Evaluation of a Barley Core Collection for Spot Form Net Blotch Reaction Reveals Distinct Genotype-Specific Pathogen Virulence and Host Susceptibility

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ABSTRACT

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Spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata* is a major foliar disease of barley (*Hordeum vulgare*) worldwide. SFNB epidemics have recently been observed in major barley producing countries, suggesting that the local barley cultivars are not resistant and that virulence of the local pathogen populations may have changed. Here we attempt to identify sources of resistance effective against four diverse

isolates of *P. teres* f. *maculata* collected from around the world. A total of 2,062 world barley core collection accessions were phenotyped using isolates of the pathogen collected in the United States (FGO), Australia (SG1), New Zealand (NZKF2), and Denmark (DEN 2.6). Isolate-specific susceptibility was identified in several of the barley accessions tested, indicating variability in both pathogen virulence and host resistance/susceptibility. Collectively, only 15 barley accessions were resistant across all isolates tested. These resistant accessions will be used to generate mapping populations and for germplasm development. Future research will involve the characterization of host resistance, pathogen virulence, and the host-pathogen interaction associated with SFNB of barley.

Barley (*Hordeum vulgare* L.) is one of the major crops of the United States and the world. Barley production faces several limiting factors including several diseases that have the potential to induce significant losses. Net blotch is a major foliar disease of barley and has two forms, net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* and spot form net blotch (SFNB) caused by *P. teres* f. *maculata*. SFNB epidemics have been reported in major barley-producing countries of the world including the United States, Canada, Australia, Denmark, Norway, and South Africa (reviewed in 18,20). SFNB yield losses have been reported up to 44% in Australia (12,15). An increase in SFNB has recently been observed in the Northern Great Plains of the United States (17). The increases in disease epidemics in these areas are likely due to shifts in the virulence within the pathogen population, which are likely in response to changes in resistance/susceptibility in the prevalent cultivars grown.

Disease management tactics for SFNB consist of fungicide application, inoculum reducing cultural practices, and the introgression of resistance into popular cultivars (22,26). Very little research has been documented to recommend specific barley accessions as effective resistance sources for SFNB. William et al. (34) evaluated 96 Australian and international accessions for SFNB resistance using a mix of south Australian isolates, identifying only a few resistant barley accessions. Likewise, McLean et al. (22) evaluated the responses of 95 barley accessions and cultivars to SFNB seedling and adult plant resistance in Australia and Canada and found that only two were resistant to all isolates tested, whereas

15 accessions, including cultivars, were resistant to the Canadian isolates tested and a mixture of Australian isolates at the adult plant stage. Gupta et al. (10) also studied the response of barley accessions to *P. teres* f. *maculata* and found that six were resistant across most of the western Australian isolates tested. In the current research project we are using the barley core collection (BCC), which consists of a diverse set of 2,062 accessions selected based on a sampling of the country of origin (33) to represent the global genetic diversity of cultivated barley and barley landraces, to identify SFNB resistance.

P. teres f. *maculata* is a sexually reproducing fungus that is pathogenically variable within and between the barley-growing regions (1,9,13,31). A given source of resistance may be effective only toward a specific pathotype and may be susceptible to other pathotypes from other regions. Karki and Sharp (13) compared the virulence of *P. teres* f. *maculata* populations collected from the Mediterranean region and Montana (United States) using 20 differential barley accessions found that approximately half of the accessions showed a differential response. Tekauz et al. (31) identified 20 different pathotypes of *P. teres* f. *maculata* by evaluating a Canadian pathogen population using 12 differential barley genotypes. Gupta et al. (10) studied the diversity of *P. teres* f. *maculata* in Australia and found that the pathogen population was highly diverse. Gupta et al. (10) also found an opposite reaction in barley cultivar Betzes, in which a pathogen population from Victoria induced a susceptible reaction and a pathogen population from Western Australia induced a resistant reaction, showing different virulence spectra was present between *P. teres* f. *maculata* populations within Australia. Collectively, these studies indicated that the virulence of *P. teres* f. *maculata* was complex and needed further investigation before we could have confidence in the durability of any source of resistance.

The nature of host resistance involved in the SFNB interaction is not fully understood. Previous studies have reported involvement of single genes (34,35), complementary genes (11), and multiple

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genes (35) in the SFNB interaction. Additionally, resistance has been mapped to several barley chromosome arms and in some cases the resistance has been shown to be only effective at specific growth stages (reviewed in 18).

The objective of this study was to identify barley lines that harbored high levels of SFNB resistance effective against a geographically and pathogenically diverse set of *P. teres* f. *maculata* isolates to be used in population development aimed at the characterization of SFNB resistance, as well as in germplasm and cultivar development.

MATERIALS AND METHODS

Plant materials. A total of 2,062 accessions of barley were evaluated for resistance to SFNB (Supplementary Table 1). This collection, referred to as BCC, is made up of barley accessions representing 10% of the U.S. Department of Agriculture–Agriculture Research Service (USDA-ARS) National Small Grain Collection within cultivated *H. vulgare*, chosen based on country of origin (USDA-ARS, National Genetic Resources Program, and Germplasm Resources Information Network, National Germplasm Resources Laboratory) (23,33). The BCC was obtained from H. Bockelman at the USDA-ARS Small Grains Collection in Aberdeen, ID. Barley lines CIho 14219 and PI 67381 were used as resistant checks and Robust as a susceptible check.

Disease evaluations. All 2,062 barley lines were evaluated using four *P. teres* f. *maculata* isolates including FGOB10Ptm-1

(FGO) collected in North Dakota (from a 2010 collection housed in the Friesen lab), SG1 collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia), NZKF2 collected in New Zealand, and DEN 2.6 collected in Denmark (NZKF2 and DEN 2.6 isolates were obtained from B. Steffenson, University of Minnesota). These isolates were chosen due to their different geographical distribution and therefore likely harbored different virulence.

