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

L. Lamari, S. E. Strelkov, A. Yahyaoui, J. Orabi, and R. B. Smith

First, second, and fifth authors: Department of Plant Science, University of Manitoba, Winnipeg, Canada, R3T 2N2; third author: International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria; and fourth author: Directorate of Agricultural Scientific Research, Damascus, Syria.

Accepted for publication 11 October 2002.

ABSTRACT

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.

Pyrenophora tritici-repentis, causal agent of tan spot, induces necrosis and chlorosis in its wheat host. The tan spot system conforms to the toxin model and three host-specific toxins have been identified (Ptr ToxA, Ptr ToxB, and putative Ptr ToxC). Processing of a collection of isolates, obtained in the Fertile Crescent and Caucasus regions, yielded two new virulence patterns. Isolate Az35-5 combined the virulences of races 2 and 5 and was classified in the new race 7. Isolates TS93-71B and TS93-71F

had a virulence pattern that combined those of races 2, 3, and 5 and were grouped in the new race 8. Southern analysis revealed that all three isolates possessed copies of the *ToxA* and *ToxB* genes, the first time the genes were found in a common background. The production of Ptr ToxA and Ptr ToxB by the isolates was confirmed by western blotting. Virulence patterns suggested that TS93-71B and TS93-71F may also produce Ptr ToxC, even though it was not present at detectable levels in culture filtrates. The identification of races 7 and 8 complete the theoretical maximum number of races that can be differentiated by three loci in the host ($2^3 = 8$), assuming a one-to-one relationship. It appears that the wheat/*P. tritici-repentis* system is a mirror image of the classical gene-for-gene relationship.

Pyrenophora tritici-repentis, the causal agent of tan spot, is a major pathogen of wheat worldwide. The pathogen induces two distinct symptoms, necrosis and chlorosis, in its wheat host (14). The induction of the two symptoms is the result of specific interactions between individual isolates of the pathogen and wheat lines/cultivars. Lamari and Bernier (15) reported the existence of physiologic variation in the pathogen and classified isolates into four pathotypes based on their ability to induce necrosis and chlorosis in specific wheat differential lines. The pathotype classification system, limited to a theoretical maximum of four groups, was later extended to account for cases where isolates induced, differentially, the same symptom in different wheat lines/cultivars. A race classification system was adopted (20) when isolates from North Africa were found to cause chlorosis in cv. Katepwa and line 6B662, previously resistant to chlorosis-inducing isolates. For practical reasons, pathotypes 1 to 4 were, respectively, renamed races 1 to 4 and race 5 was introduced to account for a newly identified virulence pattern. The race classification system, based on the reaction of isolates on individual wheat genotypes, can accommodate any number of races and is only limited by the uniqueness and number of differential wheat lines/cultivars used. To date, six races have been described on the basis of their virulence on three effective differential wheat genotypes, namely 'Glenlea' (necrosis) and lines 6B365 (race 3-type chlorosis) and 6B662 (race 5-type chlorosis) (11,28).

The wheat-*P. tritici-repentis* system conforms to the toxin model, in which compatibility results from an interaction between a pathogen-produced toxin and its putative receptor in the host.

Two host-specific toxins (Ptr ToxA and Ptr ToxB) were characterized (3,27,29,30,32) and their respective coding genes were cloned (4,5,21,25). A third toxin (Ptr ToxC) was reported in culture filtrates and has been purified and partially characterized (7). Ptr ToxA causes necrosis in 'Glenlea' and Ptr ToxB and Ptr ToxC cause chlorosis in lines 6B662 and 6B365, respectively. Three dominant and independently inherited genes were identified in susceptible wheats, each controlling susceptibility to a single toxin (11). 'Glenlea' carries the gene controlling sensitivity to Ptr ToxA (races 1 and 2), line 6B662 carries the gene for sensitivity to Ptr ToxB (races 5 and 6), and line 6B365 carries the gene for sensitivity to Ptr ToxC (races 1, 3, and 6). Ptr ToxC, the least known of the *P. tritici-repentis* toxins, is not readily produced in culture filtrate. To date, only a single race 1 isolate is known to produce it in vitro (7). However, the existence of Ptr ToxC and its role in the establishment of compatibility with its wheat host (i.e., consistent with a host-specific toxin) were predicted by several studies (10,11,17,18).

