Gene Expression in the *Triticum aestivum–Puccinia recondita* f. sp. *tritici* Gene-for-Gene System

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ABSTRACT

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The progeny of a selfed, single-uredinial isolate of *Puccinia recondita* f. sp. *tritici* segregated for virulence on near-isogenic Thatcher wheat lines with leaf rust resistance genes *Lr2a, Lr2c, Lr3, Lr3ka, Lr11, Lr17,* and *Lr30*. Progeny isolates that produced the lowest avirulent infection types on the homozygous Thatcher lines were assumed to be homozygous for avirulence alleles; isolates that produced intermediate infection types were assumed to be heterozygous; and isolates that produced high infection types were assumed to be homozygous for virulence alleles. The seven homozygous Thatcher lines were crossed with Thatcher to produce F_1 plants. The F_1 plants were selfed, and the F_2 plants were inoculated with the three different progeny isolate genotypes, such that host lines and rust isolates were evaluated for infection type in all nine genotype combinations for each of the seven corresponding gene pairs in *T. aestivum* and *P. r. tritici*. The expression of the resistance genes ranged from complete dominance to complete recessiveness, whereas expression of the avirulence genes ranged from nearly complete dominance to complete recessiveness. The expression of resistance and avirulence genes in the wheat leaf rust gene-for-gene system was found to be highly dependent on the genotypes of the rust isolates and host lines involved in the interactions.

The gene-for-gene relationship in plant-pathogen interactions has often been described using the "quadratic check" (1,2,11,16) in which pairs of host lines and pathogen isolates differ by only one gene for resistance (in the host) and avirulence (in the pathogen) (Fig. 1). This representation was developed to provide a genetic model for physiological and molecular studies of host-parasite interactions (11). In this model resistance genes (R) in the host and avirulence genes (A) in the pathogen are dominant, while susceptibility and virulence genes are recessive. Incompatible interactions (−) occur only when homozygous or heterozygous resistant (R−) hosts are challenged with homozygous or heterozygous avirulent (A−) pathogens. The other three combinations of genotypes yield compatible (+) interactions. Given the assumed dominance of resistance and avirulence, no distinction is made in this model between interactions involving host and pathogen genotypes that are homozygous or heterozygous for resistance and avirulence (1,2,16).

The purpose of this study was to examine gene expression in the *Triticum aestivum* L. (hexaploid wheat)–*Puccinia recondita* Roberge ex Dezmaz. f. sp. *tritici* (Eriks. & Henn.) (wheat leaf rust) gene-for-gene system by testing host and pathogen genotypes in all nine possible combinations for seven different corresponding gene pairs in wheat and *P. r. tritici*.

MATERIALS AND METHODS

Urediniospores from a single-uredinial field isolate of *P. r. tritici* designated as phenotype NBB in the *Prt* nomenclature (8) were purified by three successive isolations and increases from single uredinia on seedlings of the wheat cultivar Little Club (CI 4066). Inoculation and storage of rust isolates were carried out as in previous studies (4–6). Purity of urediniospores from the final single-uredinia isolation and increase was verified on the 12 differential lines in the *Prt* nomenclature. Teliospores of isolate NBB were then produced by inoculating urediniospores on adult plants of the Thatcher near-isogenic wheat line with resistance gene *Lr16* (RL 6005). Teliospores formed on the underside of the flag leaves after 28–35 days. The flag leaves with teliospores were harvested while still green, soaked for 2 wk continuously in running tap

Pathogen

Fig. 1. The quadratic check, initially described by Rowell et al (11): − = incompatibility; + = compatibility.

