x]	Net gene contribution Expected[y]	Observed[z]
*sr*6 + *sr*8 + *sr*9a	132,236 a	...	...
*Sr*6	90,908 c	...	31.25
*Sr*8	101,608 b	...	23.16
*Sr*9a	83,668 d	...	36.73
*Sr*6 + *Sr*8	62,400 e	54.41	52.81
Sr6 + *Sr*9a	53,352 f	67.98	59.65
*Sr*8 + *Sr*9a	65,260 e	59.89	50.65
*Sr*6 + *Sr*8 + *Sr*9a	30.732 g	91.14	76.76

[w] Data are averages of nine replicates. In each replicate urediospores were collected from all pustules occurring on an 8-cm section of leaf surface from four leaves (8–10 pustules per leaf). Spores were collected four times at 2-day intervals beginning 10 days after inoculation.

[x] Numbers followed by a common letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

[y] Net expected contribution was calculated by summing contributions of the values for single genes.

[z] Net observed contribution = $100\ (Ss - Sr)/Ss$ in which Ss = number of spores produced per pustule on the line with no resistance gene and Sr = number of spores produced per pustule on the line with the indicated resistance gene(s).

"Defeated" resistance genes that are retained in a host population may thus contribute residual effects that may be beneficial in protecting the host against plant pathogens as shown in this study and by Nass et al (6) and Royer et al (12).

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