Virulence data obtained to date indicated that some individual races possess more than one host-specific toxin or virulence factor: race 1 appears to combine the virulence of race 2 (Ptr ToxA) and race 3 (Ptr ToxC), whereas race 6 combines the virulence of races 5 (Ptr ToxB) and 3 (Ptr ToxC). In spite of numerous surveys conducted over the past 2 decades in western Canada and two limited surveys in North Africa, races combining Ptr ToxA and Ptr ToxB have not been identified. Furthermore, Southern analysis of DNA from the currently known races of *P. tritici-repentis* indicated that the *ToxA* locus (Ptr ToxA) did not occur in races containing *ToxB* (Ptr ToxB) and vice-versa (4,25).

A collection trip was undertaken in the Fertile Crescent (Syria-Turkey) and the Caucasus region (Azerbaijan), considered to be the center of origin of wheat. Processing of the collection yielded two new virulence patterns. The objectives of this study were (i)

Corresponding author: L. Lamari; E-mail address: l_lamari@umanitoba.ca

Publication no. P-2003-0122-01R

© 2003 The American Phytopathological Society

to characterize two new races of *P. tritici-repentis*, and (ii) to discuss the implication of our finding on the general model of host-pathogen interactions in tan spot of wheat.

MATERIALS AND METHODS

Terminology. In the context of this manuscript, susceptibility and sensitivity refer to reactions of the host to the fungus and to the toxin, respectively. The reference to Ptr ToxC is based on the report of Effertz et al. (7) and inferred from host genetic and physiological studies (10,11,17,18,28). In this context, the term virulence refers to the ability of an isolate to establish a compatible interaction with its host and implies a qualitative nature (i.e., the presence or absence of necrosis/chlorosis).

Isolates. Three single-spore isolates with previously undescribed virulence patterns were selected for characterization. Two of these isolates were from a collection made near the Turkish-Syrian border (TS93-71B and TS93-71F) in the Fertile Crescent and the third (Az35-5) from a collection made in Azerbaijan (Caucasus region). Isolates ASC1, 86-124, Hy331-9, 90-2, Alg3-24, and AlgH2 were used as controls and represent races 1 to 6, described previously (15,19,20,28). An additional race 5 isolate (Az35-1) from Azerbaijan (same field as Az35-5) was also included as a control.

Differential set. A differential set consisting of four hexaploid lines/cultivars ('Glenlea', 6B365, 6B662, and 'Salamouni') and two tetraploid lines/cultivars ('Coulter' and 4B1149) with known reaction to all characterized races of *P. tritici-repentis* was used (19,28). One cultivar or line was planted per pot as five to six evenly spaced seeds, and all treatments were replicated three times. The choice of line 6B662 over 'Katepwa' in the differential set is justified by the fact that line 6B662 is sensitive to Ptr ToxB only, whereas 'Katepwa' is sensitive to both Ptr ToxA and Ptr ToxB. Genetic studies (11) revealed that line 6B662 had a single locus controlling sensitivity to Ptr ToxB and 'Katepwa' had two independently inherited loci controlling reactions to Ptr ToxA and Ptr ToxB (one locus for each toxin).

Inoculum and inoculation. Conidial inoculum was produced on V8-potato dextrose agar as described previously (14). Seedlings were inoculated at the 2-leaf stage with a suspension of 3,500 conidia per ml, to which 10 drops per liter of Tween 20 (polyoxyethylene sorbitol monolaurate) were added. Seedlings were sprayed until runoff with a deVilbiss-type sprayer connected to an air line and operated at a pressure of approximately 67 kPa. After inoculation, plants were placed in a misting chamber for 24 h at 22/18°C (day/night) and a 16-h photoperiod. The misting chamber consisted of a polyvinyl chloride frame (2 × 1.5 × 1.5 m³) covered with a clear plastic sheet and kept on a growth room bench. Continuous leaf wetness was provided by two ultrasonic humidifiers mounted outside the chamber and controlled by an electronic circuit attached to a leaf wetness sensor suspended approximately 10 cm above the seedlings (L. Lamari, unpublished data). The mist was injected into the chamber through each of two

openings in the plastic sheet on opposite sides of the chamber. After incubation under continuous leaf wetness, seedlings were moved to a growth room bench in the same growth room as the misting chamber and observed daily for symptom development. A treatment consisting of water and Tween 20 (10 drops per liter) was used as a negative control throughout this study.

