Inheritance of Septoria Leaf Blotch (*S. tritici*) and Pyrenophora Tan Spot (*P. tritici-repentis*) Resistance in *Triticum aestivum* cv. Carifen 12

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ABSTRACT

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Wheat cultivar Carifen 12 from Chile possesses resistance to Septoria leaf blotch (*S. tritici*) and to Pyrenophora tan spot (*P. tritici-repentis*). Resistance of Carifen 12 to a mixture of two cultures of *S. tritici* was conditioned by a single dominant gene in the F_2 and F_3 from crosses with susceptible cultivars Triumph 64 and TAM W-101. Resistance of Carifen 12 to one culture of *P. tritici-repentis* was conditioned by a single recessive gene pair in the F_3 of Carifen 12 × TAM W-101. A chi-square test for independence indicated a probable association ($P = 0.05$–0.10) between genes for resistance to Septoria leaf blotch and Pyrenophora tan spot.

Additional key words: *Drechslera tritici-repentis*, genetics, *Mycosphaerella graminicola*

Septoria leaf blotch of wheat, caused by *S. tritici* Rob. ex Desm. (perfect state *Mycosphaerella graminicola* (Fckl.) Sanderson), has been reported in more than 50 countries (34). Severity of the disease is closely related to local rainfall frequency and cool weather (31). Consequently, severe epidemics have been sporadic in most geographic areas. Epidemic or near-epidemic levels of infection commonly cause losses ranging from 10 to 20% (2,32), but losses occasionally may be as high as 40% (5) or even 70% (12). Yield losses are often accompanied by corresponding decreases in grain weight (2,5,34).

Sources of resistance to Septoria leaf blotch are adequate to substantially reduce grain and forage losses (10,17,29), but only two cultivars, Oasis and Auburn, have been developed specifically for resistance to Septoria leaf blotch (22,23) and only eight reports (11,19,20,26–28,30,36) relative to inheritance of resistance have been published.

P. tritici-repentis (Died.) Drechs. (syn. *P. trichostoma* (Fr.) Fckl., conidial state *Drechslera tritici-repentis* (Died.) Shoem.) is pathogenic to many grasses throughout the world (13,14,16,21). The disease caused by *P. tritici-repentis* on wheat, commonly called tan spot, reduces both yield (1,15,33) and kernel weight (33). Only recently, tan spot has risen from a position of minor importance on wheat to one of high priority because of changing cultivar genotypes (3,8) and cultural practices (3,8,25,35). Preliminary genetic studies have indicated that resistance of wheat to tan spot may be conditioned either monogenically (6,7) or polygenically (21).

This paper reports the mode of inheritance of resistance to Septoria leaf blotch and Pyrenophora tan spot from a Chilean wheat cultivar, Carifen 12, in crosses with susceptible North American cultivars, Triumph 64 and TAM W-101. Preliminary reports were published earlier (7,9).

MATERIALS AND METHODS

Seed of *Triticum aestivum* L. 'Carifen 12,' 'Triumph 64,' and 'TAM W-101' were obtained from the Department of Agronomy, Oklahoma State University. Carifen 12 was used as the female parent in crosses with Triumph 64 and TAM W-101.

Septoria leaf blotch. Vernalized plants of the parent cultivars, F_1s, and F_2s were grown to maturity in deep (15-cm) plant flats. These flats were prepared by first filling a common flat (7.5 cm deep) to capacity with soil, then side frames of a second flat were set on those of the first and secured by nailing wood laths over the contact lines along the sides and ends. One hundred thirty plant bands were placed in the upper frame and filled with soil. A single parent, F_1, or F_2 seed was sown in each plant band. Twenty days after sowing, emerged plants were moved to cold frames, where nightly temperatures were near 0 C. After 43 days, they were returned to the greenhouse, where temperatures fluctuated between 10 and 30 C. Just before emergence of flag leaves of Triumph 64 and TAM W-101 and the penultimate leaf of Carifen 12, the plants were inoculated with a mixture of conidia from two cultures of *S. tritici*.

