Genetic analysis of wheat-*Pyrenophora tritici-repentis* interactions

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ABSTRACT

Tan spot of wheat, caused by the fungus *Pyrenophora tritici-repentis*, is a destructive disease worldwide that can cause serious losses in quality and quantity of wheat grain production. The fungus induces two distinct symptoms; tan necrosis and extensive chlorosis, on susceptible wheat cultivars. The major objective of this study was to determine the genetic control of resistance to tan spot, caused by multiple races of *P. tritici-repentis* in the newly identified sources of resistance. Plants were inoculated at the two-leaf stage under controlled environmental conditions and disease assessment was based on lesion-type rating scale. The segregating generations of each cross were analysed for the allelism and/or inheritance studies. A single recessive gene controlled resistance to tan necrosis caused by race 1 in both tetraploid and hexaploid resistant genotypes studied. The lack of segregation in the inter- and intra-specific crosses between the resistant tetraploid and hexaploid genotypes indicates that they possess the same genes for resistance to tan necrosis and extensive chlorosis induced by *P. tritici-repentis* race 1. Two independent genes, a single dominant gene for extensive chlorosis in hexaploid wheat and a single recessive gene for tan necrosis in tetraploid wheat, controlled resistance to tan spot induced by race 5. Genetic analysis of inter- and intra-specific crosses among *Triticum* species confirms that wheat-*P. tritici-repentis* host-pathosystem follows the toxin model of gene-for gene hypothesis.

INTRODUCTION

Tan spot, a foliar disease of wheat, is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs (*PTR*). The tan spot disease syndromes induced on susceptible durum and common wheat consist of two phenotypically distinct and independent symptoms: tan necrosis and extensive chlorosis. On an average, tan spot causes 5 to 15% yield losses; however, under conditions favourable for disease development, it can cause up to 50% yield loss (1). Tan spot also adversely affects the quality of grain by causing grain shrivelling, dark smudge and black point (1).

Currently, eight races of *PTR* have been identified based on their ability to induce necrosis and/or chlorosis on a set of differential wheat cultivars (6). Resistance is expressed as small, dark brown lesions that do not increase in size, while susceptibility is expressed as dark brown spots surrounded by tan necrosis and/or extensive chlorosis that may involve the entire leaf. To date, three host-specific toxins (e.g., Ptr ToxA, Ptr ToxB, and Ptr ToxC) produced by the tan spot fungus have been identified and well-characterized. Genetic studies have established that the same gene controls sensitivity to the toxins and susceptibility to their producer races (2,4).

Information coming out of genetic studies reveals which germplasm and breeding strategy is to be adopted to develop cultivars containing the desired traits. Several independent studies on tan spot host-pathogen interactions revealed that resistance is inherited qualitatively (2,4,8) or quantitatively (2,7). Singh et al. (9) screened 975 accessions of wheat and its related species and identified new sources of resistance effective against *PTR* races 1, 2, 3, and 5. The objectives of this study were to i) determine the genetic control of resistance to *PTR* races 1 and 5 in the newly identified sources of resistance coming from different *Triticum* species, and ii) determine through allelic/genic studies the relationship between resistance genes.

MATERIALS AND METHODS

Genetic material and population development. Eight cultivars, selected for this study based on their reaction to *PTR* races 1 and 5 (9), were used to produce 10 cross populations. Each F_1 plant grown to produce F_2 seed was harvested separately and the F_2 populations derived from each F_1 plant were tested individually for disease reaction. Similarly, all $F_{2:3}$ and $F_{2:5}$ families were produced by single seed descent.

Disease screening. The isolates Ptr D-2000-HT VI-5 (*PTR* race 1) and Ptr DW-13 (*PTR* race 5) were used to produce spore inoculum for disease induction. The conidial inoculum production, disease screening, and its assessment were done following the procedures described by Lamari and Bernier (4). The seedlings were rated for disease reaction based on the 1 to 5 lesion-type rating scale, with plants having ratings of 1 and 2 considered resistant and those with ratings of 3 to 5 classified as susceptible.

Statistical analysis. The chi-square test for goodness-of-fit to various genetic models was applied to the segregation data observed for the F_2 plants and the $F_{2:3}$ or $F_{2:5}$ families to determine the number of genes controlling resistance to tan spot of wheat.

RESULTS

Genetics of resistance to *PTR* race 1

Inheritance studies. All resistant/susceptible (R/S) crosses studied were observed to segregate for the necrosis component of tan spot caused by race 1 and segregation did not occur for the chlorosis component. All the F_1 plants of the R/S crosses were susceptible, indicating that resistance to necrosis was recessive

(Table 1). The F_2 generation of the R/S crosses segregated in a 1 resistant: 3 susceptible ratio, indicating that a single gene controlled resistance and confirming that resistance to necrosis was recessive in all crosses studied (Table 1). The $F_{2:3}$ families of all R/S crosses segregated in the ratio 1 homozygous resistant: 2 segregating: 1 homozygous susceptible, thus confirming the hypothesis of monogenic control of resistance to necrosis caused by race 1 (Table 2).