Culture filtrates. Isolates were grown in Fries medium amended with 1.5% yeast extract (6) to produce culture filtrates. Desalting and partial cleaning of culture filtrates was performed as described previously for Ptr ToxA (16) and Ptr ToxB (27). Mycelial-free culture filtrates were diluted 1:50 (vol/vol) with sterile distilled water and tested for the presence of Ptr ToxA and Ptr ToxB by infiltrating approximately 20 µl of the diluted filtrates into leaves of 'Glenlea' (sensitive to Ptr ToxA), 'Salamouni' (insensitive to all known Ptr toxins), 6B662 (sensitive to Ptr ToxB), and 6B365 (sensitive to putative Ptr ToxC only). Four leaves of each of the cultivars/lines were separately infiltrated with culture filtrate from each of the tested isolates. After infiltration, plants were kept in a growth room under the conditions described previously. Precautions were taken to avoid shading due to overcrowding, because chlorosis caused by Ptr ToxB is light-dependent (26). Plants were rated 72-h postinfiltration as sensitive (+) or insensitive (−) to each of Ptr ToxA and Ptr ToxB. Leaves from plants used as controls received 20 µl of sterile distilled water. A Hagborg device (12) was used to infiltrate toxins or water into the leaves.

Southern analysis. Genomic DNA was extracted from mycelia of 9-day-old liquid cultures of *P. tritici-repentis* using cetyltrimethylammonium bromide according to established protocols (2). For Southern analysis, 4 µg of genomic DNA from each isolate were digested to completion with *Eco*RI or *Hind*III and size-fractionated on a 0.8% agarose gel. Separated DNA was transferred to Hybond N⁺ membranes (Amersham Canada, Oakville, Ontario) with a VacuGeneXL Vacuum Blotting System (Pharmacia Biotech, Baie d'Urfe, Quebec, Canada) as per manufacturer's instructions. Hybridizations were carried out over 16 h at 62°C in a solution composed of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's reagent, 0.5% (wt/vol) sodium dodecyl sulfate (SDS), and 25 µg of denatured salmon sperm DNA per ml (23). Membranes were probed either with an 892-bp cDNA clone of Ptr ToxA (GenBank Accession No. U79662) (4) or a 375-bp cDNA fragment of Ptr ToxB (GenBank Accession No. AF483831) (25). Probe DNA (25 ng) was labeled with [α -³²P]ATP following the random primer procedure described by Feinberg and Vogelstein (9). Washes of the membranes were performed at 62°C, initially in 2× SSC and 0.1% SDS, then in 1× SSC and 0.1% SDS, and finally in 0.5× SSC and 0.1% SDS. Washed blots were exposed at −70°C to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) with intensifying screens for varying periods of time.

Western analysis. Total soluble protein from concentrated culture filtrates of isolates Az35-5, TS93-1B, and TS93-1F was subjected to sequential salt fractionation with ammonium sulfate as

TABLE 1. Reaction of six wheat differential lines/cultivars to various isolates of *Pyrenophora tritici-repentis*

Cultivar or line ^a	Disease rating ^b									
	Race 1 ASC1	Race 2 86-124	Race 3 Hy331-9	Race 4 90-2	Race 5 Alg3-24	Race 6 Alg-H2	Race 5 Az35-1	Race 7 Az35-5	Race 8 TS93-71B	Race 8 TS93-71F
'Glenlea'	4-5 (N)	4-5 (N)	1-2	1	1	1	1	4 (N)	4 (N)	4 (N)
6B365	5 (C)	1-2	5 (C)	1	1	5 (C)	1-2	1-2	4-5 (C)	4-5 (C)
6B662	2	1	1-2	1	4 (C)	4-5 (C)	4-5 (C)	4-5 (C)	4-5 (C)	4-5 (C)
'Salamouni'	1	1	1	1	1	1	1	1	1	1
'Coulter'	4-5 (N)	4-5 (N)	4 (N)	1	4 (N)	4 (N)	4 (N)	4 (N)	4 (N)	4 (N)
4B1149	1	1	1	1	1	1	1	1	1	1

^a All cultivars and lines listed are hexaploid except, 'Coulter' and line 4B1149, which are tetraploid.