water, then dried and wet repeatedly for 2 days and placed over young leaves of *Thalictrum speciosissimum* Loefl, an alternate host for the wheat leaf rust fungus, overnight in chambers having 100% humidity. The teliospores were placed nightly over the *Thalictrum* plants until abundant pycnial infections were visible on the leaves. Pycnia were randomly intermated within infections on each individual leaf. The aeciospores were bulked together and increased for one uredinial generation on seedlings of Little Club. Urediniospores from each of 150, 21-day-old uredinia were isolated and increased on seedlings of Little Club. The 150 aeciospore-derived, single-uredinial isolates were evaluated for infection type on 20 Thatcher backcross wheat lines near-isogenic for different leaf rust resistance genes. Infection types were rated on a 0–4 scale 12 days after inoculation (8). The progenies segregated for avirulence/virulence on the near-isogenic lines with resistance

genes *Lr2a* (RL 6000), *Lr2c* (RL 6047), *Lr3* (RL 6002), *Lr3ka* (RL 6007), *Lr11* (RL 6053), *Lr17* (RL 6008), and *Lr30* (RL 6049). Progenies that produced lowest infection types (0 to ;1−) on the homozygous Thatcher lines were assumed to be homozygous for avirulence alleles; progenies that produced intermediate infection types (;1+ to 2+) were assumed to be heterozygous; and progenies that produced high compatible infection types (3 to 4) were assumed to be homozygous for virulence alleles. Previous studies have shown that alternate alleles at single loci in *P. r. tritici* condition avirulence/virulence to *Lr2a*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr11*, *Lr17*, and *Lr30* (13,14). By using progenies from a single selfed isolate, the effect of virulence heterozygosity between isolates of similar genetic background was determined.

The seven Thatcher near-isogenic lines for which the *P. r. tritici* progenies segregated for virulence, were crossed with the sus-
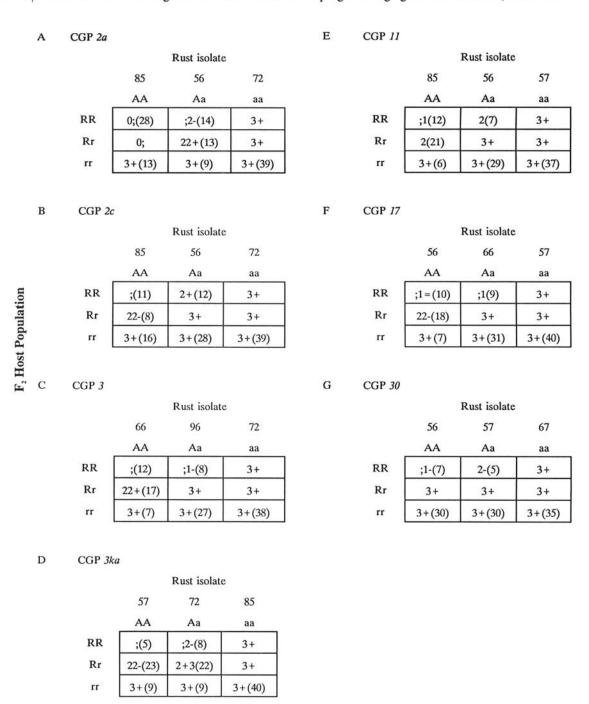
A   CGP *2a*

	Rust isolate		
	85	56	72
	AA	Aa	aa
RR	0;(28)	;2-(14)	3+
Rr	0;	22+(13)	3+
rr	3+(13)	3+(9)	3+(39)

B   CGP *2c*

	Rust isolate		
	85	56	72
	AA	Aa	aa
RR	;(11)	2+(12)	3+
Rr	22-(8)	3+	3+
rr	3+(16)	3+(28)	3+(39)

C   CGP *3*

	Rust isolate		
	66	96	72
	AA	Aa	aa
RR	;(12)	;1-(8)	3+
Rr	22+(17)	3+	3+
rr	3+(7)	3+(27)	3+(38)

D   CGP *3ka*

	Rust isolate		
	57	72	85
	AA	Aa	aa
RR	;(5)	;2-(8)	3+
Rr	22-(23)	2+3(22)	3+
rr	3+(9)	3+(9)	3+(40)

E   CGP *11*

	Rust isolate		
	85	56	57
	AA	Aa	aa
RR	;1(12)	2(7)	3+
Rr	2(21)	3+	3+
rr	3+(6)	3+(29)	3+(37)

F   CGP *17*

	Rust isolate		
	56	66	57
	AA	Aa	aa
RR	;1=(10)	;1(9)	3+
Rr	22-(18)	3+	3+
rr	3+(7)	3+(31)	3+(40)