Allelism studies. All the resistant parents involved in development of the crosses studied with race 1, were resistant to chlorosis. In each of the crosses, which were resistant/resistant (R/R) crosses for segregation to chlorosis by race 1, all F_1 and F_2 plants tested were resistant (Table 1). Confirmation of the lack of segregation of single-plant data was done by screening $F_{2:3}$ families of all the crosses. The lack of segregation for chlorosis confirms that the resistant sources from both tetraploid and hexaploid wheat shared the resistance gene(s) controlling chlorosis induced by race 1 (Table 2). All F_1 plants of the R/R crosses of resistant hexaploid genotypes with the resistant hexaploid check cultivar Erik were resistant to necrosis induced by race 1 (Table 1). However, while most F_2 plants of these crosses were resistant, a few plants in four of the five crosses gave a moderately susceptible necrotic reaction (rating 3). None of the segregations of the R/R crosses fit a ratio, indicating that the resistant sources possessed different resistance genes. All the $F_{2:3}$ families of each of the crosses tested were homozygous resistant, thus confirming that all the six resistant parents shared the same resistance gene (Table 2). All F_1 and F_2 generations and $F_{2:3}$ families of the cross between resistant tetraploid genotype *T. turgidum* # 283 (TT# 283) and resistant hexaploid cultivar Erik did not segregate for resistance (Tables 1,2). These results confirm that the tetraploid and hexaploid resistant genotypes shared the same single gene for resistance to necrosis induced by race 1.

Genetics of resistance to *PTR* race 5

Inheritance studies. All F_1 plants of the R/S crosses among the hexaploid wheat genotypes were resistant, indicating that resistance to chlorosis caused by race 5 was dominant (Table 1). The F_2 generations of the R/S intra-specific crosses segregated in a 3 resistant: 1 susceptible ratio, indicating that a single gene controls resistance and confirming that resistance to chlorosis was dominant in all the crosses studied. The $F_{2:5}$ families of all four R/S crosses segregated in the ratio 7 homozygous resistant: 2 segregating: 7 homozygous susceptible, thus confirming the hypothesis of monogenic control of resistance to chlorosis induced by race 5 in hexaploid wheat (Table 2). In the tetraploid cross *T. turgidum* # 283/Coulter, which was segregating for necrosis component of tan spot, the F_1 and F_2 generations and $F_{2:5}$ families indicated that a single recessive gene control (Tables 1,2).

Allelism studies. All F_1 and F_2 generations of the R/R crosses involving hexaploid wheat genotypes when tested with race 5 showed a resistant reaction (Table 1). Individual plant data were confirmed by screening $F_{2:3}$ families of each of the R/R crosses which gave resistant reaction confirming that all resistant sources possessed the same gene for resistance to chlorosis induced by race 5 (Table 2). All the F_1 and F_2 plants and $F_{2:3}$ families of the inter-specific cross between the resistant tetraploid and the resistant hexaploid source showed a resistant reaction confirming that resistance to race 5 in both tetraploid and hexaploid wheat were controlled by the same gene (Tables 1,2). The genetic studies with race 5 between susceptible genotypes Coulter (tetraploid) and Katepwa (hexaploid) gave interesting results. Coulter is susceptible to necrosis while Katepwa is susceptible to chlorosis caused by race 5. All F_1 plants of the cross Coulter/Katepwa were susceptible. Plants of the F_2 generation of this cross segregated for both necrosis and chlorosis. Rating plants for both necrosis and chlorosis components separately are error prone and hence disease scoring for resistant and susceptible (necrosis and/or chlorosis) reaction was performed. The F_2 generation of this inter-specific S/S cross segregated in a 3 resistant: 13 susceptible ratio, indicating that different genes control susceptibility to race 5 in this cross (Table 1). The $F_{2:3}$ families of this cross segregated for 1 homozygous resistant: 15 susceptible (homozygous and heterozygous families), confirming that different genes control susceptibility to necrosis (tetraploid) and chlorosis (hexaploid) in this cross (Table 2).

DISCUSSION

The resistant parents analysed in this study were genetically diverse but the allelic studies for resistance to *PTR* races 1 and 5 indicate that all the resistant sources possess the same gene(s) for resistance to tan spot. These findings agree with earlier studies (3,5,8) wherein different sources of resistance had no allelic differences. The allelic findings suggest that the wheat gene pool possesses very narrow genetic diversity for resistance to tan spot and hence rigorous efforts should continue to seek new and different genes for resistance.

Lamari et al. (6) suggested that the wheat-*PTR* interaction conforms to the toxin model of gene-for-gene hypothesis. The susceptibility interaction occurs due to the production of a toxin by the pathogen and the presence of a 'toxin-receptor' site in the host. If the presence of such a site is considered, the product of a susceptibility gene, then the resistance in the wheat-*PTR* pathosystem is due to lack of a susceptibility gene(s) rather than the presence of a resistance gene(s). This could be the reason why most studies have identified the same resistance gene(s) using the known races of *PTR*.