The 2,062 BCC lines were divided into 35 blocks; each block contained 60 barley accessions that fit into one rack surrounded by a border row of Robust barley to reduce any edge effect. Three barley seeds per line per replicate were sown into a single cone containing Metro mix soil (Sun Gro Hort. Co.). Plants were grown in the greenhouse until inoculation. Cones of 1-week-old seedlings were fertilized with approximately 2 g of Osmocote controlled release fertilizer (Scotts, Marysville, OH) having 15:9:12 NPK content. Barley accessions were inoculated as previously described by Friesen et al. (8). Briefly, dried fungal mycelial plugs were placed in petri plates containing V8-PDA media (150 ml of V8 juice, 10 g of Difco PDA, 3 g of CaCO₃, 10 g of agar, and 850 ml of distilled water). The plates were incubated in the dark at room temperature for 5 days and then subjected to 24 h continuous light at room temperature followed by another 24 h in the dark at 15°C to enhance sporulation. Conidia were harvested by scraping the surface of the plates with a sterile inoculating loop and the conidial concentration was adjusted to 2,000 conidia/ml. Before inoculation, Tween 20 was applied at 2 drops/100 ml to enhance uniform distribution of the spores and adherence to the leaf surface. Two-week-old seedlings were spray-inoculated to just before runoff using an air sprayer at 15 to 20 psi. Following inoculation, plants were incubated in a mist chamber for 22 to 24 h at 100% relative humidity under 24 h light to facilitate infection. The inoculated plants were then placed in a growth chamber under a 12 h photoperiod for 6 days at 21°C.

Disease reactions were evaluated 7 days postinoculation (dpi) using a 1 to 5 rating scale (Fig. 1). This rating scale categorizes the disease reaction based on lesion size and characteristic (Fig. 1). Three seedlings planted in a single cone were evaluated collectively as one replicate. Plants having approximately equal amounts of two reaction types were given an intermediate rating (e.g., equal amounts of reaction type 2 and 3 resulted in a 2.5 rating). The overall experiment consisted of three replicates.

Since little information has been reported on the progression of SFNB disease induced by *P. teres* f. *maculata*, we evaluated disease progression in a single susceptible barley cultivar (Tradition) with a single *P. teres* f. *maculata* isolate (FGO) (Fig. 2). Leaves were inoculated and kept in 100% relative humidity for the first 24 h as described above. Disease lesion progression was evaluated visually on the fully expanded secondary leaves (approximately 14-day-old seedlings at inoculation) at 24 h intervals until the 7th day (Fig. 2).

Additionally, using the 2,062 accessions in the BCC we photographed accessions that showed phenotypic differences (Fig. 3) including combinations of necrosis and chlorosis as well as regular and irregular lesion shapes. This allowed us to best identify a standard disease reaction type scale to evaluate levels of resistance/susceptibility across different barley backgrounds.

When evaluating reaction type differences between the four isolates used, a least significant difference (LSD) value of 1.055 ($P = 0.001$) was calculated between the four isolates used. A disease reaction type difference of 1.8 was chosen to identify lines that could be used to functionally differentiate the virulence of the four isolates that were used in this study. The 1.8 value was chosen for two reasons: (i) to obtain a manageable number of lines with differences and (ii) to guarantee that major virulence-resistance differences were being observed. Since the scale that we chose to use for evaluation (Fig. 1) ranges from 1 to 5, a difference of 1.8 gives us an extremely high degree of confidence that major differences are being observed, making the results found in the tables useful information in drawing conclusions about the

Fig. 1. Spot form net blotch disease rating scale. Disease reactions were scored on a 1 (top) to 5 (bottom) reaction type scale. Two leaves are shown for each reaction type. A disease reaction type of 1 (resistant) consisted of small pin-point lesions with no surrounding tan necrosis or yellow chlorosis, 2 consisted of pin-point lesions with a small amount of necrosis and/or chlorosis around the penetration point, 3 consisted of necrotic or chlorotic lesions of 2 to 3 mm in size with little or no coalescence of the lesions, 4 consisted of necrotic and chlorotic lesions of greater than 3 mm in diameter with moderate amounts of coalescing between lesions and covering less than 70% of the leaf area, and 5 consisted of necrotic or chlorotic lesions coalescing and covering greater than 70% of the leaf area.

Fig. 2. Spot form net blotch disease progression. Disease progression was from 1 day to 7 days postinoculation using the *Pyrenophora teres* f. *maculata* isolate FGO inoculated on the susceptible barley cultivar Tradition.

Fig. 3. Different lesion characteristics expressed by barley lines 7 days post-inoculation using the *Pyrenophora teres* f. *maculata* isolate FGO. **A and C,** Leaf show necrosis, **B,** leaf shows chlorosis, **D, E, and F,** leaves show a combination of both necrosis and chlorosis. Leaf A, B, C, and F have circular to elliptical lesions while D and E have elongated/irregular-shaped lesions. Additionally, leaf D and E show similar reactions, however, leaf D shows the adaxial leaf surface, while leaf E shows the abaxial surface of the leaf indicating that both leaf surfaces show a similar phenotype.

Fig. 4. Uniformly resistant barley lines 7 days postinoculation using isolates FGO, SG1, NZKF2, and DEN 2.6. Barley accessions used were the five most resistant accessions across all four *Pyrenophora teres* f. *maculata* isolates, including **A,** PI 61584, **B,** PI 296996, **C,** PI 640382, **D,** PI 84314, and **E,** PI 67381 for each isolate inoculation.

complexity of pathogen virulence and host resistance in this interaction.

Data analysis. Disease scores were calculated by taking the arithmetic mean of the disease scores across replicates. Standard deviation was calculated to show the variability in disease scoring between replicates. We considered the four isolates as different treatments applied to each barley accession and we performed a mean separation test using LSD. The LSD value was calculated using SAS 9.3 to evaluate the difference in virulence among the isolates (27).

RESULTS

Disease progression and phenotypes. SFNB disease progress was evaluated at 24 h intervals (Fig. 2). At 1 dpi, the inoculated leaves had a water-soaked appearance where the fungus had initiated penetration but no yellow or brown necrosis had developed. At 2 dpi, diffuse chlorosis was beginning to develop and at 3 dpi, lesions were becoming more distinct but continued to grow. Between 4 and 6 dpi the lesion size expanded slightly but after 6 days, lesion size changes were not obvious. Therefore, disease reaction types were evaluated at 7 dpi when the lesion size had become static but before leaf senescence.

When evaluating the BCC, several different phenotypes were identified. Several of these lesion phenotypes are presented in Figure 3. These phenotypes included various forms of necrosis (Fig. 3A and C) and chlorosis (Fig. 3B) and combinations of the two (Fig. 3D, E, and F). Most commonly, susceptible reactions were displayed as circular to oval lesions that consisted of light brown to brown necrosis similar to the leaves in Figure 3A and C.