^b Plants were rated on a scale of 1 to 5 based on lesion type, where 1 and 2 represent resistance and 3 to 5 represent susceptibility. N, tan necrosis; C, chlorosis.

described previously (27). Material precipitating out at 0 to 25% salt saturation was discarded, and material precipitating out at 25 to 80% saturation was retained. Precipitates were dissolved in 10 ml each of 20 mM sodium acetate buffer (pH 4.6) and dialyzed against the same buffer. This material was used to test for the presence of Ptr ToxA, but to test for Ptr ToxB, the redissolved and dialyzed culture filtrates were further purified by passage through a carboxymethyl-Sephadex C25 cation exchange column as described previously (27). Toxic fractions were pooled, concentrated, and desalted. Thirty microliters of each sample was subjected to SDS-polyacrylamide gel electrophoresis (PAGE) in a Mini-Protean II electrophoresis cell (Bio-Rad, Mississauga, Ontario, Canada) with a Tris-tricine buffer system (2). Blotting of SDS-PAGE gels and immunological detection of proteins were performed as described previously (28) using 1/100 and 1/200 dilutions of the immunoglobulin G (IgG) fraction of Ptr ToxB and Ptr ToxA antiserum, respectively. Polyclonal antibodies

against Ptr ToxA and Ptr ToxB were raised in rabbits at the NaH Biological Lab (Dugald, Man., Canada) and the IgG fractions of the crude antisera were purified by affinity chromatography with a Protein A Antibody Purification Kit (Sigma-Aldrich, St. Louis).

RESULTS

Isolates. The control isolates (ASC1, 86-124, Hy331-9, 90-2, Alg3-24, and AlgH2) produced symptoms typical for the races they represent (Table 1). All isolates were avirulent on 'Salamouni', 'Erik', and 4B1149. Isolate Az35-1 produced, as expected, a virulence pattern identical to that of Alg3-24 (race 5). Isolates Az35-5, TS93-71B, and TS93-71F produced novel virulence patterns (Table 1). All three isolates induced necrosis in 'Glenlea' (Fig. 1) and 'Coulter' (data not shown) and chlorosis in line 6B662 (Fig. 1). They were differentiated by their

Fig. 1. Reactions of 'Glenlea' and lines 6B365 and 6B662 to inoculation with isolates Az35-5 (race 7) and TS93-71B (race 8) of *Pyrenophora tritici-repentis*. **A**, Reaction of line 6B365 to isolate Az35-5 (note the absence of chlorosis); **B**, necrosis induced by isolate Az35-5 in 'Glenlea'; **C**, chlorosis induced by isolate Az35-5 in 6B662; **D**, chlorosis induced by isolate TS93-71B in 6B365; **E**, necrosis induced by isolate TS93-71B in 'Glenlea'; and **F**, chlorosis induced by isolate TS93-71B in 6B662. Isolate TS93-71F (data not shown) produced reactions identical to TS93-71B.

TABLE 2. Toxic activity of culture filtrates from *Pyrenophora tritici-repentis* isolates Az35-1, Az35-5, TS93-71B, and TS93-71F on various wheat cultivars and lines

Isolate	Host reaction ^a					
	'Glenlea'	6B365	6B662	'Salamouni'	'Coulter'	4B1149
Az35-1	—	—	C	—	—	—
Az35-5	N	—	C	—	N	—
TS93-71B	N	—	C	—	N	—
TS93-71F	N	—	C	—	N	—

^a N, necrosis; C, chlorosis; —, no symptoms.

Fig. 2. Southern blot analysis of genomic DNA from various *Pyrenophora tritici-repentis* isolates with a Ptr ToxA cDNA probe. Genomic DNA was digested with *Eco*RI, separated on agarose gels, and blotted onto Hybond N⁺ membranes. Blots were probed with a radiolabeled cDNA clone of Ptr ToxA. **A**, Isolates Az35-5 (lane 1, race 7) and Az35-1 (lane 2, race 5) and **B**, isolates Alg-H2 (lane 1, race 6), TS93-71B (lane 2, race 8), TS93-71F (lane 3, race 8), and ASC1 (lane 4, race 1).

ability to cause chlorosis in line 6B365. Isolates TS93-71B and TS93-71F induced chlorosis in line 6B365, whereas Az35-5 did not (Fig. 1). No symptoms developed in seedlings sprayed with water.

