

Gene Action and Linkage of Avirulence Genes to DNA Markers in the Rust Fungus *Puccinia graminis*

P. J. Zambino, A. R. Kubelik, and L. J. Szabo

Cereal Disease Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1551 Lindig Ave., St. Paul, MN 55108.

Current address of P. J. Zambino: Forestry Sciences Laboratory, USDA Forest Service, North Central Forest Experiment Station, 5985 Highway K, Rhinelander, WI 54501-9128.

Current address of A. R. Kubelik: Department of Biology, Hiram College, Hiram, OH, 44234.

Accepted for publication 13 April 2000.

ABSTRACT

Zambino, P. J., Kubelik, A. R., and Szabo, L. J. 2000. Gene action and linkage of avirulence genes to DNA markers in the rust fungus *Puccinia graminis*. *Phytopathology* 90:819-826.

Two strains of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, were crossed on barberry, and a single F₁ progeny strain was selfed. The parents, F₁, and 81 F₂ progeny were examined for virulence phenotypes on wheat differential cultivars carrying stem rust resistance (*Sr*) genes. For eight *Sr* differentials, phenotypic ratios are suggestive of single dominant avirulence genes *AvrT6*, *AvrT8a*, *AvrT9a*, *AvrT10*, *AvrT21*, *AvrT28*, *AvrT30*, and *AvrTU*. Avirulence on the *Sr*; (*Sr* 'fleck') differential showed phenotypic ratios of approximately 15:1, indicating epistatic interaction of two genes dominant for avirulence. Avirulence on

Sr9d favored a 3:13 over a 1:3 ratio, possibly indicating two segregating genes—one dominant for avirulence and one dominant for avirulence inhibition. Linkage analysis of eight single dominant avirulence genes and 970 DNA markers identified DNA markers linked to each of these avirulence genes. The closest linkages between *AvrT* genes and DNA markers were between *AvrT6* and the random amplified polymorphic DNA marker crl34-155 (6 centimorgans [cM]) *AvrT8a* and the amplified fragment length polymorphism marker eAC/mCT-197 (6 cM) and between *AvrT9a* and the amplified fragment length polymorphism marker eAC/mCT-184 (6 cM). *AvrT10* and *AvrTU* are linked at distance of 9 cM.

Additional keywords: AFLP, avirulence gene nomenclature, molecular map, RAPD, *Triticum aestivum*, *T. durum*.

Puccinia graminis Pers. is a heteroecious rust fungus, with uredinial (asexual) and telial stages on cereal and forage grass species (Poaceae, tribe Hordeae) and pycnial and aecial stages on members of the barberry family (Berberidaceae). In the early part of the century, stem rust of wheat (*Triticum aestivum* L. em Thell. and *Triticum durum* L.), caused by *P. graminis* f. sp. *tritici*, was often the major limiting factor in North American wheat production. Disease losses have been greatly reduced by the introduction of wheat cultivars with stem rust resistance (*Sr*) genes and the near eradication of barberry (*Berberis vulgaris* L.), the alternate *P. graminis* f. sp. *tritici* host in the central Great Plains (37). The latter measure had two effects: it slowed the development of epidemics by reducing and delaying the arrival of inoculum to the fields, and it extended the useful life of wheat cultivars by minimizing sexual recombination in the rust fungus and the consequent development of races with virulence to new *Sr* gene combinations (38). Despite these measures, the evolution of *P. graminis* f. sp. *tritici* races with new virulence remains a challenge to wheat and barley production. New sexual races with new combinations of virulence still become established in the Great Plains; wheat stem rust race Pgt-QCC, which overcame 50 years of resistance in barley conferred by the barley T-gene (42), is a recent example. Furthermore, new patterns of virulence may originate in the asexually reproducing stem rust population during dikaryotic (func-

tionally diploid) growth on its wheat host through mutation and other asexual processes, allowing changes in virulence to *Sr* genes to accumulate in clonally related rust fungus lineages (40).

Understanding the function and expression of rust fungus genes that control stem rust avirulence is important for understanding genetic changes that allow stem rust strains to overcome *Sr* resistance genes. Current interpretations of the gene-for-gene model of host parasite interactions (11,13) suggest that resistance to rust and other fungal diseases is caused by host recognition of one or more fungal gene products (elicitors) encoded at avirulence gene loci (9,10,14,19,23). In support of this model, avirulence on most wheat *Sr* genes is expressed as a dominant or partially dominant trait (15,16,20-22,31,32,36,46). However, avirulence on at least one wheat stem rust resistance gene, *Sr9d*, has been reported to be recessive (15,16,21,22,32) and, in one study, to segregate as if recessive in some and dominant in other crosses of the stem rust fungus (20).

Detailed analysis of unusual segregation of avirulence and studies on the effects of mutation on avirulence in the flax rust fungus, *Melampsora lini* (Ehrenb.) Lév. (12,13,28,48,49); the wheat leaf rust fungus, *P. triticea* Erikss. (formerly considered part of the *P. recondita* complex [2]) (17,47,50); and the rice blast pathogen, *Magnaporthe grisea* (Hebert) Barr (8,9,26), have indicated that in other fungal pathosystems avirulence may frequently be under the control of two types of pathogen genes: avirulence (elicitor) genes and inhibitor genes that suppress or inhibit the host's phenotypic response to fungal avirulence gene products by various mechanisms (4,14,18,19,23). Elicitor and suppressor compounds have been isolated from apoplastic fluids of wheat leaves infected with the stem rust fungus (4). It is not, however, currently known to what extent race-specific virulence and avirulence in the wheat stem rust system is mediated by production or lack of production of race-specific or general elicitors versus

Corresponding author: L. J. Szabo; E-mail address: lszabo@cdl.umn.edu

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Publication no. P-2000-0526-01R

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2000.

mechanisms that suppress, compete with, or modify the effects of such elicitors.

Understanding the functions that avirulence alleles of the rust fungus have in compatible interactions is important for understanding the nature of host-parasite interactions in wheat stem rust and assuring that efforts to maintain resistance to sexual and asexual populations of the fungus will be effective. Towards this goal the following need to be determined: the number of fungal genes that control avirulence and virulence on different *Sr* genes, the dominant versus recessive expression of avirulence, and genetic linkage. This paper reports the development of an F₂ population of the wheat stem rust fungus and the expression and estimation of the number of segregating genes controlling avirulence on 10 host-resistance differentials. We report the first random amplified polymorphic DNA (RAPD) (54) and amplified fragment length polymorphism (AFLP) (53) markers linked to avirulence loci in *P. graminis* and propose a new system of nomenclature for defining avirulence genes. The F₂ population will be used as a basis to positionally clone avirulence genes and develop a genetic map of the *P. graminis* genome.

MATERIALS AND METHODS

Parental strains. Parents for genetic crosses were selected from stored uredinial isolates of *P. graminis* f. sp. *tritici* at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN, based on the following criteria: (i) differences in their avirulence versus virulence (*avr/vir*) phenotypes; (ii) their ability to complete the sexual cycle; and (iii) their ability to produce abundant teliospores on adult wheat plants. Each parental strain was obtained from a single uredinium, presumed to have originated from a single urediniospore. Parental strains P1 (CRL 78-21-BB463, race Pgt-DFBJ as designated according to the modified [42] system of Roelfs and Martens [43]) and P2 (CRL 75-36-700-3, race Pgt-SCCL) were from the sexually reproducing North American population of *P. graminis* f. sp. *tritici* and had recently arisen from natural or artificial infections on common barberry.