Numerical disease reaction type scale. Based on the disease progress observed in the previous section, disease reaction types were evaluated at 7 dpi based on a 1 to 5 reaction type scale, where 1 was the most resistant and 5 was the most susceptible (Fig. 1). Due to the differences identified in lesion types across the BCC, we based this disease scale primarily on lesion size and disease coverage on the leaf. A disease reaction type of 1 (resistant) consisted of small pinpoint lesions with no surrounding tan necrosis or yellow chlorosis. A disease reaction type of 2 (moderately resistant) consisted of pinpoint lesions with a small amount of necrosis and/or chlorosis around the penetration point. A disease reaction type of 3 (moderately susceptible) consisted of necrotic or

chlorotic lesions of 2 to 3 mm across with little or no coalescence of the lesions. A disease reaction type of 4 (susceptible) consisted of necrotic and chlorotic lesions of greater than 3 mm in diameter with moderate amounts of coalescing between lesions and covering less than 70% of the leaf area. A disease reaction type of 5 (highly susceptible) consisted of necrotic or chlorotic lesions coalescing and covering greater than 70% of the leaf area.

Pathogen virulence. One of the objectives of this study was to identify barley accessions that showed a differential response among the four isolates used in this study for use in characterization of the genetics of pathogen virulence. When using a differential cutoff of 1.8, 13 accessions showed a differential reaction between FGO and SG1 with 12 of those being more susceptible to FGO than to SG1 and only one being more susceptible to SG1 than FGO. Similarly, 42 accessions showed a differential reaction between FGO and NZKF2 and 27 accessions showed a differential reaction between FGO and DEN 2.6 with only one and five of those being more susceptible to NZKF2 and DEN 2.6, respectively. When comparing SG1 with NZKF2 and DEN 2.6, SG1 showed higher virulence on 37 and 29 accessions, respectively, with NZKF2 and DEN 2.6 showing higher virulence to SG1 on only 8 and 10 accessions, respectively. When comparing NZKF2 and DEN 2.6, three accessions showed a differential reaction with all three accessions being more susceptible to DEN 2.6.

Additionally, we analyzed the phenotypic data to identify which barley lines showed differential reactions for more than one isolate combination. Using this information, we can speculate about which isolates contain unique or common virulence or avirulence factors based on the corresponding host resistance or susceptibility genes. Group I contains a single barley accession that indicates that the FGO isolate harbors a unique virulence not present in the other three isolates. Group II contains five barley accessions that indicate that FGO and SG1 harbor a common virulence not present in NZKF2 or DEN 2.6. Group III contains five barley accessions that indicate that SG1 likely harbors a unique virulence that is not present in NZKF2 or DEN 2.6; however, FGO is not clear in this interaction. Group IV contains two barley accessions that indicate that DEN 2.6, although less virulent overall, harbors virulence not present in the two most virulent isolates FGO and SG1. Group V contains five barley accessions that show that FGO and SG1 harbor virulences not present in NZKF2 with DEN 2.6 being less clear on these lines. Group VI contains six barley accessions that indicate that FGO

TABLE 1. Resistant barley lines having mean disease reaction scores of less than or equal to 2 across the four *Pyrenophora teres* f. *maculata* isolates tested^a

Barley accession	Country of origin	Row type	Accession type	Disease scores			
				FGO ^b	SG1 ^c	NZKF2 ^d	DEN 2.6 ^e
PI 67381	Turkistan	2	Breeding line	1.5 ± 0.4	1.0 ± 0.4	1.3 ± 0.2	1.5 ± 0.4
PI 84314	Uzbekistan	6	Landrace	1.2 ± 0.2	1.7 ± 0.4	1.0 ± 0.0	1.5 ± 0.4
PI 640382	United States	6	Breeding line	2.0 ± 0.7	1.2 ± 0.2	1.4 ± 0.4	1.0 ± 0.0
PI 296996	Argentina	2	Breeding line	1.7 ± 0.5	1.3 ± 0.4	1.7 ± 0.6	1.3 ± 0.5
PI 61584	Mongolia	6	Landrace	1.0 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	2.0 ± 0.0
PI 84311	Uzbekistan	6	Landrace	1.7 ± 0.6	1.3 ± 0.4	1.5 ± 0.5	1.5 ± 0.4
CIho 14214	Mongolia	6	Landrace	1.2 ± 0.2	1.3 ± 0.2	1.9 ± 0.2	1.6 ± 0.5
PI 84312	Uzbekistan	6	Landrace	2.0 ± 0.4	1.5 ± 0.0	1.3 ± 0.2	1.2 ± 0.2
PI 182625	Japan	6	Cultivar	1.7 ± 0.2	2.0 ± 0.0	1.3 ± 0.0	1.2 ± 0.2
CIho 14219	Mongolia	6	Landrace	1.5 ± 0.4	1.8 ± 0.5	1.2 ± 0.2	1.8 ± 0.2
PI 290316	Hungary	6	Cultivar	2.0 ± 0.8	1.0 ± 0.0	1.8 ± 0.6	1.5 ± 0.0
PI 28624	Iran	2	Landrace	1.3 ± 0.2	1.5 ± 0.4	1.8 ± 0.2	1.8 ± 0.6
PI 296998	Argentina	2	Breeding line	1.3 ± 0.2	1.6 ± 0.4	1.8 ± 0.2	1.8 ± 0.5
PI 60205	China	6	Landrace	1.5 ± 0.4	2.0 ± 0.0	2.0 ± 0.4	1.5 ± 0.4
CIho 15270	Canada	6	Landrace	1.7 ± 0.5	2.0 ± 0.3	2.0 ± 0.7	1.8 ± 0.5

^a Row type and accession type data were obtained from The Triticeae Tool Box (available at <http://triticeaetoolbox.org/>). Barley accessions were ordered based on disease reaction score.

^b FGO (FGOB10Ptm-1) collected in North Dakota.

^c SG1 = *P. teres* f. *maculata* isolate collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia).

^d NZKF2 = *P. teres* f. *maculata* isolate collected in New Zealand (obtained from B. Steffenson, University of Minnesota).

