Identification of Powdery Mildew Resistance Genes in Soft Red Winter Wheat Cultivars and Ohio Breeding Lines

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ABSTRACT

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Eight-day-old seedlings of seven soft red winter wheat cultivars and four elite breeding lines were tested for the presence of powdery mildew resistance genes with 14 isolates of *Blumeria graminis* f. sp. *tritici*. These isolates were characterized for their virulence on differential wheat cultivars and lines with single genes for powdery mildew resistance. Phenotypic reactions of the cultivars and lines to each isolate were assessed 8 days after inoculation. Differential reactions and pedigree information were used to determine the presence of putative powdery mildew resistance genes. The *B. g. tritici* isolates detected powdery mildew resistance gene *Pm*3a in AGRA brand GR915, and pedigree information indicated that Freedom contained *Pm*8. No *Pm* genes were detected in Cardinal, Clark, Dynasty, or Titan. The gene *Pm*3a was present in breeding lines OH470 and OH493-1, and gene *Pm*17 was detected in OH464. OH490 contained gene *Pm*2 or *Pm*6, or possibly both; the isolates could not differentiate these two genes.

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal, is an important disease of wheat (*Triticum aestivum* L.) in the United States (7,13,14,17,20,23,26,29) and in other areas of the world (12,16, 34,35). Over a 3-yr period in Ohio, yield losses on susceptible and moderately susceptible cultivars ranged from 5 to 27%, depending on the cultivar and year (20).

The use of host resistance has been cited as the most effective, economical, and environmentally safe method of managing this disease (3,22,24). A disease management program that relies on host resistance requires information on the type of host resistance deployed and virulence spectrum of the pathogen population. This information will enable the replacement of ineffective host resistance genes with those that are currently effective. It will also enable close monitoring of changes in virulence in the pathogen population relative to deployment of known resistance genes in the host.

Genes for powdery mildew resistance (*Pm*) have been described at 17 different loci in wheat (8,21,29–31), and most have been used in spring and winter wheat cultivars to manage this disease (1,18,21). However, there is a lack of information on the presence of genes in specific cultivars being used in the United States (18). This lack of information has hindered breeding and disease management efforts in the United States.

Powdery mildew resistance genes have been identified in many winter and spring wheat cultivars grown in Germany (9,10,18) and for some cultivars grown in the United States (1,18,21,28). Identification of resistance genes in some soft red winter wheat cultivars has been based on the use of isolates of *B. g. tritici* characterized for their virulence and pedigree relationships (9,10,18). This information has been useful to European wheat breeders and is essential in developing management strategies for powdery mildew (18).

The objective of this study was to evaluate certain commercial cultivars grown in Ohio and lines from the Ohio State University wheat breeding program for the presence of specific powdery mildew resistance genes. Cultivars and lines were inoculated with selected *B. g. tritici* isolates with known genes for virulence. Differential reactions and pedigree relationships were used to postulate the presence of powdery mildew resistance genes under the assumptions of Flor's gene-for-gene concept (6).

MATERIALS AND METHODS

Inoculum production. Fourteen *B. g. tritici* isolates, obtained from wheat fields in Ohio, were chosen on the basis of their differential response on lines known to carry certain resistance genes (25) (Table 1). Isolates were chosen to differentiate between their corresponding—and in some cases the combination of the corresponding—resistance genes in their host. Several isolates were similar in their virulence spectrum but were included to confirm the accuracy of these tests.