Crosses. Teliospores for inoculating barberry were obtained 28 to 30 days after injecting suspensions of urediniospores in sterile water into the immature boot (31) and between the leaf sheath and stem of 6-week-old plants of the susceptible wheat cvs. Morocco (PI 431591) and Line E (PI 357308). Teliospore dormancy was broken by cycling telia-laden straw between 7 and -4°C three times daily and soaking in distilled water two to three times a week. A small piece of the straw was sampled every 2 to 3 weeks to assess whether teliospores would germinate at 18°C (1). Once dormancy was broken (3 to 5 months), the straw was soaked in distilled water for 7 days at 4°C, blotted partially dry, and suspended over barberry plants in sealed chambers at 18°C with 12 h of light and 100% relative humidity (RH). Pycnia began to form on barberry leaves after 5 to 8 days, at which time the straw and the most heavily infected barberry leaves were removed. The humidity was then reduced to ambient conditions. To prevent self-fertilization, leaves bearing multiple pycnia that might enlarge and coalesce were removed approximately 5 to 6 days after immature aecia from overlapping pycnia were first noted.

A sterile toothpick moistened in sterile water was used to transfer pycniospores between pairs of well-isolated pycnia from the two parental strains to produce F₁ progeny. Selves from the single F₁ strain CRL ZX107-1 were recovered by inoculating barberry plants as mentioned above and harvesting aecia either from the confluent edge of pairs of adjacent pycnia that were well separated from other pycnia or from crosses between selected pycnia. Individual aecia from crosses and selves were cut from barberry leaves with surrounding uninfected leaf tissue before aecia had begun to shed aeciospores and held in individual petri plates at 4°C until the aecia matured.

To obtain uredinial strains, excised aecia were brought to room temperature at 100% RH, and loose aeciospores from individual aecia were dabbed onto primary leaves of a pot of 7-day-old susceptible plants (cvs. Morocco and McNair 701) that had been treated after emergence with maleic hydrazide to enhance uredinial development (45). After inoculation, pots were individually bagged in clear plastic and incubated at 20°C under 12 h of illumination for 3 to 7 days, then transferred to isolation chambers in a greenhouse. Urediniospores collected 14 to 21 days after inoculation were suspended in light mineral oil (Soltröl 170, Phillips Petroleum Company, Bartlesville, OK), and several dilutions of the spores were sprayed onto fresh pots of maleic hydrazide-treated wheat plants. Infection occurred in an 18°C dew chamber (41). To ensure purity of uredinial strains, each was derived from a single uredinial pustule presumed to originate from a single urediniospore. Up to three single pustule isolates were obtained per strain and were checked on wheat stem rust differentials for purity. Strains suspected of being a mixture of genotypes, based on either pathogenicity or DNA marker data and for which duplicate isolates were not available, were not used for analysis.

Infection phenotypes on differential cultivars. Forty-six wheat cultivars for which avirulence and virulence infection phenotypes (*avr/vir* phenotypes) could be readily differentiated were used as stem rust differentials (Table 1). Twenty-five F₂ progeny were used in initial tests to determine if the stem rust F₂ population segregated for *avr/vir* phenotypes on any of these differential cultivars. Subsequent tests used 81 F₂ progeny on a smaller set of differentials that included 10 *Sr* genes (*Sr*6, *Sr*8a, *Sr*9a, *Sr*9d, *Sr*10, *Sr*21, *Sr*28, *Sr*30, *Sr*; [*Sr* 'fleck'], and *Sr*U) on which the stem rust fungus F₂ population showed segregation for avirulence and *Sr* differentials that determine the standard 16-gene race designations used by the Cereal Disease Laboratory to categorize races of North American stem rust fungus (42). Because the *avr/vir* phenotypes of the parental, F₁, and F₂ strains used in this study all differ from prevalent North American races on several of these differentials, the purity of these strains was checked with each set of differentials.

The *avr/vir* phenotypes were judged by comparing uredinial pustule size on each differential cultivar with previously published reports of infection types for compatible (high-infection type) and incompatible (low-infection type) interactions (39,41,44). Differentials were inoculated as 7-day-old plants (not treated with maleic hydrazide) (44), using suspensions of urediniospores in mineral oil as previously described. After infection, plants were placed in a greenhouse at 18 to 28°C under 160 W VHO fluorescent tubes with a 12 h photoperiod. Infection types of 0 to 4 were assigned 14 to 21 days after inoculation based on the size and appearance of uredinia (34,43). Infection types of each strain were judged after comparing the results of two or more inoculations on sets of differential cultivars, with each replication inoculated on a different day and at least one replication grown in a separate greenhouse. Infection types of 0 (no observable infection), fleck (a small amount of necrosis without spore production), and 1, 2, and 3C (small uredinial pustules, often with chlorosis or necrosis) were considered avirulent (incompatible) phenotypes, while infection type 4 (large uredinial pustules lacking chlorosis and necrosis) was considered a virulent (compatible) phenotype. For a few rust fungus strains where fully compatible pustules developed slower than expected on McNair 701 and other standard differentials with known compatibility, infection types of size 3 to 3+ were equivalent to the fully compatible infection type 4.

DNA preparation and amplification. Urediniospores of *P. graminis* f. sp. *tritici* were germinated as described by Liu et al. (30). DNA was prepared as described by Kubelik and Szabo (24) from lyophilized mats of germinated urediniospores. RAPD analysis was performed using PTC-100 thermocyclers (MJ Research, Watertown, MA) as described by Kubelik and Szabo (24). RAPD oligonucleotide primer kits (10-mer) were purchased from Operon

Technologies (op) (Alameda, CA) and Genosys (gen) (The Woodlands, TX). Custom-designed RAPD primers (crl) with a guanine + cytosine content of 80 to 100% were as described (24). Amplification products were electrophoresed in 1.4% agarose gels containing ethidium bromide at 0.8 µg/ml. AFLP analysis was performed as described by Vos et al. (53) using a commercially available kit (Gibco BRL, Grand Island, NY) and primers with two base instead of three base extensions. Amplified products were separated in 5% Hydro-link Long Ranger acrylamide sequencing gels (FMC Bioproducts, Rockland, MD) using Sequi-Gen electrophoresis units (Bio-Rad Laboratories, Hercules, CA).

Data analysis. Chi-square values corrected for continuity (51, 55) were calculated to compare observed ratios of avirulent versus virulent phenotypes against Mendelian ratios of 3:1, 15:1, 1:3, and 3:13 that would be expected if avirulence phenotypes were controlled by a single dominant gene for avirulence, two dominant genes for avirulence, a single recessive gene for avirulence, or an epistatic interaction between a dominant gene for avirulence and a dominant gene inhibiting avirulence, respectively (Table 2). The methods of NaNagara (35) and Mather (33) were employed to distinguish whether data better fit a 1:3 or 3:13 ratio when neither ratio could be rejected on the basis of *P* value. Chi-square was also used to compare the ratio between presence and absence of markers among strains to a 3:1 ratio expected for dominant markers. In these chi-square analyses, *P* values identified the probability that even greater deviations from the test ratio than those observed could be expected by chance alone (51). *P* < 0.05 was selected as the level for rejecting the null hypothesis that data approximated a tested ratio.