Culture filtrates. Culture filtrates from isolates Az35-5, TS93-71B, and TS93-71F induced necrosis in 'Glenlea' and 'Coulter' and chlorosis in line 6B662, suggesting the presence of Ptr ToxA and Ptr ToxB, respectively (Table 2). Culture filtrate of isolate Az35-1 induced chlorosis in line 6B662 and failed to induce necrosis in 'Glenlea' and 'Coulter', the two Ptr ToxA-sensitive genotypes (Table 2). Culture filtrates from all of the isolates tested failed to produce any visible symptoms in cultivars/lines Salamouni, 'Erik', 4B1149, and 6B365 (Table 2).

Southern analysis. Southern blotting analysis using a Ptr ToxA cDNA clone as a probe revealed the presence of a single hybridizing fragment in genomic DNA from isolate Az35-5 (Fig. 2A, lane 1). A single hybridizing fragment was also observed in genomic DNA digests from isolates TS93-71B and TS93-71F (Fig. 2B, lanes 2 and 3). As expected, one fragment hybridized with the Ptr ToxA cDNA clone in genomic DNA from isolate ASC1 (Fig. 2B, lane 4), but none hybridized in DNA from isolates Az35-1 (Fig. 2A, lane 2) and Alg-H2 (Fig. 2B, lane 1).

When utilizing a Ptr ToxB cDNA clone as a probe, Southern blotting analysis revealed the presence of multiple hybridizing fragments in genomic DNA from isolates Az35-5 (Fig. 3, lane 2), TS93-71B (Fig. 3, lane 4), and TS93-71F (Fig. 3, lane 5). No hybridization signal was observed in genomic DNA from isolate ASC1 (Fig. 3, lane 6), but bands were detected in DNA from isolates Alg-H2 (Fig. 3, lane 1) and Az35-1 (Fig. 3, lane 3).

Western analysis. Western blotting analysis of protein precipitated from concentrated culture filtrates of isolates Az35-5, TS93-71B, and TS93-71F at 25 to 80% ammonium sulfate saturation revealed that the IgG fraction of rabbit Ptr ToxA antiserum reacted with a protein of approximately 14 kDa (Fig. 4, lanes 2, 3, and 4). Similarly, western blotting analysis of concentrated toxic fractions from carboxymethyl-Sephadex C25 chromatography revealed that

Fig. 3. Southern blot analysis of genomic DNA from various *Pyrenophora tritici-repentis* isolates with a Ptr ToxB cDNA probe. Genomic DNA was digested with *Hind*III, separated on an agarose gel, and blotted onto a Hybond N⁺ membrane. The blot was probed with a radiolabeled cDNA clone of Ptr ToxB. Shown are isolates Alg-H2 (lane 1, race 6), Az35-5 (lane 2, race 7), Az35-1 (lane 3, race 5), TS93-71B (lane 4, race 8), TS93-71F (lane 5, race 8), and ASC1 (lane 6, race 1).

the IgG fraction of rabbit Ptr ToxB antiserum reacted with a protein approximately 6 kDa in size in the same isolates (Fig. 5, lanes 2, 3, and 4).

DISCUSSION

It was known from previous studies that physiologic variation existed in *P. tritici-repentis* and that virulence of the pathogen was qualitative and highly specific to host genotypes. Prior to this study, six races were described based on a set of differential wheat lines (15,20,28), three of which (6B365, 6B662, and 'Glenlea') had single loci controlling reaction to the known races of *P. tritici-repentis* (10,11).

Two new virulence patterns were characterized in this study: (i) isolate Az35-5 combines the virulences of races 2 and 5 in that it causes necrosis in 'Glenlea' (similar to race 2) and chlorosis in line 6B662 (similar to race 5), and (ii) the virulence of isolates TS93-71B and TS93-71F appears to be a combination of the virulences of races 2 (necrosis on 'Glenlea'), 3 (chlorosis on 6B365), and 5 (chlorosis on 6B662). Because these two virulence patterns are reported for the first time, we propose that the pattern represented by isolate Az35-5 be classified as race 7 and that of TS93-71B and TS93-71F be classified as race 8.