Inoculum was increased on Becker or Chancellor seedlings grown in 10-cm-diameter plastic pots containing Baccto potting mix (Michigan Peat Company, Houston, TX). Approximately 15 seeds were planted in each pot. Pots were placed in glass cases; one side of each case was partially open to permit access to the pots. The opening was covered with two layers of cheesecloth held tightly to the case by Velcro strips. Plants were inoculated 7–8 days after planting when seedlings had one or two leaves. Just before inoculation, each pot was watered with 30 ml of 50 ppm chlormequat chloride (Cycocel) to retard plant growth. Pots were then fitted with 19-cm-tall clear plastic cylinders to prevent contamination. Cylinders were covered at the top with a 0.5-cm-thick piece of cotton held in place by a rubber band. Cylinders were secured to pots by wrapping Parafilm around the cylinder bottom and the top rim of the pot. The inoculation room was disinfested with UV light for 2 hr prior to inoculation. Conidia were transferred by tapping the tube containing each isolate over seedlings in a pot or by using a sterile camel's hair brush to transfer conidia onto plants in a pot. Inoculated plants were then placed in a growth chamber maintained at 16 C and at least 85% RH under a 12-hr photoperiod with illumination of 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the top of the cylinder. Inoculum was ready for use in 8–10 days. The same procedure was performed on control plants except that an uninfected seedling was used as a test to ensure that contamination did not occur.

Identification of resistance genes. Three seeds of seven wheat cultivars grown in Ohio, four elite breeding lines developed by the Ohio State University's wheat breeding program, and the susceptible cultivar Chancellor (Table 2) were planted in radial rows in a 21-cm-diameter plastic pot half filled with Baccto planting mix. Each pot was then placed in a 60 × 26 cm plastic bag to prevent contamination of the seedlings. Tops of plastic bags were supported by a thin stake placed in the center of the pot. A flat piece of cotton was wound around the top of the stake, and the plastic bag was bound with a rubber band

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around the cotton at the top of the stake to allow aeration. Pots were placed in the greenhouse under temperatures varying from 18 to 23 C and a 12-hr photoperiod (approximately 415 $\mu E \cdot m^{-2} \cdot s^{-1}$) during seed germination and early seedling growth.

Eight-day-old seedlings (one- to two-leaf stage) were inoculated with conidia from a single isolate by shaking infected plants over the pot in the inoculating room under the conditions described above. After inoculation, pots were placed in growth chambers under the conditions described previously. Seedlings of the cultivars and lines were inoculated with each isolate in separate pots, and the tests were repeated at least three times. New inocula were produced for each repetition of the test. This procedure was repeated on the control plants except that noninfected plants were shaken over these pots.

Seedlings were rated for their reactions on an individual plant basis 8 days after inoculation on a scale of 0–9, where 0 = immunity, no visible sign of infection; 1–3 = highly resistant, increasing from a few flecks with no necrosis, to more flecks and tiny necrotic spots, to large necrotic spots with some chlorosis; 4–6 = intermediate, increasing from large chlorotic areas with moderate amounts of mycelium to some sporulation; and 7–9 = susceptible, increasing in amount, size, and density of mycelium and conidial production representing a compatible reaction. A score of 0–6 indicated a resistant reaction of the host to the particular isolate, while a score of 7–9 indicated a susceptible reaction (18,22, 23). An isolate that gave a rating of 7–9 on a particular differential host line or cultivar was identified as having the virulence gene that could overcome the corresponding resistance gene in the host. This test was repeated at least three times, and the mean rating was used as a basis for interpreting the results.

RESULTS AND DISCUSSION

The set of *B. g. tritici* isolates used in this study identified the powdery mildew genes *Pm*1, *Pm*3a, *Pm*3b, *Pm*4a, *Pm*17, and *Pm*2 and/or *Pm*6 and *Pm*3c and/or *Pm*5 (Table 1). The isolates used in this test could not identify genes *Pm*7 and *Pm*8 because none was avirulent on lines carrying these two genes. Also, none of the isolates could differentiate between resistance genes *Pm*3c and *Pm*5 or between genes *Pm*2 and *Pm*6. In both cases, the corresponding virulence genes occurred together in all isolates available for testing (25). Reactions of the cultivars and lines were consistent across the three experiment replications. Reactions were either resistant type 0–3 or susceptible type 7–9 (Table 2). No intermediate types of reaction were observed. The highly susceptible Chancellor plants in each pot had profuse conidial development in response to all isolates. The uninoculated control plants remained symptomless during the entire study, indicating that contamination or isolate mixing did not occur. The use of more than one isolate with the same genes for virulence (i.e., isolates 40 and 257, 310 and 351, 339 and 362, and 63 and 313) corroborated these results (Tables 1 and 2).