^e DEN 2.6 = *P. teres* f. *maculata* isolate collected in Denmark (obtained from B. Steffenson, University of Minnesota).

harbors virulence that is not present in NZKF2 or DEN 2.6 with SG1 being less clear on these lines. Group VII contains a single barley accession that shows that FGO and SG1 harbor virulences not present in DEN 2.6 with NZKF2 not being clearly defined. Group VIII contains two barley accessions that indicate that FGO and DEN 2.6 harbor a virulence that is not present in SG1, however, it is not clear from the data whether or not NZKF2 harbors the same virulence. Group IX contains a single barley accession that indicates that FGO and DEN 2.6 harbor a common virulence not present in NZKF2, with SG1 showing an intermediate reaction on this accession. Group X contains a single barley accession that shows that the two least virulent isolates overall harbor at least one virulence factor not present in the two most virulent isolates FGO and SG1. Group XI shows that NZKF2 and DEN 2.6 harbor a virulence that is not present in SG1 but FGO is less clear on this barley accession. Overall, these data show that each isolate used in this study likely harbor unique virulences but also virulences that are common with the other three isolates used.

Host line responses and identification of resistant lines.

The disease score patterns observed were distributed in a bell-shaped pattern across the BCC with each of the four *P. teres* f. *maculata* isolates. The disease reaction type score was categorized into five groups based on a numerical disease reaction; 1 to less than 1.5, 1.5 to less than 2.5, 2.5 to less than 3.5, 3.5 to less than 4.5, and 4.5 to 5. We found that only a few barley accessions fell under the resistant extreme (<1.5) or over the susceptible extreme (>4.5), with most of the accessions falling in an intermediate range of reaction types creating a bell-shaped distribution (Fig. 4). Out of the 2,062 barley accessions evaluated, only 50 showed mean disease reaction scores of less than 2 using the FGO isolate, similarly only 79, 182, and 244 accessions showed mean disease reactions of less than 2 to SG1, DEN 2.6, and NZKF2, respectively. Only 12, 21, 42, and 66 accessions showed mean disease reactions of 1 to less than 1.5 with FGO, SG1, DEN 2.6, and NZKF2 isolates, respectively. Additionally, only 15 accessions showed a disease reaction of less than or equal to 2 for all four isolates tested (Table 1).

DISCUSSION

Disease development and disease reaction characteristics. The development of the SFNB disease results from the infection process carried out by the pathogen *P. teres* f. *maculata*. The infection process includes the attachment of the spore to the leaf surface followed by spore germination, appressoria and penetration peg formation, the subsequent disease development, and finally sporulation (reviewed in 18). We observed

TABLE 2. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates FGO and SG1^a

Barley accession	Country of origin	FGO ^b	SG1 ^c
CIho 3694	Egypt	<u>3.5</u> ± 0.3	1.3 ± 0.4
PI 138711	Iran	<u>3.9</u> ± 0.2	1.8 ± 0.6
CIho 14882	Eritrea	<u>4.3</u> ± 0.3	2.3 ± 0.6
CIho 4050	Mongolia	<u>2.9</u> ± 0.5	1.0 ± 0.0
PI 410646	Pakistan	<u>3.8</u> ± 0.2	2.0 ± 0.7
CIho 4056	Mongolia	<u>3.5</u> ± 0.4	1.7 ± 0.6
PI 290366	Hungary	<u>3.2</u> ± 0.5	1.3 ± 0.2
PI 292019	Israel	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 138726	Iran	<u>4.0</u> ± 0.3	2.2 ± 0.6
PI 410483	Pakistan	<u>4.1</u> ± 0.2	2.3 ± 0.2
CIho 15339	Tunisia	<u>4.1</u> ± 0.6	2.3 ± 0.2
PI 573662	Georgia	<u>3.1</u> ± 0.6	1.4 ± 0.4
PI 533205	Pakistan	<u>1.0</u> ± 0.0	<u>2.8</u> ± 0.2

^a Underlined numbers indicate higher differential virulence for that isolate.

^b FGO (FGOB10Ptm-1) collected in North Dakota.

^c SG1 = *P. teres* f. *maculata* isolate collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia).

a periodic development of the disease beginning at 24 h after inoculation and progressing up to 7 days after inoculation (Fig. 2). Karki and Sharp (13) also noted that disease development started from the appearance of small water-soaked areas on the leaves 24 h after inoculation followed by the development of visible necrotic lesions 3 to 4 days later. Keon and Hargreaves (14) noted that the susceptible host cells started dying near or adjacent to the intracellular hyphae 2 days after inoculation (Fig. 2). A chlorotic area starts to develop around the initial necrotic lesion and is thought to be due to the secretion of diffusible toxins or effectors that induce programmed cell death (PCD) similar to other necrotrophic systems involving closely related fungi (18,19,24,25).

Karki and Sharp (13) noted different lesion characteristics in specific barley genotype–isolate combinations, where in general, Montana isolates induced more chlorotic and necrotic type lesions in several barley genotypes compared with Mediterranean isolates. In our study, we also found different lesion characteristics in specific barley accession–isolate combinations (Fig. 3). It is likely that specific isolates have different virulence factors that interact with corresponding host factors that confer a distinct visible symptom. Distinctly different visible symptoms have also been shown in the tan spot of wheat pathosystem caused by the closely related pathogen, *P. tritici-repentis*. In the tan spot of wheat interaction, depending on the host selective toxin (HST)–host gene combination, infection results in necrotic or chlorotic spots, spreading chlorosis, or a combination of necrosis and chlorosis (reviewed in 3). These different symptoms have been traced back to three HSTs including Ptr ToxA, Ptr ToxB, and Ptr ToxC and the corresponding host sensitivity genes, *Tsn1*, *Tsc2*, and *Tsc1*, respectively (2–4,6). The different symptoms present in the SFNB pathosystem also indicate the involvement of different pathogen–host combinations that result in different visible phenotypes resulting from a complex system of host–pathogen interactions.