Thirteen seeds of individual F_3 families, descended from 72 randomly selected and tested F_2 plants from Carifen 12 × Triumph 64, and 13 seeds per F_3 family, descended from 387 similarly treated F_2 plants from Carifen 12 × TAM W-101, were sown in plant bands in deep flats. The plants were grown in a greenhouse and inoculated with a mixture of conidia from the same cultures used to test the F_2 when the third leaves were fully extended.

One of the two cultures (MT-5) used in the inoculum was obtained from A. L. Scharen (ARS, USDA, Department of Plant Pathology, Montana State University, Bozeman 59715). The second culture (ST-22) was isolated from a single pycnidium in an infected wheat plant grown near Stillwater, OK.

Inoculum preparation and application was essentially the same for both the F_2 and F_3 tests. Cultures were grown singly in 250-ml flasks containing 25 ml of liquid medium (2 g of yeast extract [U.S. Biochemical Corp., Cleveland, OH] and 4 g of malt extract [Difco] per liter of distilled water) for 8–10 days on a laboratory bench at 20–25 C. During incubation, they were shaken for a few seconds once or twice daily. Conidia and the medium were filtered through cheesecloth to remove mycelial fragments. After filtering, conidia and the medium of the two cultures were combined on an equal medium-volume basis to produce the inoculum. Counts using a hemacytometer indicated that, on the average, cultures MT-5 and ST-22 produced, respectively, 8.5×10^6 and 2.4×10^6 conidia per milliliter of medium used in the F_2 test and 10.7×10^6 and 5.7×10^6

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conidia per milliliter of medium used in the F_3 test. Unflavored gelatin (0.5 g dissolved in 25 ml of warmed, distilled water) was added to each 100 ml of inoculum as a sticker. Plants were inoculated three times at 24-hr intervals with 250 ml of inoculum per 1,000 plants. Inoculum was applied with a DeVilbiss atomizer at 5 psi of air pressure. Plants were then placed inside an opaque polyethylene chamber and kept moist for 96 hr with a timeclock-controlled mist blower. Temperatures in the chamber varied from 15 to 30 C. Plants were classified for reactions to *S. tritici* 19–21 days after inoculation.

Pyrenophora tan spot. From crosses of Carifen 12 × TAM W-101, residual seed of 141 F_3 families, derived from four F_1 plants, were available for analyzing inheritance of tan spot resistance. Seed of parent cultivars (not actual parent plants) and of each F_3 family were sown in plastic pots (5 × 5 cm). Ten days after sowing, the seedlings were inoculated with an isolate (designated PYOK-2) of *P. tritici-repentis* obtained from a naturally infected wheat leaf.

Inoculum was prepared by growing PYOK-2 in 25 ml of liquid V-8 medium in 250-ml flasks. When 12 days old, the cultures were macerated in the medium in groups of four in a Waring Blendor. Tap water was added to each group to bring the volume to 200 ml. The resulting suspensions of mycelial fragments were mixed and sprayed onto the seedlings with a DeVilbiss atomizer until runoff. Inoculated seedlings were kept moist for 48 hr at 20 C as described for inoculation with *S. tritici*, then moved to a greenhouse bench.

Ten days after inoculation, the plants were classed as either resistant or susceptible, and families as homozygous resistant, segregating resistant and susceptible, or homozygous susceptible. Plants per family ranged from 10 to 30, with an estimated mean near 15. (If all plants in a family were susceptible, the number was not recorded.) Chi-square tests were used to analyze data from the segregating populations.

RESULTS

Septoria leaf blotch. *Reactions of parents and F_1 plants.* Fifteen to 17 days after inoculation, ellipitical lesions 3–10 mm long and containing pycnidia in necrotic centers had developed in the inoculated leaves of Triumph 64 and TAM W-101. Within 20 days of inoculation, the lesions often coalesced to form irregular patterns of chlorosis and necrosis containing numerous pycnidia. Lesions did not develop in leaves of Carifen 12 or the F_1 plants. Small chlorotic flecks were observed on Carifen 12 and F_1 plants, but it was not determined that they were caused by *S. tritici*. The absence of lesion development in the F_1 plants indicated that resistance of Carifen 12 to cultures MT-5 and ST-22 of *S. tritici* was conditioned by one or more dominant gene(s).