The program Mapmaker, version 3.09 (Whitehead Institute for Biomedical Research, Cambridge, MA) (25) was used for multipoint linkage analysis of eight avirulence genes and 970 DNA markers. This set of DNA markers included 354 RAPD markers (348 dominant and 6 codominant markers) and 616 AFLP markers (607 dominant and 9 codominant markers). Each marker considered codominant satisfied three criteria: (i) there were no recombinant genotypes in repulsion (i.e., absence of both amplified products) identified in the *F*₂ population; (ii) each pair of amplified products was amplified by a single RAPD primer or AFLP primer pair; and (iii) at least one of the amplified products (allele) was in each of the two parents. Data was scored as an *F*₂ intercross and a threshold log₁₀ of the likelihood ratio (lod) score of 3.0 was used. Genetic distance, expressed in centimorgans, was calculated using the Haldane mapping function (6). Given the size of the *F*₂ population, all distance values (centimorgans) have been rounded to the nearest whole number. In addition, two-point recombinant fraction values (*r*) were calculated for *AvrT* loci and closely linked DNA markers from the same parent and between two linked *AvrT* loci. Chi-square was used to test the null hypothesis of random assortment for pairs of loci, with *P* < 0.05 selected as the level for rejecting the null hypothesis, indicating statistically significant linkage.

Avirulence gene and DNA marker designations. For discussion and mapping purposes, each single gene presumed to control an avr/vir phenotype has been designated with the prefix “*AvrT*” indicating avirulence on *Triticum* stem rust differential and a suffix corresponding to the name of an *Sr* gene published in McIntosh (34) and Roelfs and Groth (41). Rust fungus genes presumed to inhibit or suppress expression of avirulence are designated with a prefix of “*T*” plus the *Avr* abbreviation for the avirulence gene(s) whose avirulence phenotype is presumed to be inhibited—a convention previously used in the flax rust fungus (28). Each DNA marker has been designated by the name of the RAPD primer or AFLP primer pair used and the size(s) of the amplified fragment(s) to the nearest unit of 10 bp for RAPDs and single bases for AFLPs. For example, the RAPD marker opAJ18-090 is a 0.9-kb DNA segment amplified using the AJ18 primer from Operon, and codominant AFLP marker eAG/mCC-173/247 is a pair of

DNA segments of 173 and 247 bases amplified using primers *EcoRI*-AG and *MseI*-CC.

RESULTS

Genetic analysis of avir/vi phenotypes. To determine if any of the genes controlling the avirulence phenotype were segregating in the *P. graminis* f. sp. *tritici* *F*₂ population, we tested 25 *F*₂ progeny on 46 wheat stem rust differential cultivars (Table 1). These *F*₂ progeny showed segregation for avr/vir phenotype on 10 *Sr* differential cultivars but had a nonvariable avirulence phenotype on 22 *Sr* differentials and a nonvariable virulence phenotype on 14 additional *Sr* differentials. To obtain *F*₂ segregation data, we tested 81 *F*₂ progeny on a smaller set of differentials that included the 10 *Sr* genes on which the *P. graminis* f. sp. *tritici* *F*₂ population had shown segregation for avirulence.

Although different sizes of avirulent infection types have been suggested as an indication of whether avirulence alleles are present in the homozygous versus heterozygous state (39,41), the differences are often minute. In some cases intermediate infection types were observed, which may represent the heterozygotes. However, there was enough variation to make it difficult to reliably score the intermediate classes. Therefore, we categorized infection phenotypes of *P. graminis* f. sp. *tritici* strains into only two classes: fully compatible interactions that produced large uredinial pustules, indicating virulence phenotypes versus interactions that were not fully compatible and produced smaller and more chlorotic or necrotic pustules, indicating avirulence phenotypes (39,41,44).

On most *Sr* differentials, the avirulence and virulence phenotypes of parents, *F*₁, and *F*₂ progeny fit patterns that would be expected if the avirulence phenotype were dominant, e.g., when an *F*₁ was virulent, all *F*₂ progeny were virulent, and when an *F*₁ was avirulent, *F*₂ progeny would either be avirulent or segregate for avirulence (Table 1). Exceptions were found with the *Sr9d* and *SrTt-3* differentials. On *Sr9d*, the *F*₁ was virulent but the *F*₂ progeny segregated for avr/vir phenotypes. On *SrTt-3*, parent P2 was virulent, but parent P1, *F*₁, and *F*₂ progeny were all avirulent. In both of these cases, the data suggests the possibility of a dominant gene, that suppresses the expression of avirulence. For example, the data indicate that parent P2 was heterozygous for a dominant suppressor of avirulence on *SrTt-3*, and the *F*₁ is homozygous for the recessive allele of this gene.

Segregation data from eight differentials supported 3:1 avr/vir phenotype ratios, indicating eight single *P. graminis* f. sp. *tritici* avirulence genes with dominant gene expression (*AvrT6*, *AvrT8a*, *AvrT9a*, *AvrT10*, *AvrT21*, *AvrT28*, *AvrT30*, and *AvrTU*; Table 2). Segregation data from the *Sr*; (*Sr*“fleck”) differential supported a 15:1 ratio (avr/vir) (*P* > 0.8), but not a 3:1 ratio (*P* < 0.05), indicating the likelihood that two dominant unlinked *P. graminis* f. sp. *tritici* avirulence genes interact epistatically to control phenotype on the *Sr*; differential cv. 8N221*Sr*;RHR. For segregation data from the *Sr9d* differential, chi-square tests of 1:3 (one recessive gene conditioning avirulence) and 3:13 ratios (epistatic interaction of one gene dominant for avirulence and one gene dominant for suppression of avirulence) gave *P* values of 0.19 and 0.95, respectively. Since the results from the two ratios did not reach the *P* < 0.05 needed to reject the null hypothesis, additional tests were used to determine whether the data better fit a 1:3 or 3:13 ratio. Although the *F*₂ population size is not large enough to unequivocally differentiate between these hypotheses, both Mather’s (33) “ambiguous ratio” criterion and NaNagara’s (35) “dividing line” favor the 3:13 ratio over the one gene 1:3 ratio.

Identification of DNA markers linked to avirulence genes. To identify DNA markers linked to the eight single dominant avirulence genes segregating in this *F*₂ population, RAPD and AFLP analyses were employed. Using 694 RAPD oligonucleotide primers (620 op, 40 gen, and 34 crl) yielded 360 reproducible amplification products that were detected in one or both of the

parents and segregated in the F₂ population. In addition, 21 AFLP primer pairs produced 625 polymorphic amplification products that segregated. In this set of RAPD and AFLP amplification products, 30 were identified that behaved as pairs of alleles to 15 codominant loci and were scored as codominant markers. Twenty-nine percent of the segregating DNA markers were found in both parents, indicating that at least one of the parents was heterozygous for each of these markers. This heterokaryotic nature was also observed with respect to the avirulence loci (Table 2) and indicates the high level of genetic heterogeneity that may be obtained between the two nuclei in the dikaryotic asexual stage of this fungus.

Linkage analysis of the 970 DNA markers and 8 avirulence genes identified DNA markers linked to each of the avirulence genes (Fig. 1). The closest DNA markers to avirulence genes are

cr134-155, 6 cM from *AvrT6*; eAC/mCT-197, 6 cM from *AvrT8a*; and eAC/mCT-184, 6 cM from *AvrT9a* (Table 3). Two avirulence genes, *AvrT10* and *AvrTU*, were linked (9 cM). Occasional minor differences in distance values given in Table 3 versus those given in Figure 1 are due to differences in methods of calculating distances. Two-point linkage analysis was used for data in Table 3, but multipoint linkage analysis was used for data in Figure 1. For example, the distance between the RAPD marker cr134-155 and *AvrT6* is 5 cM on the genetic linkage map (Fig. 1) and 6 cM in the linkage data (Table 3).