TABLE 3. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates FGO and DEN 2.6^a

Barley accession	Country of origin	FGO ^b	DEN 2.6 ^c
PI 138711	Iran	<u>3.9</u> ± 0.2	1.0 ± 0.4
PI 327667	Tajikistan	<u>4.5</u> ± 0.4	2.0 ± 0.0
PI 410451	Pakistan	<u>4.3</u> ± 0.6	1.8 ± 0.5
PI 410525	Pakistan	<u>3.7</u> ± 0.6	1.5 ± 0.4
PI 38305	Russian Federation	<u>4.3</u> ± 0.2	2.2 ± 0.5
PI 244823	Pakistan	<u>3.0</u> ± 0.0	1.0 ± 0.0
PI 64004	Algeria	<u>3.8</u> ± 0.2	1.8 ± 0.8
CIho 7491	Mexico	<u>3.2</u> ± 0.2	1.2 ± 0.2
PI 565826	China	<u>3.7</u> ± 0.2	1.7 ± 0.5
PI 402037	Colombia	<u>2.9</u> ± 0.5	1.0 ± 0.0
PI 190790	Korea, North	<u>3.6</u> ± 0.2	1.8 ± 0.3
PI 243184	France	<u>3.3</u> ± 0.2	1.5 ± 0.4
PI 611592	Tajikistan	<u>3.8</u> ± 0.2	2.0 ± 0.7
PI 34424	Venezuela	<u>3.3</u> ± 0.5	1.5 ± 0.4
PI 145693	Saudi Arabia	<u>3.7</u> ± 0.5	1.8 ± 0.6
PI 190786	Korea, North	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 327833	Uzbekistan	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 478431	Bolivia	<u>3.0</u> ± 0.4	1.2 ± 0.2
PI 564649	Oman	<u>3.7</u> ± 0.2	1.8 ± 0.6
PI 39592	Venezuela	<u>3.5</u> ± 0.4	1.7 ± 0.6
PI 328632	Greece	<u>4.3</u> ± 0.2	2.5 ± 0.4
PI 57065	Algeria	<u>4.3</u> ± 0.3	2.5 ± 0.8
PI 467731	Norway	<u>2.2</u> ± 0.6	<u>4.0</u> ± 0.4
PI 548761	Israel	<u>2.2</u> ± 0.5	<u>4.0</u> ± 0.4
PI 573659	Georgia	<u>1.3</u> ± 0.2	<u>3.3</u> ± 0.2
PI 356226	Morocco	<u>1.3</u> ± 0.5	<u>3.3</u> ± 0.2
PI 304912	Morocco	<u>1.2</u> ± 0.2	<u>3.3</u> ± 0.2

^a Underlined numbers indicate higher differential virulence for that isolate.

^b FGO (FGOB10Ptm-1) collected in North Dakota.

^c DEN 2.6 = *P. teres* f. *maculata* isolate collected in Denmark (obtained from B. Steffenson, University of Minnesota).

Tekauz (30) proposed an SFNB rating scale that included ratings of 1 to 9, with 1 to 3 being resistant, 5 being intermediate, and 7 to 9 being susceptible; however, in this scale no 4 or 6 ratings were described, making statistical analysis problematic. Karki and Sharp (13) proposed a 1 to 10 rating scale that was based on a combination of lesion size and leaf tip necrosis that we rarely observed. Therefore, we have proposed an SFNB disease rating scale (Fig. 1) that is based on the 1 to 5 disease rating scale proposed by Lamari et al. (16) for use with *P. tritici-repentis*, causal agent of tan spot of wheat, where 1 is most resistant and 5 is most susceptible. Our proposed SFNB scale takes into consideration primarily lesion size but also percent leaf area coverage in the higher reaction types.

Based on reports in the literature of proteinaceous and non-proteinaceous toxins being present in the SFNB and NFNB systems (reviewed in 18), it is likely that underlying this quantitative trait, there are several effector–host gene interactions that, in combination with one another, result in the different visual phenotypes and varying disease reaction scores. Several of the Pleosporales pathogens in the Dothideomycete class of fungi produce necrotrophic effectors that interact with dominant host susceptibility gene products (reviewed in 28,36). The work

presented here lays the groundwork for evaluating the genetics of this host–pathogen interaction. Based on this work and the work presented in the adjoining paper (29), we have provided information about specific accessions and pathogen isolates that are currently being used in host and pathogen population development. The genetic characterization of both virulence and resistance is critical to a complete understanding of this system.

Diversity in pathogen virulence. The isolates used in this study were collected from four different barley-growing regions on three different continents and were therefore expected to have some genetic diversity. Several of the barley accessions evaluated showed a differential reaction between the isolates tested, indicating that each isolate used contained a different but functionally overlapping set of virulence factors. Interestingly, the FGO isolate collected in North Dakota, where epidemics have recently occurred, harbored the broadest virulence, with only a few accessions showing lower levels of susceptibility to FGO than the other three. The epidemic in North Dakota has been fairly recent and may be a result of a virulence shift where the local *P. teres* f. *maculata* population has adapted to current local barley cultivars. Previous studies have also reported a higher virulence of isolates collected in the Northern Great Plains of the United States and the Canadian prairie region compared with other regions (13,31,32). SFNB epidemics have also recently been reported in Australia (20,21) and we found that the average virulence of the Australian isolate SG1 was somewhat lower than the U.S. isolate but higher than isolates collected in New Zealand and Denmark (Tables 2 to 7). Based on our results collected for these four isolates across the 2,062 barley accessions, it is likely that the four isolates harbor several common virulence factors; however, each isolate also harbors at least one unique factor with the FGO isolate harboring the most unique virulence factors followed

TABLE 4. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates FGO and NZKF2^a