The differential reactions of cultivars and lines to each isolate clearly identified resistance gene *Pm*3a in lines OH470, OH493-1, and AGRA brand GR915; *Pm*17 in line OH464; and *Pm*2 and/or *Pm*6 in line OH490 (Table 2). GR915

Table 1. Reactions of 12 wheat cultivars and lines with known genes for powdery mildew resistance to 14 isolates of *Blumeria graminis* f. sp. *tritici* from Ohio

Cultivar or line	Resistance gene	Isolate number of *B. g. tritici*													
		38	40	63	239	257	290	299	310	313	318	320	339	351	362
Chancellor	None	S[a]	S	S	S	S	S	S	S	S	S	S	S	S	S
Axminster/8c[b]	*Pm*1	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Ulka/8c	*Pm*2	S	R-I	R-I	S	R	S	R	S	R	R-I	S	S	S	S
Tyler	*Pm*3a	R	R-I	R	R	R	S	R	R	R	R	R	R	R	R
Chulu/8c	*Pm*3b	R	R	R	R	R	R	S	R	R	R	R	R	R	R
Sonora/8c	*Pm*3c	S	R	S	R	R	S	S	S	S	S	S	S	S	S
Khapli/8c	*Pm*4a	S	R-I	R	R	R	R	S	S	R	S	S	R	S	R
Hope/8c	*Pm*5	S	R-I	S	R	R	S	S	S	S	S	S	S	S	S
PI 405718	*Pm*2 + *Pm*6	S	R	R	S	R	S	R	S	R	I	S	S	S	S
Tanzec	*Pm*7	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Kavkaz	*Pm*8	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Amigo	*Pm*17	S	R	R	R	R	R	R	R	R	R-I	R	R	I	R

[a] R = resistant, I = intermediate, and S = susceptible; R-I = resistant reaction predominated.
[b] Line or cultivar followed by /8c was crossed to Chancellor, then seven backcrosses to Chancellor were made (4,19).

Table 2. Reactions of 11 soft red winter wheat cultivars and breeding lines to 14 isolates of *Blumeria graminis* f. sp. *tritici* with known virulence to powdery mildew resistance genes

Cultivar or line	Putative resistance gene(s)	Isolate number of *B. g. tritici*													
		38	40	63	239	257	290	299	310	313	318	320	339	351	362
Chancellor	None	S[a]	S	S	S	S	S	S	S	S	S	S	S	S	S
OH470[b]	*Pm*3a	R	R	R	R	R	S	R	R	R	R	R	R	R	R
OH464[b]	*Pm*17	S	R	R	R	R	R	R	R	R	R	R	R	R	R
OH490[b]	*Pm*2 + *Pm*6	S	R	R	S	R	S	R	S	R	R	S	S	S	S
OH493-1[b]	*Pm*3a	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Titan[c]	None	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GR915[c,d]	*Pm*3a	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Freedom[c]	*Pm*8[e]	S	S	S	S	S	S	S	S	S	S	S	S	S	S
GR863[c,d]	None	S*	S*	S*	S*	S*	S*	S*	S*	S*	S*	S*	S*	S*	S*
Dynasty[c]	None	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cardinal[c]	None	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Clark[c]	None	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a] R = resistant and S = susceptible; S* = susceptible reaction with some necrotic spots.
[b] Elite lines in Ohio State University breeding program.
[c] Commercial wheat cultivars grown in Ohio.
[d] AGRA brand cultivar.
[e] Freedom has been reported to carry the 1*BL*/1*RS* wheat/rye chromosome translocation (2) on which *Pm*8 is located (21).

gave mainly reaction type 0 in almost all cases where a resistant reaction was recorded. The reaction type expressed by AGRA brand GR863 was susceptible, as evidenced by the amount of mycelial growth and conidia being produced on this cultivar by all isolates. However, a few necrotic spots were detected on leaves when tested against each isolate (Table 2). This may indicate the presence of some resistance, but the nature of this reaction could not be determined in this study. Major powdery mildew resistance genes could not be identified in the other six commercial cultivars because of a lack of differential reactions to the isolates tested.