For three of the avirulence loci (*AvrT6*, *AvrT28*, and *AvrT30*), each of the parents carry at least one avirulent allele (Table 2). This provides an opportunity to test whether the closest linked DNA marker provides a useful tag for these avirulence genes rather than an indication of parentage. In the case of the *AvrT6*

TABLE 1. Avirulence and virulence phenotypes of parental, F₁, and F₂ progeny strains from a cross of *Puccinia graminis* f. sp. *tritici* on wheat stem rust differential cultivars

Sr gene ^a	Cultivar	Accession no. ^b	Phenotypes of strains ^c			
			P1	P2	F ₁	F ₂
Sr5	LCSr5R1	CRL 1-1	Avr	Vir	Vir	Vir
Sr6	Isr6-Ra, W2691Sr6	Cltr 14163, CRL 1-22	Avr	Avr	Avr	SEG
Sr7b	Isr7b-Ra	Cltr 14165	Avr	Avr	Avr	Avr
Sr8a	Isr8-Ra	Cltr 14167	Vir	Avr	Avr	SEG
Sr8b	Barletta Benvenuto	Cltr 14196	Vir	Avr	Vir	Vir
Sr9a	Isr9a-Ra	Cltr 14169	Avr	Vir	Avr	SEG
Sr9b	W2691Sr9b	Cltr 17386	Avr	Avr	Avr	Avr
Sr9d	Isr9d-Ra	Cltr 14177	Vir	Avr	Vir	SEG
Sr9e	Vernal	Cltr 3686	Vir	Vir	Vir	Vir
Sr9g	CnsSr9g	CRL 5-63	Vir	Vir	Vir	Vir
Sr10	W2691Sr10	Cltr 17388	Vir	Avr	Avr	SEG
Sr11	Isr11-Ra	Cltr 14171	Avr	Avr	Avr	Avr
Sr13	W2691Sr13	Cltr 17387	Avr	Avr	Avr	Avr
Sr14	W2691Sr14, Line A sel.	CRL 5-187, CRL 5147B	Avr	Avr	Avr	Avr
Sr15	W2691Sr15 Nk	CRL 1-13	Vir	Vir	Vir	Vir
Sr18	LCSr18 RI	CRL 5-20	Vir	Vir	Vir	Vir
Sr20	LCSr20Mq, LCSr20 RI	CRL 5-22, CRL 5-46	Vir	Vir	Vir	Vir
Sr21	Einkorn	Cltr 2433, W 3586	Avr	Vir	Avr	SEG
Sr22	SwSr22T.B.	CRL 3-21	Avr	Avr	Avr	Avr
Sr24	BtSr24Ag	CRL 3-22	Avr	Avr	Avr	Avr
Sr25	LCSr25Ars	Cltr 17474	Avr	Avr	Avr	Avr
Sr26	Eagle (A)	PI 365582	Avr	Avr	Avr	Avr
Sr27	WRT 238-5	Cltr 14141	Avr	Avr	Avr	Avr
Sr28	W2691Sr28Kt	CRL 1-20	Avr	Avr	Avr	SEG
Sr29	Pusa/Etirole de Choisy	CRL 5-55B	Avr	Avr	Avr	Avr
Sr30	BtSr30Wst	PI 442897	Avr	Avr	Avr	SEG
Sr31	Line E/Kavkaz	CRL 5-161B	Avr	Avr	Avr	Avr
Sr32	Er5155	CRL 5-203	Avr	Avr	Avr	Avr
Sr33	Tetra Canthatch	RL 5405	Avr	Avr	Avr	Avr
Sr35	Mq(2)5XG2919	CRL 5-234	Vir	Avr	Vir	Vir
Sr36	W2691SrTt-1	Cltr 17385	Avr	Avr	Avr	Avr
Sr37	W2691SrTt-2	CRL 5-171B	Avr	Avr	Avr	Avr
Srdp 2	Media Ag9d	Cltr 3255	Avr	Avr	Avr	Avr
Sr;	8N221Sr;RHR	CRL 8-80	Avr	Avr	Avr	SEG
SrGt	BtSrGtGt	CRL 3-5	Avr	Avr	Avr	Avr
SrH	H44 deriv.	CRL 5-167	Avr	Avr	Avr	Avr
SrKt2	Line AE sel.	CRL 1-20	Vir	Vir	Vir	Vir
SrLC	Little Club	Cltr 4066	Vir	Vir	Vir	Vir
SrMcN	McNair 701	Cltr 15288	Vir	Vir	Vir	Vir
SrPI	Peliss	Cltr 1584	Vir	Vir	Vir	Vir
SrPt	Peterson ML68-14	PI 355090	Vir	Vir	Vir	Vir
SrTmp	Triumph 64	Cltr 13679	Avr	Avr	Avr	Avr
SrTt-3	Federation *2/SrTt-3	CRL5-64D	Avr	Vir	Avr	Avr
SrU	SrUAc	CRL 5-182	Vir	Avr	Avr	SEG
SrWld	BtSrWld-1	CRL 3-25	Avr	Avr	Avr	Avr
SrWst-2	LcSrWst-2Wst	CRL 5-228	Vir	Vir	Vir	Vir

^a Sr = Stem rust resistance.

^b Original sources for stored derivative lines used as differentials: CRL = USDA, ARS, Cereal Disease (Rust) Laboratory, St. Paul, MN; Cltr and PI = USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network, National Germplasm Resources Laboratory (Beltsville, MD); W = Plant Breeding Institute, The University of Sydney, Sydney, Australia; RL = Agriculture Canada, Winnipeg.

^c Parental strains: P1 = CRL 78-21-BB463, P2 = CRL 75-36-700-3. F₁ strain = CRL ZX107-1. Avirulence (Avr) and virulence (Vir) determined by comparison with previously reported compatible and incompatible infection types (39,41,44). SEG indicates mixtures of avirulent and virulent phenotypes among the F₂ progeny strains.

locus, the closest DNA marker, crl34-155 (6 cM), was also found in both parents (Table 3). This was also true for the *AvrT28* locus, in that both parents carry the DNA marker eAC/mTA-107 (9 cM). However, in the case of the *AvrT30* locus, the closest DNA marker (eAC/mCT-448, 12 cM) is found in only one parent (P2). Although these DNA markers (crl34-155 and eAC/mCT-488) are not very tightly linked to the corresponding avirulence genes, it is possible that these markers may be useful for determining the presence of avirulent alleles for *AvrT6* and *AvrT28* loci.

Overall, the dominant DNA markers and the individual alleles of the codominant markers showed normal segregation. A frequency distribution, plotting the individual segregation ratios for all of the dominant DNA markers approximated a monomodal curve fitting the expected 3:1 ratio (data not shown). Approximately 16% of the markers (42 RAPDs and 113 AFLPs) showed segregation ratios that fell within the tails of the curve and would be rejected at the $P < 0.05$ level for fitting a 3:1 ratio. Linkage analysis of these markers failed to identify any specific regions of the genome that showed distorted segregation. In addition, none of the markers having skewed segregation ratios showed linkage to genes conditioning avirulence on *Sr9d* and *Sr*; differentials.