Barley accession	Country of origin	FGO ^b	NZKF2 ^c
PI 410525	Pakistan	<u>3.7</u> ± 0.6	1.2 ± 0.2
PI 145693	Saudi Arabia	<u>3.7</u> ± 0.5	1.4 ± 0.4
Ciho7491	Mexico	<u>3.2</u> ± 0.2	1.0 ± 0.0
PI 327833	Uzbekistan	<u>3.2</u> ± 0.2	1.0 ± 0.0
PI 138711	Iran	<u>3.9</u> ± 0.2	1.8 ± 0.2
PI 328498	Greece	<u>4.3</u> ± 0.2	2.2 ± 0.8
PI 410451	Pakistan	<u>4.3</u> ± 0.6	2.2 ± 0.6
PI 38305	Russian Federation	<u>4.3</u> ± 0.2	2.2 ± 0.8
Ciho 4072	Mongolia	<u>3.3</u> ± 0.3	1.3 ± 0.5
PI 327667	Tajikistan	<u>4.5</u> ± 0.4	2.5 ± 0.4
PI 328976	Ethiopia	<u>3.5</u> ± 0.4	1.5 ± 0.4
PI 429314	Yemen	<u>4.0</u> ± 0.4	2.0 ± 0.8
PI 564477	Bulgaria	<u>3.5</u> ± 0.0	1.5 ± 0.4
PI 584971	China	<u>3.3</u> ± 0.8	1.3 ± 0.5
PI 55551	Chile	<u>3.3</u> ± 0.5	1.3 ± 0.5
PI 414082	Bolivia	<u>4.3</u> ± 0.2	2.3 ± 0.2
Ciho 15355	Tunisia	<u>4.3</u> ± 0.2	2.3 ± 0.2
PI 289805	Iran	<u>3.6</u> ± 0.2	1.7 ± 0.6
PI 402078	Colombia	<u>3.8</u> ± 0.3	1.8 ± 0.2
PI 157884	Italy	<u>3.3</u> ± 0.3	1.3 ± 0.5
PI 134636	Afghanistan	<u>3.7</u> ± 0.3	1.8 ± 0.2
PI 223136	Jordan	<u>3.3</u> ± 0.2	1.5 ± 0.7
PI 356741	Morocco	<u>4.0</u> ± 0.4	2.2 ± 0.2
PI 610230	China	<u>3.3</u> ± 0.2	1.5 ± 0.7
PI 611480	Korea, North	<u>3.8</u> ± 0.2	2.0 ± 0.7
PI 611592	Tajikistan	<u>3.8</u> ± 0.2	2.0 ± 0.4
PI 414083	Bolivia	<u>4.0</u> ± 0.0	2.2 ± 0.6
PI 429526	Nepal	<u>4.0</u> ± 0.4	2.2 ± 0.5
Ciho 3512	China	<u>3.7</u> ± 0.2	1.8 ± 0.6
PI 222508	Croatia	<u>3.2</u> ± 0.6	1.3 ± 0.5
PI 328577	Greece	<u>3.5</u> ± 0.0	1.7 ± 0.2
PI 565826	China	<u>3.7</u> ± 0.2	1.8 ± 0.2
PI 70853	China	<u>3.2</u> ± 0.6	1.3 ± 0.2
PI 429510	Nepal	<u>3.2</u> ± 0.2	1.3 ± 0.5
PI 434852	Canada	<u>3.0</u> ± 0.4	1.2 ± 0.2
PI 328632	Greece	<u>4.3</u> ± 0.2	2.5 ± 0.4
PI 129484	Finland	<u>4.2</u> ± 0.2	2.4 ± 0.5
PI 328924	Italy	<u>3.6</u> ± 0.4	1.8 ± 0.2
PI 159126	Mexico	<u>3.0</u> ± 0.0	1.3 ± 0.3
PI 270683	Peru	<u>3.8</u> ± 0.3	2.0 ± 0.4
PI 270687	Peru	<u>4.3</u> ± 0.3	2.5 ± 0.4
PI 548761	Israel	<u>2.2</u> ± 0.5	<u>4.0</u> ± 0.0

^a Underlined numbers indicate higher differential virulence for that isolate.

^b FGO (FGOB10Ptm-1) collected in North Dakota.

^c NZKF2 = *P. teres* f. *maculata* isolate collected in New Zealand (obtained from B. Steffenson, University of Minnesota).

TABLE 5. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates SG1 and DEN 2.6^a

Barley accession	Country of origin	SG1 ^b	DEN 2.6 ^c
PI 244823	Pakistan	<u>3.3</u> ± 0.0	1.0 ± 0.0
Ciho 2348	India	<u>3.0</u> ± 0.4	1.0 ± 0.0
Ciho 15364	Tunisia	<u>3.3</u> ± 0.2	1.3 ± 0.2
PI 268391	Afghanistan	<u>3.0</u> ± 0.0	1.0 ± 0.0
Ciho 7492	Mexico	<u>3.7</u> ± 0.2	1.7 ± 0.6
PI 584977	China	<u>3.2</u> ± 0.5	1.2 ± 0.2
PI 29004	Turkmenistan	<u>3.4</u> ± 0.2	1.5 ± 0.0
PI 57089	China	<u>3.3</u> ± 0.2	1.5 ± 0.0
PI 361711	Denmark	<u>2.8</u> ± 0.2	1.0 ± 0.0
PI 410525	Pakistan	<u>3.3</u> ± 0.2	1.5 ± 0.4
PI 611592	Tajikistan	<u>3.8</u> ± 0.8	2.0 ± 0.7
Ciho 7491	Mexico	<u>3.0</u> ± 0.0	1.2 ± 0.2
Ciho 14291	China	<u>3.5</u> ± 0.4	1.7 ± 0.6
PI283433	Cyprus	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 327833	Uzbekistan	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 361929	Romania	<u>3.0</u> ± 0.2	1.2 ± 0.2
PI 566140	China	<u>3.0</u> ± 0.4	1.2 ± 0.2
PI 584971	China	<u>3.5</u> ± 0.4	1.7 ± 0.5
PI 189770	Tunisia	<u>3.0</u> ± 0.0	1.3 ± 0.3
Ciho 4050	Mongolia	<u>1.0</u> ± 0.0	<u>2.8</u> ± 0.2
PI 467731	Norway	<u>2.2</u> ± 0.2	<u>4.0</u> ± 0.4
PI 548760	Syria	<u>1.5</u> ± 0.4	<u>3.3</u> ± 0.5
PI 94806	Former Soviet Union	<u>2.2</u> ± 0.2	<u>4.0</u> ± 0.0
PI 294747	Bulgaria	<u>2.2</u> ± 0.2	<u>4.0</u> ± 0.4
Ciho 14250	Afghanistan	<u>1.5</u> ± 0.5	<u>3.5</u> ± 0.4
PI 356226	Morocco	<u>1.0</u> ± 0.0	<u>3.3</u> ± 0.2
PI 573662	Georgia	<u>1.4</u> ± 0.4	<u>4.0</u> ± 0.5
PI 292017	Israel	<u>1.2</u> ± 0.2	<u>3.8</u> ± 0.6
PI 548761	Israel	<u>1.0</u> ± 0.0	<u>4.0</u> ± 0.4

^a Underlined numbers indicate higher differential virulence for that isolate.

^b SG1 = *P. teres* f. *maculata* isolate collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia).

^c DEN 2.6 = *P. teres* f. *maculata* isolate collected in Denmark (obtained from B. Steffenson, University of Minnesota).

by the Australian isolate SG1. It is likely that the local barley cultivars are driving adaptation in each local pathogen population; however, additional isolates from each of these regions will need to be tested to identify which virulences are unique to each region.