G   CGP *30*

	Rust isolate		
	56	57	67
	AA	Aa	aa
RR	;1-(7)	2-(5)	3+
Rr	3+	3+	3+
rr	3+(30)	3+(30)	3+(35)

F₂ Host Population

Fig. 2. Infection types of F₂ Thatcher wheat line populations and *P. r. tritici* progeny rust isolates in all nine possible genotype combinations at corresponding gene pair (CGP) loci *2a*, *2c*, *3*, *3ka*, *11*, *17*, and *30* in the host and pathogen. Infection types 0 to 2+ are avirulent; infection types from 3 to 4 are virulent. Numbers in parentheses indicate number of F₂ plants with the indicated infection type. AA = homozygous avirulent; Aa = heterozygous avirulent; aa = homozygous virulent.

ceptible recurrent parent Thatcher (RL 6101) to produce F_1 seeds that were heterozygous for the resistance genes. F_1 plants from each cross were grown to maturity. F_1 seedlings, 35–40 F_2 seedlings, the parental Thatcher backcross lines, and Thatcher were inoculated with different combinations of seven progeny rust isolates that had produced either the lowest infection types (putatively homozygous avirulent); intermediate infection types (putatively heterozygous avirulent); or high infection types (putatively homozygous virulent) on the near-isogenic Thatcher backcross lines. The F_1 and F_2 plants and their respective parents were evaluated for infection type in separate tests. The ambient greenhouse temperatures were 15–20 C during the F_1 tests and 18–20 C when the F_2 populations were evaluated. Infection types from the F_2 tests were determined to be more reliable since these tests could be repeated. The F_2 plants were rated for segregation of infection type: if an infection type distinct from Thatcher and the Thatcher near-isogenic line parent could be repeatedly distinguished, it was assumed the progeny isolate could discriminate between homozygous and heterozygous resistant plants. If an infection type different from either of the two parents could not be reliably distinguished, infection types of the host heterozygotes were grouped with one of the parental infection types. These combinations were tested three times to confirm the infection types of the host heterozygotes. Other genotype combinations of host line and rust isolate were tested twice to confirm the infection types. Representative data from the F_2 tests are summarized in Figure 2.

RESULTS

Infection types for the nine genotype combinations for each of the seven corresponding gene pairs in wheat and *P. r. tritici* are shown in Figure 2. Resistance gene *Lr2a* expressed complete dominance when tested with a homozygous avirulent isolate (Fig. 2A). When tested with a heterozygous isolate, resistance gene *Lr2a* expressed incomplete dominance. Conversely, the gene conditioning avirulence to *Lr2a* expressed incomplete dominance with homozygous and heterozygous resistant hosts. The *Lr2c* gene expressed incomplete dominance when tested with a homozygous avirulent isolate and was recessive when tested with a heterozygous isolate (Fig 2B). Avirulence to *Lr2c* expressed incomplete dominance when tested with homozygous resistant hosts and was recessive with heterozygous hosts. Resistance gene *Lr3* expressed incomplete dominance when tested with a homozygous avirulent isolate and was recessive when tested with a heterozygous isolate (Fig. 2C). Avirulence to gene *Lr3* expressed nearly complete dominance when tested with homozygous resistant hosts and was recessive when tested with heterozygous hosts. Gene *Lr3ka* expressed incomplete dominance when tested with homozygous and heterozygous avirulent isolates (Fig. 2D). Avirulence to gene *Lr3ka* was incompletely dominant when tested with homo- and heterozygous hosts. Resistance genes *Lr11* and *Lr17* were incompletely dominant when tested with homozygous avirulent isolates and recessive with heterozygous isolates. (Fig. 2E and F). Avirulence to *Lr11* was incompletely dominant with homozygous resistant hosts and recessive with heterozygous hosts. Avirulence to *Lr17* was nearly completely dominant when tested with homozygous resistant hosts and recessive with heterozygous hosts. Gene *Lr30* was recessive when tested with homo- and heterozygous avirulent isolates, whereas avirulence to this gene was incompletely dominant with homozygous resistant hosts and was not expressed with heterozygous hosts (Fig. 2G).