DISCUSSION

In this study, we developed a segregating F_2 population for *P. graminis* f. sp. *tritici* consisting of 81 F_2 progeny of a single cross. Of the 10 avr/vir phenotypes segregating on *Sr* differentials, segregation analysis indicated that 8 were due to single, dominant *AvrT* genes (Table 2). Thus, the results of this study support observations that avirulence is dominant for most *P. graminis* f. sp. *tritici* genes involved in avirulence. We confirmed previous reports of dominant avirulence for each of the following genes: *AvrT6* (as avirulent on Eureka [22,32]); *AvrT8a* (synonym avirulence on *Sr8* [15,16,22]); *AvrT9a* (15,16,22); *AvrT10* (15); and *AvrT21* (synonym avirulence on Einkorn [Citr2433]; 20,31,32). *AvrT28*, dominant for avirulence in this study, may have been referred to by Loegering and Powers (31) when they mentioned two dominant genes for avirulence detected in progeny of a rust cross using the differential cv. Kota (Citr5878), a cultivar later determined to carry resistance genes *Sr28* and *Sr'Kt2'*. Dominant avirulence of *AvrT30* and *AvrTU* had not been previously documented.

A 15:1 avr/vir segregation ratio indicated that two dominant unlinked genes control avirulence on *Sr*; (*Sr* 'fleck') differential cv. 8N221Sr;RHR. This most likely indicates that the *Sr*; differ-

ential carries two or more *Sr* genes that have not been previously differentiated—a problem frequently encountered in the development of stem rust differentials (31). The *Sr*; differential was derived from a cross between 'Kenya 58' (Citr12471) and susceptible cv. Baart (PI5078) but had not been selected as a single gene line (A. P. Roelfs, retired, *personal communication*). However, an alternative explanation is that the gene products of two unlinked *P. graminis* f. sp. *tritici* avirulence loci are recognized by the gene product of a single resistance gene (*Sr*;) and are acting as elicitors of the host resistance response. If the latter is true, there are two possibilities: (i) there could be duplicate, unlinked copies of an avirulence gene in *P. graminis* f. sp. *tritici* mediating host recognition, or (ii) two different unlinked avirulence genes could each be producing an elicitor that is recognized by the *Sr*; gene product. The exact nature of the *AvrT* genes controlling avirulence on the *Sr*; differential will remain unknown until the two genes are studied separately.

The observed ratio of avirulent to virulent phenotypes that was obtained on *Sr9d* (synonyms *Sr1* resistance and resistance of Arnautka [Citr1493], Mindum [Citr5296]), and Spelmar [Citr6236]; [34]) was of special interest, because it provides evidence that avirulence and inhibitor genes might interact to control avir/vir phenotypes in *P. graminis*. Segregation on this *Sr* differential does not fit the 3:1 ratio expected of a dominant avirulence gene but fits 3:13 and 1:3 ratios, with a very close approximation to the 3:13 ratio. There are several possible explanations for the data: (i) two genes are segregating, one dominant for avirulence (an *AvrT* gene) and one dominant for the suppression of avirulence (an *IAvrT* inhibitor gene); (ii) a single dominant gene for suppression of avirulence (an *IAvrT* inhibitor gene) is segregating in a background fixed for avirulence; and (iii) a single avirulence gene that is genuinely recessive for avirulence is segregating. Distortions in segregation caused by linkage to lethal mutations or mating type genes might have caused a single gene, suggested in hypotheses two and three, to appear to fit a 3:13 two-gene ratio. However, regions of linkage having distorted segregation have not been identified in the current study. This and the very close approximation of the data to the 3:13 ratio suggest that the most likely explanation for the observed pattern of avir/vir phenotypes on *Sr9d* is the two-gene (avirulence gene plus inhibitor gene) hypothesis. Selves of the avirulent F_2 progeny or backcrosses will need to be done to unequivocally determine the number and type of genes that control this avirulence phenotype.

Previous studies provide additional, albeit weak, support for an inhibitor gene affecting avirulence on the *Sr9d* gene. In one study,

TABLE 2. Segregation of avirulence and virulence phenotypes (avr and vir respectively) in a cross of *Puccinia graminis* f. sp. *tritici*

<i>Sr</i> gene ^a	Infection types of strains ^b					Analysis of Segregation Ratios in F_2 ^c				
	P1	P2	F_1	F_2 avr	F_2 vir	Observed Ratio (avr/vir)	Expected Ratio (avr/vir)	χ^2	<i>P</i>	Model for avirulence
<i>Sr6</i>	0-;1	0-;1	0-;1	0-;1 ⁺	4	58:21	3:1	0.038	0.867	<i>AvrT6</i>
<i>Sr8a</i>	4	2 ⁻ 2	;1-2	1-2 ⁺	(4 ⁻)4	55:19	3:1	0.000	1.000	<i>AvrT8a</i>
<i>Sr9a</i>	2 ⁻ 2	4	;2-2	1-2	4	54:24	3:1	1.094	0.245	<i>AvrT9a</i>
<i>Sr9d</i>	4	;2	4	;2	4	14:64	1:3	1.709	0.194	1 gene
							3:13	0.001	0.975	2 genes
<i>Sr10</i>	4	0-13c	;31c	;31c	4	53:24	3:1	1.251	0.268	<i>AvrT10</i>
<i>Sr21</i>	1-2	4	2-2 ⁺	1 ⁻ 2	(3c)4	63:14	3:1	1.562	0.213	<i>AvrT21</i>
<i>Sr28</i>	4	0-0;	0-0;	0-0;	4	60:19	3:1	0.004	0.949	<i>AvrT28</i>
<i>Sr30</i>	2 ⁻ 2	2c-23	2 ⁻ 2	1-2 ⁺	4	63:17	3:1	0.417	0.528	<i>AvrT30</i>
<i>SrU</i>	4	;1c-23c	2c-23c	;23c	4	55:19	3:1	0.000	1.000	<i>AvrTU</i>
<i>Sr</i> ; ('fleck')	;2-23	2 ⁻ 2 ⁺	;2	;2 ⁺	(3c)4	74:6	15:1	0.053	0.844	2 genes

^a *Sr* = stem rust resistance.

^b Parental strains: P1 = CRL 78-21-BB463, P2 = CRL 75-36-700-3; F_1 strain: ZX107-1. Infection types are according to Roelfs (39), Roelfs and Groth (41) and Roelfs and McVey (44). Infection type: 0 = no observable symptoms; ; (i.e. 'fleck') = small amount of necrosis without spore production; 1, 2, and 3 = pustules of increasing size that produce urediniopores with or without chlorosis (c); 4 = fully compatible infection type with large uredinial pustules lacking chlorosis and necrosis. +, -, and = indicate pustule sizes slightly larger and smaller than standard infection types, respectively. Parentheses indicate rare infection types.

^c Chi-square values corrected for continuity (51,55). *P* value represents probability of greater chi-square. The null hypothesis (that the ratio is correct) is rejected at $P < 0.05$. Only ratios that cannot be rejected by chi-square analysis are presented.

small numbers of progeny were obtained from each of four selfed strains (20). Segregation for avirulence on *Sr9d* from three strains appeared to approximate a 1:3 ratio expected for the recessive avirulence phenotype, but the fourth approximated a 3:1 ratio for the dominant avirulence phenotype, indicating segregation of a second gene. Other genetic studies suggested that the avirulence phenotype on *Sr9d* was a recessive trait controlled by a single gene (15,16,21,32). Kao and Knott (22) suggested that avirulence on cv. Mq-Sr1, which carries *Sr9d* and another uncharacterized resistance gene, was a recessive trait controlled by two genes. In each case, the observed ratio of 1 avirulent/3 virulent could be explained by either a dominant inhibitor of avirulence or a recessive avirulence gene.