Fig. 4. Western blot analysis of protein from various *Pyrenophora tritici-repentis* isolates with polyclonal antibodies specific for Ptr ToxA. Thirty microliters of the redissolved and desalted 25 to 80% ammonium sulfate fraction from culture filtrates of the isolates was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to protein gel blot analysis with the immunoglobulin G fraction of Ptr ToxA antisera. Shown are isolates Az35-5 (lane 2, race 7), TS93-71B (lane 3, race 8), and TS93-71F (lane 4, race 8). Lane 1 contained pure Ptr ToxA (30 ng).

The identification of races 7 and 8 is highly significant in that these races possess both *ToxA* and *ToxB* loci, associated with, respectively, Ptr ToxA (4,5) and Ptr ToxB (21,25; G. M. Ballance, *personal communication*). Although Ptr ToxC (or the factor associated with chlorosis on 6B365) was previously found in combination with Ptr ToxA (necrosis on 'Glenlea') in race 1 (15) or Ptr ToxB in race 6 (28), Ptr ToxA and Ptr ToxB always occurred in separate backgrounds and appeared to be mutually exclusive. Southern analysis was used to confirm the presence in races 7 and 8 of *ToxA* and *ToxB* (Figs. 2 and 3). We have also confirmed the production of Ptr ToxA and Ptr ToxB (or similar proteins) in the culture filtrates of isolates Az35-5 (race 7) and TS93-71B and TS93-71F (race 8) using bioassays (Table 2) and western blotting techniques (Figs. 4 and 5). Of the eight races described to date, race 8 is the only one that combines virulence to all three effective differential genotypes (6B365, 6B662, and 'Glenlea') and is regarded as the most complex race identified so far.

In spite of sustained annual surveys of the pathogen in western Canada over almost 2 decades and the presence of the *ToxA* and *ToxB* loci in the pathogen's population (15,19,20,28), these two loci were never reported to occur together in single isolates, such as in isolates of races 7 and 8 described in this study. We believe that this may be due, at least in part, to the selection pressure exerted by the host on the pathogen. Cultivars grown in western Canada, and probably elsewhere in North America, are mainly susceptible to necrosis. This is consistent with our findings that races 1 and 2, which produce Ptr ToxA, the toxin responsible for inducing necrosis, are the predominant races in the Prairies (19). A study conducted by our group (1) using random amplified polymorphism DNA analysis suggested that races 1 and 2 were closely related and, as a group, distinct from races 3, 4, 5, and 6. Based on the evidence available to us prior to this study, we hypothesized that more complex races were likely to be found in or near the center of origin of wheat, which is by definition the region of greatest variability of the species (13,31). We believe that the presence of these complex races in Syria (Fertile Crescent) and Azerbaijan (Caucasus) is intimately associated with the variability of the host. It is likely that the local wild host population and the cultivation of land races in some areas exerted selection pressure to maintain the various virulence factors (such as Ptr ToxA, Ptr ToxB, and Ptr ToxC) in high frequency. Further investigation on the genetic structure of the pathogen and the host in those areas is needed.

Ptr ToxC, which is assumed to be responsible for inducing chlorosis in line 6B365, is not readily produced in culture filtrates and is believed to be highly unstable (S. W. Meinhardt, *personal communication*). However, Ptr ToxC was predicted (11,17) and isolated from a single isolate (race 1) by Effertz et al. (7). The fact that Ptr ToxC was not produced in culture filtrates under our

Fig. 5. Western blot analysis of protein from various *Pyrenophora tritici-repentis* isolates with polyclonal antibodies specific for Ptr ToxB. The redissolved and desalted 25 to 80% ammonium sulfate fraction from culture filtrates of each isolate was further purified by passage through a carboxymethyl-Sephadex C25 chromatography column and toxic fractions were pooled and concentrated. Thirty microliters of each sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to protein gel blot analysis with the immunoglobulin G fraction of Ptr ToxB antisera. Shown are isolates Az35-5 (lane 2, race 7), TS93-71B (lane 3, race 8), and TS93-71F (lane 4, race 8). Lane 1 contained pure Ptr ToxB (1 µg).

conditions does not negate its existence in the isolates used in this study. Compounds capable of differentially inducing chlorosis in line 6B365 were present in intercellular washing fluid (in planta) of wheat cultivars infected by races known to cause chlorosis on line 6B365 (L. Lamari, *unpublished data*). Irrespective of the nature of the factor associated with the differential induction of chlorosis in line 6B365, it appears to be responsible for the establishment of compatibility (similar to a host-specific toxin).