Reactions of F_2 and F_3 populations. A total of 159 F_2 plants derived from three F_1 plants of Carifen 12 × Triumph 64 were classified for reactions to *S. tritici* (Table 1). The chi-square test for heterogeneity indicated that distribution of reaction types among progeny of the three F_1 plants was homogeneous ($0.8 < P < 0.9$), and the data were combined. Fifty-two plants were as resistant as Carifen 12 and the F_1, 74 developed small chlorotic spots or necrotic lesions (especially near the leaf tips) that in some instances contained pycnidia, and 33 developed large, spreading lesions with numerous pycnidia similar to those in Triumph 64. Seventy-two randomly selected F_3 families, descended from about half of the tested F_2 progeny of each F_1 plant, were tested and their reactions classified as follows: 14 homozygous resistant; 44 segregating resistant, moderately resistant, and susceptible; and 14 homozygous susceptible. These results of testing the F_2 and F_3 of Carifen 12 × Triumph 64 indicated that resistance of Carifen 12 to the combined cultures MT-5 and ST-22 of *S. tritici* was conditioned by a single, incompletely dominant gene.

The reactions of F_2 plants derived from five F_1 plants of Carifen 12 × TAM W-101 were similar to those in the preceding cross in appearance and distribution (Table 1). The chi-square test for heterogeneity indicated that the F_2 plants derived from the five F_1 plants may have been samples from the same population ($0.3 < P < 0.5$) and that the data could be combined. Of 387 F_2 plants tested, 89 were highly resistant, 199 moderately resistant, and 99 susceptible.

Thirty-eight F_3 families, representing about 1/10 of the F_2 population, were randomly selected for testing. Of these, 5 were homozygous for resistance to the combined cultures; 23 segregated for resistance, moderate resistance, and susceptibility; and 10 were homozygous for susceptibility (Table 1).

Pyrenophora tan spot. *Reactions of F_3 families.* Leaf reactions of F_3 plants closely resembled those of either resistant Carifen 12 or susceptible TAM W-101. Resistant plants developed dark brown or gray flecks occasionally surrounded by a chlorotic band and little if any tip chlorosis-necrosis. Susceptible plants developed spreading, often coalescing, chlorotic-necrotic spots and progressive tip necrosis that often extended to 50–90% of the leaf.

Homozygous resistant families were easily discernible; however, segregating and homozygous susceptible families could not always be separated with confidence for two reasons. First, reactions of presumably heterozygous plants in segregating families often failed to differ discretely from reactions of susceptible ones. Second, the small sizes of some families enhanced the probability that susceptible plants were erroneously classed resistant when they had merely escaped inoculum in quantities sufficient to cause necrosis. Consequently, when the data were analyzed, families classed as segregating and homozygous susceptible were combined (Table 2). The number of families classed as homozygous resistant and the combined segregating and homozygous susceptible ones were a good fit to a 1:3 ratio, indicative of a single gene pair for resistance. In a group of 30 clearly segregating families (among the 97 segregating ones), 285 plants were classed as resistant and 148 as susceptible. These numbers are skewed toward expectations for a 3:1 ratio (324.75:108.25) and indicate that resistance was dominant. Because evidence for a dominant gene was apparent in less than one-third of the segregating families, we concluded that resistance was recessive under conditions of our test.

Association of resistance genes. Fifty-one of the F_3 families tested for resistance to PYOK-2 were descendants of F_2 plants that had been classified for reactions to *S. tritici*. These families were divisible into four groups. Of 27 F_2 plants resistant to *S. tritici*, four F_3 families were homozygous for resistance to culture PYOK-2

Table 1. Segregation for resistance and susceptibility to *Septoria tritici* among F_2 plants and F_3 families from crosses of resistant cultivar Carifen 12 with susceptible cultivars Triumph 64 and TAM W-101

Cross and generation	Numbers of F_2 plants and F_3 families			
	Resistant	Intermediate (F_2) segregating (F_3)	Susceptible	P[a]
Carifen 12 × Triumph 64				
F_2	52	74	33	0.05–0.1
F_3[b]	14	44	14	0.10–0.2
Carifen 12 × TAM W-101				
F_2	89	199	99	0.5–0.7
F_3[c]	5	23	10	0.2–0.3

[a] Probability of fit to a 1:2:1 ratio of resistant, intermediate, and susceptible F_2 plants and homozygous resistant, segregating, and homozygous susceptible F_3 families.
[b] About half of the F_3 families from tested F_2 plants were randomly selected for testing.
[c] About 1/10 of the F_3 families from tested F_2 plants were randomly selected for testing.

and 23 were either segregating or homozygous susceptible. Of 24 F_2 plants either moderately resistant or susceptible to *S. tritici*, 10 were homozygous for resistance to culture PYOK-2 and 14 were either segregating or homozygous susceptible. A chi-square test for independence (Fisher's formula corrected for continuity) indicated that resistances to Septoria leaf blotch and Pyrenophora tan spot were associated ($P = 0.05–0.10$).