The discovery of inhibitor genes in fungal pathosystems has provided new insights into host-parasite interactions at a molecular level for some pathosystems. Inhibitor genes that control avirulence have been demonstrated in wheat leaf rust (17,48,50), flax rust (12,13,27,49), and rice blast disease caused by the ascomycetous fungus *Magnaporthe grisea* (8,9,26). For example, it has been postulated that there are separate inhibitor genes in *M. grisea* for each of 17 avirulence loci (9) and that some inhibitor genes in the flax rust fungus (28) and the wheat leaf rust fungus (47) affect the phenotypic expression of several avirulence genes. It is likely that there are genes that inhibit the expression of avirulence in *P. graminis*. However, the high frequency with which avirulence behaves as a single dominant trait in *P. graminis*

indicates that inhibitor genes may be more rare or affect fewer avirulence genes than in other fungi.

The seven genetic linkage groups, containing 52 DNA markers and 8 avirulence genes, represent the first partial genetic map of *P. graminis*. These seven linkage groups cover only a portion of the 18 chromosomes contained in the haploid genome (5), which is estimated to contain 67 million base pairs (3). Development of a complete genetic map has been hindered by several factors, including the heterokaryotic nature of the asexual uredinial stage of *P. graminis* and the dominant nature of the markers scored. To complete this genetic map, a polymerase chain reaction-based, codominant marker system such as simple sequence length polymorphisms or single nucleotide polymorphisms will need to be developed. We have begun to clone and characterize RADP and AFLP markers linked to these eight avirulence genes, which will be used to develop codominant markers and physical maps of these regions. Given the difficulty of making crosses, it is unlikely that a significantly larger F_2 population will be generated. Therefore, the exact location of the avirulence genes on a physical map may depend on characterization of induced virulent mutants as well as transformation experiments with cloned segments of *P. graminis* f. sp. *tritici* DNA.

Though most avirulence genes did not appear to be linked to each other, *AvrT10* and *AvrTU* were linked at ≈ 9 cM. Green (15) previously suggested that both *AvrT7a* and *AvrT11* (as virulence to cv. Lee) are also linked to *AvrT10*, although this could not be

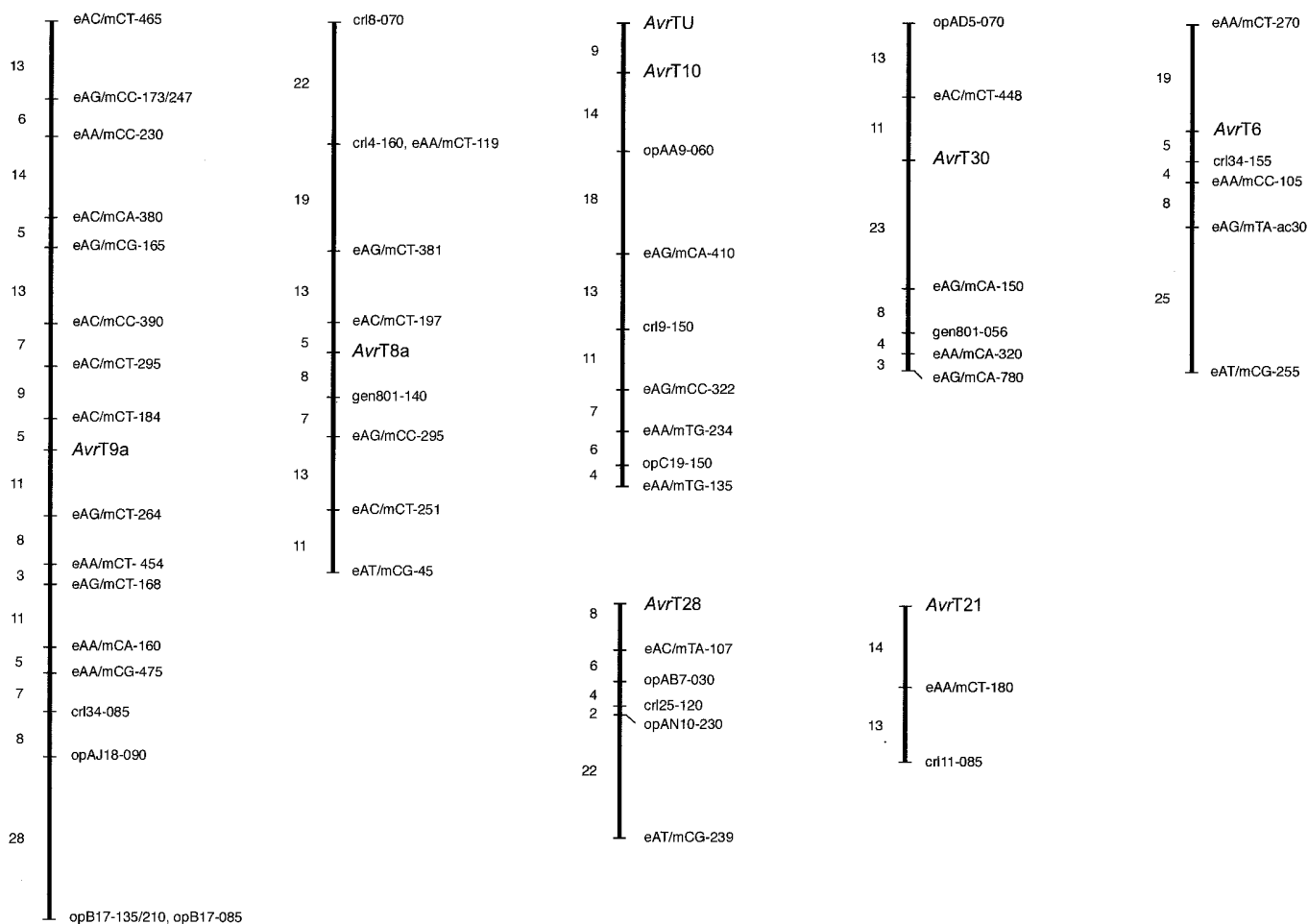


Fig. 1. Genetic maps of chromosomal regions containing avirulence genes *AvrT6*, *AvrT8a*, *AvrT9a*, *AvrT10*, *AvrT21*, *AvrT28*, *AvrT30*, and *AvrTU*. Segregation data were obtained from an F_2 intercross of *Puccinia graminis* f. sp. *tritici*. Markers are indicated on the right; distances between markers (in centimorgans) are indicated on the left. Markers prefixed with op, gen, and crl indicate random amplified polymorphic DNA (RAPD) markers obtained using RAPD primer kits from Operon and Genosys, and primers designed at the Cereal Disease Laboratory (24), respectively. Markers prefixed with eNN/mNN (e.g., eAG/mCG) indicate amplified fragment length polymorphism markers obtained with *EcoRI* and *MseI* primers with two base extensions. Mapmaker (25) version 3.09 was used for multipoint linkage analysis.

tested in this study. Genetic linkage, with distances of 20 cM or more (7,29,52), has been shown between avirulence genes in *Magnaporthe grisea*. Linkages between avirulence genes have also been observed in *Melampsora lini*, which in some cases are tightly linked (28), indicating the possibility that avirulence genes in fungal genomes, like resistance genes in plants, may be clustered. At present only a few of the more than 60 wheat stem rust avirulence genes have been genetically analyzed. Therefore, the distribution of these genes within the *P. graminis* genome remains unknown.