Pedigree information was helpful in identifying the source of the resistance in OH470 and OH464. OH470 was selected from the cross Tyler/Pioneer brand 2550. Tyler carries the gene *Pm*3a (21), and it may be the donor of the gene *Pm*3a. The pedigree for OH464 is OH115/Pike//Adena/Amigo. The gene *Pm*17 is carried on a rye (*Secale cereale* L.) translocation from chromosome 1R of rye to chromosome 1A of wheat in the cultivar Amigo (11). Thus, *Pm* 17, identified in OH464, was possibly derived from the cultivar Amigo.

Pedigree information did not identify the source of gene *Pm*3a in OH493-1 and GR915 because there are no reports of any powdery mildew resistance genes in the parents of these lines. Pedigree information on OH490 also did not provide any insight into the source of *Pm*2 and/or *Pm*6. The source of the factor causing necrotic spotting on GR863 was unknown. This cultivar resulted from the cross Xelaju 66/Logan//Abe. Abe is reported to have the gene *Pm*6 (18) and *Pm*6 + *Pm*2 (1). However, these genes were not identified in this line, suggesting that resistance in Abe was not transferred to GR863. The cultivar Logan was reported to be susceptible to all races of powdery mildew in Ohio (15), and the powdery mildew resistance present in Xelaju 66, if any, is not known. It is possible that this type of reaction may be more fully expressed in the adult plant or may be conditioned by genes with smaller effects (18).

The cultivar Freedom gave a susceptible reaction to all isolates tested even though it exhibits considerable resistance in the field. Freedom possesses the 1BL/1RS wheat/rye translocation inherited from Kavkaz (2). The 1BL/1RS translocation contains the gene *Pm*8 (21). Unfortunately, none of the isolates used in this study could differentiate *Pm*8. On the basis of disease severity in field plots, Freedom is resistant to *B. g. tritici* at a level similar to GR876, which also carries the 1BL/1RS translocation (2). In recent years, more powdery mildew has been detected on GR876 than on Freedom. This suggests that Freedom may possess other resistance factors in addition to the gene *Pm*8. Previous studies have revealed that all *B. g. tritici* isolates collected over a 2-yr period throughout Ohio were virulent on *Pm*8 (25). Thus, it appears that the gene *Pm*8 has been defeated in Ohio and is expected to provide little protection if used in cultivars in the future.

Little information was available on powdery mildew resistance genes present in the parental wheat lines of cultivars Cardinal, Clark, Dynasty, and Titan. The cultivar Arthur is a parent of Dynasty and is reported to have resistance genes *Pm*5 and *Pm*6 (18) and *Pm*2 and *Pm*6 (1). Thus, our results indicate that the resistance genes present in Arthur were not transferred to Dynasty during crossing. It also appears that no resistance genes were present in Cardinal, Clark, or Titan, since none could be identified by the isolates used in our test nor could any be postulated from pedigree information.

Recent studies indicated that few *B. g. tritici* isolates were capable of overcoming the genes *Pm*1, *Pm*3b, and *Pm*17 in Ohio (25). Of these genes, only *Pm*17 was detected in one of the 11 cultivars and lines tested in this study. The need for greater diversity for resistance to powdery mildew in winter wheat has been expressed by other researchers (9,18). If single-gene resistance is to be used in cultivars for this area, resistance sources with *Pm*1 and *Pm*3b could be exploited (9,10,18,21). At least in the short term, these genes, along with *Pm*17, could provide some protection against the races of powdery mildew in Ohio (25). Long-term strategies for powdery mildew management should, however, involve a quantitative type of resistance (5,27,32,33), since *B. g. tritici* is known to overcome single-gene resistance and to increase the frequency of its virulence on these genes within only a few years.

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