The identification of races 7 and 8 complete the theoretical maximum number of races that can be differentiated by three loci in the host ($2^3 = 8$), assuming a one-to-one relationship. These three independently inherited loci, found in 'Glenlea', 6B365, and 6B662, were previously shown to control reactions to race 2/Ptr ToxA, race 3/Ptr ToxC, and race 5/Ptr ToxB, respectively (11). Using an analysis similar to the "ideal gene-for-gene" model described by Person (22), we expect the races to carry combinations of virulence factors (toxins) as predicted by the binomial series:

$$(x + y)^n = \sum_{i=0}^n C_n^i x^i y^{n-i}$$

where n is the total number of virulence factors (toxins), i is the number of toxins in each race, $C_n^i = n!/(n-i)!i!$, and x and y represent the tox^+ and tox^- states, respectively. By making x and y each equal to 1, the binomial coefficient C_n^i represents the number of races that carry i toxins ($i = 0, 1, 2, \dots, n$) and, therefore, lack $(n-i)$ toxins. Based on the above binomial expansion with three virulence factors matching three loci in the host, we should expect a total of eight races $[(x + y)^3 = (1 + 1)^3 = 2^3 = 8]$, with a 1:3:3:1 distribution for the number of races carrying 0, 1, 2, and 3 toxins, respectively. The eight currently characterized races of *P. tritici-repentis* fit this distribution perfectly: race 4 carries no toxin/virulence factor and is avirulent, races 2, 3, and 5 carry one toxin/virulence factor, races 1, 6, and 7 carry two toxins/virulence factors, and race 8 carries three toxins/virulence factors (Table 3). Similarly, crosses made among hexaploid wheat genotypes resulted in progeny that possessed all possible combinations of loci controlling reaction to the three toxins of *P. tritici-repentis* (11).

Reaction to *P. tritici-repentis* is controlled by major genes, inherited in a Mendelian fashion (8,10,11,16,17,24). Similarly, sensitivity to the toxins is controlled by single, dominant, and independently inherited genes, with one gene for each toxin (10, 11). The combined results of this and previous studies, therefore, suggest that the relationship between wheat and *P. tritici-repentis* is very similar to the one reported in pathosystems that follow the classical gene-for-gene model (i.e., a one-to-one relationship). However, the basis for specificity in tan spot of wheat is compatibility, which results from interactions between individual host-specific toxins and their respective putative host receptors

TABLE 3. Relationship between races and host-specific toxins in *Pyrenophora tritici-repentis*

Cultivar or line	Race							
	4	2	1	8	7	5	6	3
'Glenlea'		Ptr ToxA						
6B662				Ptr ToxB				
6B365			Ptr ToxC ^a				Ptr ToxC ^a	
Compatible loci/number of toxins ^b	0	1	2	3	2	1	2	1

^a Putative toxin or pathogenicity factor.

^b The eight races follow a 1:3:3:1 distribution for 0, 1, 2, and 3 toxins, respectively, corresponding to the three host loci: race 4 (0 toxin); races 2, 3, and 5 (1 toxin); races 1, 6, and 7 (2 toxins); and race 8 (3 toxins).

(targets). In the classical gene-for-gene model, the basis for specificity is incompatibility. Thus, it would appear that the model we describe for tan spot is a perfect mirror image of the classical gene-for-gene relationship. To our knowledge, tan spot is the only reported system in which a single pathogen produces multiple host-specific toxins that can differentially attack different lines or cultivars of a single host species. It provides a good opportunity to further explore the interrelationship between the toxin and gene-for-gene models.

ACKNOWLEDGMENTS

This study was funded by the Natural Science and Engineering Research Council of Canada (awarded to L. Lamari), the Canadian Wheat Board and the University of Manitoba (awarded to S. E. Strelkov), and the International Center for Agricultural Research in the Dry Areas (awarded to A. Yahyaoui). We thank ICARDA for facilitating the survey of tan spot of wheat in Syria, Azerbaijan, and Central Asia, G. M. Ballance for providing the Ptr ToxA cDNA clone and the antibodies against Ptr ToxA, R. F. Kowatsch for help with the Southern blot of *ToxB*, and L. Ciuffetti (Oregon State University) for interesting comments.