We have utilized a new system of nomenclature to provide a uniform and flexible system for naming and mapping genes involved in avirulence in all formae speciales of *P. graminis* and fostering discussions of these genes. In this system, avirulence genes are named using a four letter prefix that indicates avirulence on a particular host, such as 'AvrT', 'AvrS', 'AvrH', and 'AvrA' for wheat (*Triticum*), rye (*Secalis*), barley (*Hordeum*), and oats (*Avena*), respectively, and a suffix that indicates the name of the stem rust resistance gene on which compatibility is assessed. In cases where a resistance gene has its origin in another host genus, e.g., *Sr27* and *Sr31*, translocated from rye (34), the prefix would indicate the host in which compatibility is being assessed, rather than its host of origin, i.e., *AvrT* instead of *AvrS*.

A second feature of the proposed system is that it can be used to provide different names for two types of rust fungus genes that may condition avr/vir in race-specific interactions: (i) avirulence (*Avr*) genes that may be presumed to cause avirulence by their production of an elicitor of a host resistance response and show evidence of dominant or partially dominant gene expression of avirulence; and (ii) inhibitor (*I*Avr) genes that inhibit or suppress avirulence conditioned at one or more *Avr* loci, whether the *Avr* locus whose effect it moderates is itself fixed or variable for avirulence in the rust fungus population. Such inhibitor genes might be variously and tentatively identified in the stem rust fungus by dominant expression of avirulence suppression, loss of such suppression or inhibition after mutation, or crosses that prove epistatic interactions with *Avr* genes. Providing a prefix of *I* before the list of *Avr* designations for the avirulence gene(s) whose avirulence phenotype(s) are being inhibited follows current nomenclature in the flax rust system in which avirulence suppression was first discovered (28).

Inasmuch as the proposed system accepts the proposition that more than one rust fungus gene may affect the avr/vir phenotype on an *Sr* gene stem rust differential, it is in contrast to the previous gene naming systems for stem rust that are based on a strict gene-for-gene hypothesis that refers to *P. graminis* genes that control

avr/vir phenotypes as *P* genes or pathogen genes (22,31,39,41,46). The ability of the new system to consolidate information from crosses of the rust fungus originating from the same or different host genera and to recognize potential elicitor versus inhibitor genes will allow many genes involved in avirulence to be incorporated into the same genetic map. The system, thus, may provide a platform for discussion of whether rust fungus genes can condition avirulence in more than one host genus.

In conclusion, this study has demonstrated that of the rust fungus genes affecting the avr/vir phenotype in wheat stem rust, most are dominant for avirulence and unlinked to each other. Moreover, the set of materials provided by this study will be useful for investigations of the genetic control of avirulence phenotypes in the host-parasite interactions of the wheat stem rust fungus, *P. graminis* f. sp. *tritici*. The F₂ intercross population, RAPD and AFLP markers may enable cloning and examination of gene structure of up to eight avirulence loci that may eventually provide answers to questions of how the gene products of avirulence loci function in the fungus and elicit resistance in the host.

ACKNOWLEDGMENTS

We thank A. P. Roelfs for his many suggestions and his help in determining infection types on differential wheat cultivars. We also thank A. Balmuth, D. Butler, A. Krohn, J. C. Mork, and T. Seeland for their technical assistance. Published as paper 981220052 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

LITERATURE CITED

- Anikster, Y. 1986. Teliospore germination in some rust fungi. *Phytopathology* 76:1026-1030.
- Anikster, Y., Bushnell, W. R., Eilam, T., Manisterski, J., and Roelfs, A. P. 1997. *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats, and rye. *Can. J. Bot.* 75:2082-2096.
- Backlund, J. E., and Szabo, L. J. 1993. Physical characteristics of the genome of the phytopathogenic fungus *Puccinia graminis*. *Curr. Genet.* 23:89-93.
- Beissman, B., Engels, W., Kogel, K., Marticke, K.-H., and Reisener, H. J. 1992. Elicitor-active glycoproteins in apoplastic fluids of stem-rust-infected wheat leaves. *Physiol. Mol. Plant Pathol.* 40:79-89.
- Boehm, E. W. A., Wenstrom, J. C., McLaughlin, D. J., Szabo, L. J., Roelfs, A. P., and Bushnell, W. R. 1992. An ultrastructural pachytene karyotype for *Puccinia graminis* f. sp. *tritici*. *Can. J. Bot.* 70:401-413.
- Crow, J. F., and Dove, W. F. 1990. Anecdotal, historical and critical commentaries on genetics. *Genetics* 125:669-671.
- Dioh, W., Tharreau, D., Nottoghem, J. L., Orbach, M., and Lebrun, M.-H. 2000. Mapping of avirulence genes in the rice blast fungus, *Magnaporthe grisea*, with RFLP and RAPD markers. *Mol. Plant-Microbe Interact.* 13:217-227.

TABLE 3. Genetic linkage analysis of DNA markers and avirulence genes

Avirulence gene	Marker gene ^a	r ^b	Distance (cM)	(χ ²) ^c	P	lod score ^d	P1	P2
AvrT6	crl34-155	0.05	6	57.7	3.1 E -14	12.8	+ / Avr ^e	+ / Avr
AvrT6	eAA/mCC-114	0.07	12	32.3	1.3 E -8	6.5	- / Avr	+ / Avr
AvrT8a	eAC/mCT-194	0.05	6	48.0	4.3 E -12	10.8	- / Vir	+ / Avr
AvrT8a	gen801-140	0.08	9	40.0	2.5 E -10	9.5	- / Vir	+ / Avr
AvrT9a	eAC/mCT-182	0.06	6	50.6	1.1 E -12	12.9	+ / Avr	- / Vir
AvrT9a	eAG/mCT-264	0.09	11	40.8	1.7 E -10	9.3	+ / Avr	- / Vir
AvrT10	AvrTU	0.09	9	39.3	3.6 E -10	10.2	Vir / Vir	Avr / Avr
AvrT10	opAA9-060	0.12	15	36.1	1.9 E -9	7.4	- / Vir	+ / Avr
AvrT21	eAA/mCT-180	0.12	15	22.2	5.0 E -6	5.8	+ / Avr	- / Vir
AvrT28	eAC/mTA-107	0.07	9	40.3	2.2 E -10	8.9	+ / Avr	+ / Avr
AvrT28	opAB7-030	0.12	15	25.4	4.7 E -7	6.1	+ / Avr	+ / Avr
AvrT30	eAC/mCT-448	0.10	12	39.8	2.8 E -10	8.6	+ / Avr	- / Avr

^a Markers prefixed with op, gen or crl indicate random amplified polymorphic DNA markers; markers prefixed with eNN/mNN indicate amplified fragment length polymorphism markers.

^b Recombination fraction.

^c Chi-square values corrected for continuity (51,55), testing the null hypothesis of no linkage. Values of *P* < 0.05 reject the null hypothesis.

^d Log₁₀ of the likelihood ratio.

^e +: Parental isolate amplifying the DNA marker; -: Parental isolate not amplifying the DNA marker; Avr: parental isolate showing an avirulent phenotype on wheat stem rust differential with the corresponding stem rust (*Sr*) resistance gene; Vir: parental isolate showing a virulent phenotype wheat stem rust differential with the corresponding *Sr* resistance gene.