DISCUSSION

The data shown here clearly indicate that in the *T. aestivum–P. r. tritici* gene-for-gene system, host resistance may be completely dominant, incompletely dominant, or recessive. Similarly, avirulence in the pathogen may be completely dominant, incompletely dominant or recessive. The dominance relationship of resistance and avirulence genes relative to the alternate alleles is highly dependent on the genotypes of the host lines and rust isolates. The

conceptual basis of the quadratic check was mostly derived from Flor's (3) studies with flax and flax rust, in which resistance and avirulence genes were invariably dominant.

Genetic studies with wheat leaf and stem rust resistance genes have indicated that combinations of heterozygous hosts and rust isolates condition intermediate to virulent infection types. Samborski (12) determined that *Lr9* expressed complete dominance when tested with a homozygous avirulent isolate and was recessive when tested with a heterozygous avirulent isolate. The combination of host and rust heterozygotes at corresponding gene pair 9 produced a virulent 3 infection type. Roelfs (10) has presented infection types for the nine genotype combinations at corresponding gene pairs 7b, 10, and 17 in the wheat stem rust gene-for-gene system. Heterozygous combinations of host lines and rust isolates at these loci conditioned intermediate infection types. Loegering and Sears (7) determined that wheat lines monotelodisomic for the chromosome arms with *Sr6*, *Sr8*, *Sr9a*, and *Sr11* conditioned intermediate infection types compared with the highly resistant euploid lines.

In this study, differences between host-pathogen genotype combinations were assessed by visual rating of the infection types. For a number of corresponding gene pairs, heterozygous *P. r. tritici* isolates did not distinguish different infection types between hosts heterozygous for resistance and those lacking resistance genes. However, differences between these genotype combinations may have been detected if the infection process had been examined at a physiological or histological level. Slesinski and Ellingboe (15) examined ^{35}S uptake by *Erysiphe graminis* f. sp. *tritici* in the four genotype combinations of the quadratic check. The incompatible *P1/Pm1* combination had the lowest rate of ^{35}S uptake as expected; however, among the phenotypically similar compatible combinations, *p1/Pm1* had a lower rate than *P1/pm1* or *p1/pm1*.

In general, little variation in infection types was observed between the F_1 and F_2 tests. The combinations of heterozygous rust isolates and heterozygous host lines at corresponding gene pairs *3ka* and *11* differed the most. In the F_1 tests the double heterozygote combination at corresponding gene pair *3ka* had an infection type of 3+, whereas in the F_2 tests the same genotype combination had a 2+3 infection type. In the F_1 tests the double heterozygote combination at corresponding gene pair *11* had a 2+ infection type, whereas the same genotype combination in the F_2 tests had a 3+ infection type. These variations were most likely due to the temperature differences between the F_1 and F_2 tests. Infection types conditioned by heterozygous host lines and/or rust isolates are probably more affected by differences in temperatures than combinations where both host lines and rust isolates are homozygous for resistance and avirulence.

The effect of pathogen heterozygosity on expression of host resistance genes also entails practical considerations. For example, attention should be given to the choice of rust isolates used in backcrossing resistance genes. Use of an isolate to which the resistance gene expresses complete or intermediate dominance will obviously be preferable to an isolate to which the resistance gene is recessive. In virulence surveys of *P. r. tritici* (6), the single-uredinial isolates often express intermediate infection types to the homozygous Thatcher near-isogenic lines (J. A. Kolmer, *unpublished data*). Genetic data (4) also indicate that avirulent isolates collected in the annual surveys are often heterozygous.

It is apparent that the generalization of completely dominant resistance and avirulence cannot be applied to the *T. aestivum–P. r. tritici* interaction. The quadratic check offers the simple hypothesis of actively produced resistance and avirulence gene products interacting to produce an incompatible infection type (1,2). However, this model cannot account for the compatible infection types observed with heterozygous avirulent rust isolates and heterozygous host lines at corresponding gene pairs *2c, 3, 11, 17,* and *30*. This study illustrates what Person and Mayo (9) stated, that dominance at resistance and avirulence gene loci is not an essential element in gene-for-gene relationships or host-parasite specificity. Further models and studies examining specificity and mechanisms of compatible and incompatible infection types will also need

to take into account recessive host resistance genes and pathogen avirulence genes.

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