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TABLE 1. Reaction of F_1 and F_2 seedlings of wheat crosses to *Pyrenophora tritici-repentis* race 1 and 5 evaluated in greenhouse.

Cross	F_1 R	F_1 S	F_2 R	F_2 S	Ratio tested	χ^2 value
Inheritance studies with race 1						
2000 Spelt # 20/Kenyon	0	7	74	177	1:3	2.69*
CIMMYT # 18/Kenyon	0	9	61	198	1:3	0.29*
Altar Synthetic/Kenyon	0	8	54	202	1:3	2.08*
TT # 283/Coulter	0	7	74	181	1:3	2.19*
Allelism studies with race 1						
2000 Spelt # 20/Erik	8	0	178	2	1:0	-
CIMMYT # 18/Erik	8	0	248	8	1:0	-
Altar Synthetic/Erik	9	0	255	1	1:0	-
TT # 283/Erik	7	0	192	0	1:0	-
Inheritance studies with race 5						
2000 Spelt # 20/Kenyon	7	0	149	59	3:1	1.26*
CIMMYT # 18/Kenyon	8	0	150	58	3:1	0.92*
Altar Synthetic/Kenyon	7	0	157	63	3:1	1.55*
TT # 283/Coulter	0	8	45	172	1:3	2.10*
Allelism studies with race 5						
2000 Spelt # 20/Erik	7	0	220	0	1:0	-
CIMMYT # 18/Erik	9	0	201	0	1:0	-
Altar Synthetic/Erik	8	0	212	0	1:0	-
TT # 283/Erik	9	0	221	0	1:0	-
Coulter/Katepwa	0	9	13	63	3:13	0.13*

*Probability of obtaining deviations from the expected ratio is by chance alone and the observed population does not differ significantly from expected hypothesis.

TABLE 2. Reaction of $F_{2:3}$ and $F_{2:5}$ families of wheat crosses to *Pyrenophora tritici-repentis* race 1 and 5 evaluated in greenhouse.

Cross	$F_{2:3}$ and $F_{2:5}$ families R	Seg	S	Ratio tested	χ^2 value
Inheritance studies with race 1					
2000 Spelt # 20/Kenyon	15	45	20	1:2:1	1.87*
CIMMYT # 18/Kenyon	14	44	22	1:2:1	1.50*
Altar Synthetic/Kenyon	13	42	25	1:2:1	3.13*
TT # 283/Coulter	17	33	20	1:2:1	0.36*
Allelism studies with race 1					
2000 Spelt # 20/Erik	80	0	0	1:0:0	-
Intros Line # 7/Erik	80	0	0	1:0:0	-
Altar Synthetic/Erik	80	0	0	1:0:0	-
TT # 283/Erik	70	0	0	1:0:0	-
Inheritance studies with race 5					
2000 Spelt # 20/Kenyon	33	14	33	7:2:7	1.83*
CIMMYT # 18/Kenyon	38	14	28	7:2:7	3.28*
Altar Synthetic/Kenyon	34	16	30	7:2:7	4.34*
TT # 283/Coulter	32	11	37	7:2:7	0.47*
Allelism studies with race 5					
2000 Spelt # 20/Erik	80	0	0	1:0:0	-
CIMMYT # 18/Erik	80	0	0	1:0:0	-
Altar Synthetic/Erik	80:	0	0	1:0:0	-
TT # 283/Erik	70	0	0	1:0:0	-
Coulter/Katepwa	4	---66--		1:15	0.85*

*Probability of obtaining deviations from the expected ratio is by chance alone and the observed population does not differ significantly from expected hypothesis.

REFERENCES

1. De Wolf, E. D., Effertz, R. J., Ali, S., and Francl, L. J. 1998. Vistas of tan spot research. Can. J. Plant Pathol. 20:349-370.
2. Elias, E., Cantrell, R. G., and Horsford, R. M., Jr. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Sci. 29:299-304.
3. Gamba, F. M., Lamari, L., and Brûlé-Babel, A. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Can. J. Plant Pathol. 20:401-407.
4. Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49-56.
5. Lamari, L., and Bernier, C. C. 1989. Toxin of *Pyrenophora tritici-repentis*: host specificity, significance in disease, and inheritance of host reaction. Phytopathology 79:740-744.
6. Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391-396.
7. Nagle, B. J., Frohberg, R. C., and Hosford, R. M. Jr. 1982. Inheritance of resistance to tan spot of wheat. Pages 40-45 in: Tan Spot of Wheat and Related Diseases Workshop. R. M. Hosford Jr., ed. North Dakota State University, Fargo, N.D.
8. Singh, P. K., and Hughes, G. R. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, races 1 and 2, in spring and winter wheat genotypes. Phytopathology 95:172-177.
9. Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., Elias, E. M., and Hughes, G. R. 2006. Identification of new sources of resistance to tan spot, Stagonospora nodorum blotch, and Septoria tritici blotch of wheat. Crop Sci. 46: 2047-2053.