Nature of disease resistance. Tekauz et al. (31) evaluated 42 isolates of *P. teres* f. *maculata* collected in western Canada and showed that 20 different pathotypes were present based on the use of a set of 12 barley differential lines. Similarly, Karki and Sharp (13) characterized a set of 14 *P. teres* f. *maculata* isolates collected in Montana (U.S.), Morocco, Tunisia, and Turkey on 20 barley genotypes with 12 of these showing a differential reaction between the isolates tested. These previous studies indicated that virulence variability was prevalent in the populations tested. Based on a gene-for-gene (5) or inverse gene-for-gene hypothesis (7), for each pathogen virulence there should be a distinct corresponding host resistance/susceptibility gene and therefore we expected that the BCC tested here should have a high level of resistance diversity. To our knowledge, the study we are presenting here is the first documentation of the screening of a large world barley collection using a diverse collection of *P. teres* f. *maculata* isolates. Based on the diversity of the 2,062 barley accessions tested in this study, we would expect that the vast majority of the resistance/susceptibility loci present in cultivated barley are represented here. It was interesting to note that only a very small percentage of the accessions evaluated showed high levels of resistance to all four

isolates tested (Table 2; Figs. 4 and 5). Similarly, only a few accessions showed a high level of susceptibility to all isolates tested (Fig. 4) with the remainder of the accessions falling in the moderately resistant to moderately susceptible region, resulting in a bell-shaped curve. Several accessions showed a strong differential reaction between isolates (Tables 2 to 7) indicating that these accessions harbor resistance/susceptibility that is unique to a specific isolate. We also observed some of the accessions showing differential reaction to multiple isolates (Table 8) indicating that along with some unique virulences, each isolate harbors virulences that are common with one or more of the other isolates. The observation of several sources of resistance being effective to only one, two, or three but not all of the isolates used in this study, along with the normal distribution of reaction types, and the fact that several recent outbreaks of this disease have occurred around the world (18), are all indications of a complex host–pathogen interaction that is highly variable in the natural population. This makes the complete characterization of both host resistance and pathogen virulence critical. The disease reaction information presented here lays the foundation for further studies involving the characterization of these resistance sources. Currently, barley populations are being developed using resistant accessions identified here. Additionally, resistance/susceptibility loci have been mapped by an association mapping strategy utilizing the phenotyping data generated in this study (29, adjoining manuscript). The overall goal is to characterize the different sources of broad resistance and intelligently deploy them in regional barley cultivars.

TABLE 6. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates SG1 and NZKF2^a

Barley accession	Country of origin	SG1 ^b	NZKF2 ^c
PI 87840	Korea, North	<u>3.8</u> ± 0.2	1.6 ± 0.4
CIho 14291	China	<u>3.5</u> ± 0.4	1.3 ± 0.5
PI 410525	Pakistan	<u>3.3</u> ± 0.2	1.2 ± 0.2
PI 584971	China	<u>3.5</u> ± 0.4	1.3 ± 0.5
PI 327833	Uzbekistan	<u>3.2</u> ± 0.2	1.0 ± 0.0
CIho7491	Mexico	<u>3.0</u> ± 0.0	1.0 ± 0.0
CIho 15364	Tunisia	<u>3.3</u> ± 0.2	1.3 ± 0.2
PI 222508	Croatia	<u>3.3</u> ± 0.2	1.3 ± 0.5
PI 268391	Afghanistan	<u>3.0</u> ± 0.0	1.0 ± 0.0
PI 564477	Bulgaria	<u>3.5</u> ± 0.4	1.5 ± 0.4
CIho 11533	Argentina	<u>3.2</u> ± 0.2	1.2 ± 0.2
PI 283433	Cyprus	<u>3.2</u> ± 0.2	1.2 ± 0.2
PI 55551	Chile	<u>3.3</u> ± 0.4	1.3 ± 0.5
PI 29004	Turkmenistan	<u>3.4</u> ± 0.2	1.5 ± 0.0
CIho 2353	Turkmenistan	<u>2.8</u> ± 0.2	1.0 ± 0.0
CIho 14070	Spain	<u>4.0</u> ± 0.0	2.2 ± 0.2
PI 270683	Peru	<u>3.8</u> ± 0.2	2.0 ± 0.4
PI 296192	Tajikistan	<u>4.0</u> ± 0.4	2.2 ± 0.6
PI 611592	Tajikistan	<u>3.8</u> ± 0.8	2.0 ± 0.4
PI 640390	United States	<u>3.3</u> ± 0.2	1.5 ± 0.0
CIho 2622	Israel	<u>3.2</u> ± 0.2	1.3 ± 0.5
CIho 3387	Algeria	<u>3.2</u> ± 0.2	1.3 ± 0.5
CIho 7339	Japan	<u>3.2</u> ± 0.2	1.3 ± 0.2
PI 283445	Belgium	<u>3.5</u> ± 0.0	1.7 ± 0.2
PI 296453	Eritrea	<u>3.7</u> ± 0.2	1.8 ± 0.2
PI 328924	Italy	<u>3.7</u> ± 0.5	1.8 ± 0.2
PI 402264	Colombia	<u>3.5</u> ± 0.0	1.7 ± 0.5
PI 157884	Italy	<u>3.2</u> ± 0.2	1.3 ± 0.5
PI 327831	Turkmenistan	<u>3.1</u> ± 0.4	1.3 ± 0.5
PI 436155	Chile	<u>2.5</u> ± 0.4	<u>4.3</u> ± 0.6
PI 94797	Armenia	1.3 ± 0.2	<u>3.2</u> ± 0.2
PI 190762	Japan	1.3 ± 0.2	<u>3.2</u> ± 0.2
PI 292017	Israel	1.2 ± 0.2	<u>3.0</u> ± 0.7
PI 344903	Serbia	2.0 ± 0.7	<u>3.8</u> ± 0.2
PI 428499	Sweden	2.3 ± 0.2	<u>4.2</u> ± 0.5
PI 73298	Armenia	2.3 ± 0.8	<u>4.2</u> ± 0.5
PI 548761	Israel	1.0 ± 0.0	<u>4.0</u> ± 0.0

^a Underlined numbers indicate higher differential virulence for that isolate.

^b SG1 = *P. teres* f. *maculata* isolate collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia).

^c NZKF2 = *P. teres* f. *maculata* isolate collected in New Zealand (obtained from B. Steffenson, University of Minnesota).

TABLE 7. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) between *Pyrenophora teres* f. *maculata* isolates DEN 2.6 and NZKF2^a

Barley accession	Country of origin	DEN 2.6 ^b	NZKF2 ^c
PI 270605	Peru	<u>3.5</u> ± 0.4	1.7 ± 0.6
PI 310480	France	<u>4.2</u> ± 0.2	2.3 ± 0.5
PI 289805	Iran	<u>3.7</u> ± 0.2	1.7 ± 0.6

^a Underlined numbers indicate higher differential virulence for that isolate.