DISCUSSION

The reaction of Carifen 12 to *S. tritici* was easily distinguished from reactions of Triumph 64 and TAM W-101. Carifen 12 typically developed no lesions or very small ones at the tips of a few leaves. Triumph 64 and TAM W-101 always developed large, spreading lesions containing numerous pycnidia. Also, the reaction of F_1 plants did not present a classification problem because it was very similar to the reaction of Carifen 12.

Difficulty with reaction classifications was encountered in segregating populations because some plants developed large lesions without pycnidia, whereas others developed small to medium-sized lesions with a few pycnidia. The reactions of these plants were classified as intermediate to reactions of Carifen 12 and either Triumph 64 or TAM W-101. Also, the reactions of some plants classified first as highly resistant resembled the intermediate class when observed 5–10 days later. Although the reactions of individual plants were not always discrete, occasional classification errors of F_2 plant reactions (indicated by reactions of direct-descendant F_3 families) did not significantly affect the fit of the F_2 segregation to a 1:2:1 ratio. Data derived from testing the F_2 and F_3 generations of the crosses strongly indicated that resistance of Carifen 12 was conditioned by a single, incompletely dominant gene. In contrast, reaction of the F_1, by virtue of its similarity to the reaction of Carifen 12, indicated that resistance was completely dominant. We have no firm explanation for this inconsistency. We suggest, however, that intermediate reactions in the F_2 and F_3 indicated a response of heterozygotes to either unidentified segregating modifier genes or to subtle environmental influences. The concept that dominance of resistance can depend on genetic background has been demonstrated for resistance to leaf rust (4) and stem rust (24) of wheat.

The observation that the highly resistant reactions of some plants retrogressed to an intermediate level with time is not without precedent. Rillo and Caldwell (26) studied inheritance of the resistance of wheat cultivar Bulgaria 88 to *S tritici* and noted that ". . .resistant heterozygotes declined towards intermediacy 7–10 days after the first reading, whereas that of homozygotes was maintained." At this time, it is not known why resistance of wheat leaf tissue decreases as the leaves mature and senesce.

The fact that resistance of Carifen 12 to *S. tritici* is inherited monogenically is encouraging from the standpoint of breeding resistant wheat cultivars because a single gene usually can be transferred easily by backcrossing. Neither physiologic races nor the sexual stage of *S. tritici* have been shown to exist in the United States. Consequently, the effectiveness of monogenic resistance may endure for a substantial period of time. In contrast, a wide range of virulence attributed to physiological specialization occurs in populations of *P. tritici-repentis* (18). Also, the fleck reaction conditioned by a single gene in Carifen 12 appears as a hypersensitive response. Thus we anticipate that this resistance, based on a single gene, would not endure for long periods in widely sown cultivars.

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Table 2. Segregation for seedling reactions to isolate PYOK-2 of *Pyrenophora tritici-repentis* among F_3 families from crosses of Carifen 12 with TAM W-101

F_1 plant designation and statistics	Number of F_3 families[a]			P[b]
	Homozygous resistant	Segregating	Homozygous susceptible	
1	14	29	4	0.03–0.05
2	3	20	5	0.05–0.10
3	10	41	6	0.10–0.20
4	2	7	0	0.50–0.90
Total	29	97	15	0.20–0.30
Heterogeneity chi-square (3 df = 3.87, $P = 0.20–0.30$)				

[a] Because of small family sizes and intergradation of reactions among plants, segregating and homozygous susceptible families were combined for chi-square analysis.

[b] Probability of fit to a 1:3 ratio of homozygous resistant families to combined segregating and homozygous susceptible ones.

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