8. Ellingboe, A. H. 1992. Segregation of avirulence/virulence on three rice cultivars in *Magnaporthe grisea* pathogenic on rice. *Can. J. Bot.* 69:2130-2134.
9. Ellingboe, A. H. 1996. Genic interactions in hosts and pathogens. Pages 33-46 in: *Molecular Aspects of Pathogenicity and Resistance: Requirement for Signal Transduction*: D. Mills, H. Kunoh, N. T. Keen, and S. Mayama, Eds. The American Phytopathological Society, St. Paul, MN.
10. Ellis, J., Lawrence, G., Ayliffe, M., Anderson, P., Collins, N., Finnegan, J., Frost, D., Luck, J., and Pryor, T. 1997. Advances in the molecular genetic analysis of the flax-flax rust interaction. *Annu. Rev. Phytopathol.* 35:271-291.
11. Flor, H. H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32:653-669.
12. Flor, H. H. 1965. Inheritance of smooth-spore-wall and pathogenicity in *Melampsora lini*. *Phytopathology* 55:724-727.
13. Flor, H. H. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9:275-296.
14. Gabriel, D. W., and Rolfe, B. G. 1990. Working models of the gene-for-gene interaction. *Annu. Rev. Phytopathol.* 28:365-391.
15. Green, G. J. 1964. A color mutation, its inheritance, and the inheritance of pathogenicity in *Puccinia graminis* Pers. *Can. J. Bot.* 42:1653-1664.
16. Green, G. J. 1966. Selfing studies with races 10 and 11 of wheat stem rust. *Can. J. Bot.* 44:1255-1260.
17. Hagag, M. E. A., Samborski, D. J., and Dyck, P. L. 1973. Genetics of pathogenicity in three races of leaf rust on four wheat varieties. *Can. J. Genet. Cytol.* 15:73-82.
18. Heath, M. C. 1995. Signal exchange between higher plants and rust fungi. *Can. J. Bot.* 73(Suppl. 1):S616-S623.
19. Heath, M. C. 1997. Signaling between pathogenic rust fungi and resistant or susceptible host plants. *Ann. Bot.* 80:713-720.
20. Johnson, T. 1954. Selfing studies with physiologic races of the wheat stem rust, *Puccinia graminis tritici*. *Can. J. Bot.* 32:506-522.
21. Johnson, T., and Newton, M. 1940. Mendelian inheritance of certain pathogenic characters of *Puccinia graminis tritici*. *Can. J. Res. C* 18:599-611.
22. Kao, K. N., and Knott, D. R. 1969. The inheritance of pathogenicity in races 111 and 29 of wheat stem rust. *Can. J. Genet. Cytol.* 11:266-274.
23. Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. *Annu. Rev. Genet.* 24:447-463.
24. Kubelik, A. R., and Szabo, L. J. 1995. High-GC primers are useful in RAPD analysis of fungi. *Curr. Genet.* 28:384-389.
25. Landers, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
26. Lau, G. W., Chao, C. T., and Ellingboe, A. H. 1993. Interaction of genes controlling avirulence/virulence of *Magnaporthe grisea* on rice cultivar Katy. *Phytopathology* 83:375-382.
27. Lawrence, G. J. 1988. *Melampsora lini*, rust of flax and linseed. Pages 313-331 in: *Advances in Plant Pathology*, vol. 6. Academic Press, New York.
28. Lawrence, G. J., Mayo, G. M. E., and Shepherd, K. W. 1981. Interactions between genes controlling pathogenicity in the flax rust fungus. *Phytopathology* 71: 12-19.
29. Leung, H., Borromeo, E. S., Bernardo, M. A., and Nottoghem, J. L. 1988. Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. *Phytopathology* 78:1227-1233.
30. Liu, Z., Szabo, L. J., and Bushnell, W. R. 1993. Molecular cloning and analysis of abundant and stage-specific mRNAs from *Puccinia graminis*. *Mol. Plant-Microbe Interact.* 6:84-91.
31. Loegering, W. Q., and Powers, H. R., Jr. 1962. Inheritance of pathogenicity in a cross of physiological races 111 and 36 of *Puccinia graminis* f. sp. *tritici*. *Phytopathology* 52:547-554.
32. Luig, N. H., and Watson, I. A. 1961. A study of inheritance of pathogenicity in *Puccinia graminis* var. *tritici*. *Proc. Linn. Soc. NSW* 86:217-229.
33. Mather, K. 1951. *The Measurement of Linkage in Heredity*. 2nd ed. John Wiley and Sons, New York.
34. McIntosh, R. A., Wellings, C. R., and Park, R. F. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. CSIRO Australia and Kluwer Academic Publishers, Boston.
35. NaNagara, P. 1953. *Testing Mendelian Ratios*. M.S. thesis. Cornell University, Ithaca, NY.
36. Newton, M., Johnson, T., and Brown, A. M. 1930. A study of the inheritance of spore color and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* 10:775-798.
37. Roelfs, A. P. 1978. Estimated losses caused by rust in small grain cereals in the United States—1918-76. *U. S. Dep. Agric. Misc. Publ.* 1363.
38. Roelfs, A. P. 1982. Effects of barberry eradication on stem rust in the United States. *Plant Dis.* 66:177-181.
39. Roelfs, A. P. 1988. Genetic control of phenotypes in wheat stem rust. *Annu. Rev. Phytopathol.* 26:351-367.
40. Roelfs, A. P., and Groth, J. V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Phytopathology* 70:855-862.
41. Roelfs, A. P., and Groth, J. V. 1988. *Puccinia graminis* f. sp. *tritici*, black stem rust of *Triticum* spp. *Advances Plant Pathol.* 6:345-361.
42. Roelfs, A. P., Long, D. L., and Roberts, J. J. 1993. Races of *Puccinia graminis* in the United States during 1990. *Plant Dis.* 77:125-128.
43. Roelfs, A. P., and Martens, J. W. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathology* 78:526-533.
44. Roelfs, A. P., and McVey, D. V. 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. *Phytopathology* 69:722-730.
45. Rowell, J. B. 1984. Controlled infection by *Puccinia graminis* f. sp. *tritici* under artificial conditions. Pages. 291-332 in: *The Cereal Rusts*, Vol. I. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York.
46. Rowell, J. B., Loegering, W. Q., and Powers, H. R., Jr. 1963. Genetic model for physiologic studies of mechanisms governing development of infection type in wheat stem rust. *Phytopathology* 53:932-937.
47. Samborski, D. J., and Dyck, P. L. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. *Can. J. Genet. Cytol.* 10:24-32.
48. Statler, G. D. 1990. Inheritance of pathogenicity of progeny from an F₁ culture of *Melampsora lini*. *J. Phytopathol.* 128:184-190.
49. Statler, G. D. 1994. Inheritance of virulence of a mutant isolate of *Melampsora lini*. *Phytopathology* 84:761-764.
50. Statler, G. D., and Jin, Y. 1991. Inheritance of virulence of *Puccinia recondita* culture X43. *Can. J. Plant Pathol.* 13:33-37.
51. Steele, R. G. D., and Torrie, J. H. 1980. *Principles and Procedures of Statistics*. 2nd edition. McGraw-Hill Book Co., New York.
52. Valent, B., Farrall, L., and Chumley, F. G. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. *Genetics* 127:87-101.
53. Voss, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.
54. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
55. Yates, F. 1934. Contingency tables involving small numbers and the χ^2 test. *J. R. Stat. Soc. Suppl.* 1:217-235