^b DEN 2.6 = *P. teres* f. *maculata* isolate collected in Denmark (obtained from B. Steffenson, University of Minnesota).

^c NZKF2 = *P. teres* f. *maculata* isolate collected in New Zealand (obtained from B. Steffenson, University of Minnesota).

Fig. 5. Disease reaction score distribution of the four *Pyrenophora teres* f. *maculata* isolates used. Few barley accessions are highly resistant or highly susceptible with most of the accessions showing an intermediate reaction for each isolate resulting in a bell-shaped curve.

TABLE 8. Barley core collection lines showing a differential reaction (difference of greater than or equal to 1.8) in multiple combinations between *Pyrenophora teres* f. *maculata* isolates FGO, SG1, NZKF2, and DEN 2.6^a

Barley accession	Country of origin	FGO ^b	SG1 ^c	NZKF2 ^d	DEN 2.6 ^e	Differential reaction	Group
PI 138711	Iran	3.9 ± 0.2	1.8 ± 0.6	1.8 ± 0.2	1.0 ± 0.4	FGO > SG1 FGO > NZKF2 FGO > DEN 2.6	I
PI 410525	Pakistan	3.7 ± 0.6	3.3 ± 0.2	1.2 ± 0.2	1.5 ± 0.4	FGO > NZKF2	II
CIho7491	Mexico	3.2 ± 0.2	3.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.2	FGO > DEN 2.6	
PI 584971	China	3.3 ± 0.8	3.5 ± 0.4	1.3 ± 0.5	1.7 ± 0.5	SG1 > NZKF2	
PI 611592	Tajikistan	3.8 ± 0.2	3.8 ± 0.8	2.0 ± 0.4	2.0 ± 0.7	SG1 > DEN 2.6	
PI 327833	Uzbekistan	3.2 ± 0.2	3.2 ± 0.2	1.0 ± 0.0	1.3 ± 0.2		
CIho 15364	Tunisia	2.0 ± 0.7	3.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2		III
PI 268391	Afghanistan	1.6 ± 0.4	3.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	SG1 > NZKF2	
PI 29004	Turkmenistan	2.7 ± 0.2	3.4 ± 0.2	1.5 ± 0.4	1.5 ± 0.0	SG1 > DEN 2.6	
CIho 14291	China	2.7 ± 0.5	3.5 ± 0.4	1.3 ± 0.5	1.7 ± 0.6		
PI 283433	Cyprus	2.4 ± 0.4	3.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2		
PI 467731	Norway	2.2 ± 0.6	2.2 ± 0.2	3.2 ± 0.8	4.0 ± 0.4	DEN 2.6 > FGO	IV
PI 356226	Morocco	1.3 ± 0.5	1.0 ± 0.0	2.4 ± 0.4	3.3 ± 0.2	DEN 2.6 > SG1	
PI 564477	Bulgaria	3.5 ± 0.0	3.5 ± 0.4	1.5 ± 0.4	3.2 ± 0.2	FGO > NZKF2	V
PI 157884	Italy	3.3 ± 0.8	3.2 ± 0.4	1.3 ± 0.5	2.4 ± 0.5		
PI 55551	Chile	3.3 ± 0.5	3.3 ± 0.4	1.3 ± 0.5	2.0 ± 0.0	SG1 > NZKF2	
PI 222508	Croatia	3.2 ± 0.6	3.3 ± 0.2	1.3 ± 0.5	1.8 ± 0.2		
PI 328924	Italy	3.6 ± 0.4	3.7 ± 0.5	1.8 ± 0.2	3.5 ± 0.4		
PI 327667	Tajikistan	4.5 ± 0.4	3.7 ± 0.2	2.5 ± 0.4	2.0 ± 0.0		VI
PI 410451	Pakistan	4.3 ± 0.6	3.2 ± 0.2	2.2 ± 0.6	1.8 ± 0.5	FGO > NZKF2	
PI 38305	Russian Fed	4.3 ± 0.2	3.8 ± 0.3	2.2 ± 0.8	2.2 ± 0.5	FGO > DEN 2.6	
PI 565826	China	3.7 ± 0.2	2.3 ± 0.8	1.7 ± 0.2	1.8 ± 0.5		
PI 145693	Saudi Arabia	3.7 ± 0.5	3.0 ± 0.0	1.4 ± 0.4	1.8 ± 0.6		
PI 328632	Greece	4.3 ± 0.2	3.3 ± 0.2	2.5 ± 0.4	2.5 ± 0.4		VII
PI 244823	Pakistan	3.0 ± 0.0	3.3 ± 0.2	1.8 ± 0.5	1.0 ± 0.0	FGO > DEN 2.6 SG1 > DEN 2.6	
CIho 4050	Mongolia	2.9 ± 0.5	1.0 ± 0.0	2.3 ± 0.5	2.9 ± 0.2	FGO > SG1	VIII
PI 573662	Georgia	3.1 ± 0.6	1.4 ± 0.4	3.0 ± 0.0	4.0 ± 0.5	DEN 2.6 > SG1	
PI 289805	Iran	3.6 ± 0.2	3.0 ± 0.0	1.7 ± 0.6	3.7 ± 0.2	FGO > NZKF2 DEN 2.6 > NZKF2	IX
PI 548761	Israel	2.2 ± 0.5	1.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.4	NZKF2 > FGO NZKF2 > SG1 DEN 2.6 > FGO DEN 2.6 > SG1	X
PI 292017	Israel	2.3 ± 0.2	1.2 ± 0.2	3.0 ± 0.7	3.8 ± 0.6	NZKF2 > SG1 DEN 2.6 > SG1	XI

^a Horizontal space is used to separate differential combinations.

^b FGO (FGOB10Ptm-1) collected in North Dakota.

^c SG1 = *P. teres* f. *maculata* isolate collected in Western Australia (obtained from R. P. Oliver, Curtin University, Perth, Western Australia).

^d NZKF2 = *P. teres* f. *maculata* isolate collected in New Zealand (obtained from B. Steffenson, University of Minnesota).

^e DEN 2.6 = *P. teres* f. *maculata* isolate collected in Denmark (obtained from B. Steffenson, University of Minnesota).

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