LITERATURE CITED

- Aung, T. S. T. 2001. Molecular polymorphism and virulence in *Pyrenophora tritici-repentis*. M.Sc. thesis. University of Manitoba, Winnipeg, Canada.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (eds.) 1996. Current Protocols in Molecular Biology. John Wiley & Sons, New York.
- Ballance, G. M., Lamari, L., and Bernier, C. C. 1989. Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. *Physiol. Mol. Plant Pathol.* 35:203-213.
- Ballance, G. M., Lamari, L., Kowatsch, R., and Bernier, C. C. 1996. Cloning, expression and occurrence of a gene encoding Ptr-necrosis toxin from *Pyrenophora tritici-repentis*. *Mol. Plant Pathol.* Online Publication 1996/1209ballance.
- Ciuffetti, L. M., Tuori, R. P., and Gaventa, J. M. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. *Plant Cell* 9:135-144.
- Dhingra, O. D., and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, FL.
- Effertz, R. J., Meinhardt, S. W., Andersen, J. A., Jordahl, J. G., and Franci, L. J. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. *Phytopathology* 92:527-533.
- Faris, J. D., Anderson, J. A., Franci, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology* 86:459-463.
- Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
- Gamba, F. M., and Lamari, L. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). *Can. J. Plant Pathol.* 20:408-414.
- 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.
- Hagborg, W. A. F. 1970. A device for injecting solutions and suspensions

- into thin leaves of plants. *Can. J. Bot.* 48:1135-1136.
- Harlan, J. R. 1987. Centers of origin. Pages 15-21 in: CRC Handbook of Plant Science in Agriculture. Vol. 1. B. R. Christie, ed. CRC Press, Boca Raton, FL.
- Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion types. *Can. J. Plant Pathol.* 11:49-56.
- Lamari, L., and Bernier, C. C. 1989. Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions. *Can. J. Plant Pathol.* 11:284-290.
- 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.
- Lamari, L., and Bernier, C. C. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. *Phytopathology* 81:1092-1095.
- Lamari, L., and Bernier, C. C. 1994. Temperature induced resistance to tan spot of wheat (*Pyrenophora tritici-repentis*). *Can. J. Plant Pathol.* 16:279-286.
- Lamari, L., Gilbert, J., and Tekauz, A. 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western Canada. *Can. J. Plant Pathol.* 20:396-400.
- Lamari, L., Sayoud, R., Boulif, M., and Bernier, C. C. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: Implications for the current pathotype classification system. *Can. J. Plant Pathol.* 17:312-318.
- Martinez, J. P., Ottum, S. A., Ali, S., Franci, L. J., and Ciuffetti, L. M. 2001. Characterization of the *ToxB* gene from *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 14:675-677.
- Person, C. 1959. Gene-for-gene relationships in host-parasite systems. *Can. J. Bot.* 37:1101-1129.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Stock, W. S., Brûlé-Babel, A. L., and Penner, G. A. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese Spring. *Genome* 39:598-604.
- Strelkov, S. E. 2002. Occurrence and expression of *ToxB* and comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. Ph.D. thesis. The University of Manitoba, Winnipeg, Canada.
- Strelkov, S. E., Lamari, L., and Ballance, G. M. 1998. Induced chlorophyll degradation by a chlorosis toxin from *Pyrenophora tritici-repentis*. *Can. J. Plant Pathol.* 20:428-435.
- Strelkov, S. E., Lamari, L., and Ballance, G. M. 1999. Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 12:728-732.
- Strelkov, S. E., Lamari, L., Sayoud, R., and Smith, R. B. 2002. Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. *Can. J. Plant Pathol.* 24:29-35.
- Tomás, A., Feng, G. H., Reeck, G. R., Bockus, W. W., and Leach, J. E. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Mol. Plant-Microbe Interact.* 3:221-224.
- Tuori, R. P., Wolpert, T. J., and Ciuffetti, L. M. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 8:41-48.
- Vavilov, N. I. 1951. The Origin, Variation, Immunity and Breeding of Cultivated Plants: Selected Writings. Chronica Botanica Co., Waltham, MA.
- Zhang, H.-F., Franci, L. J., Jordahl, J. G., and Meinhardt, S. W. 1997. Structural and physical properties of a necrosis-inducing toxin from *Pyrenophora tritici-repentis*. *Phytopathology* 87:154-160.