Assessing the genetic and pathogenic variability of *Pyrenophora teres* f. *maculata* (spot form net blotch of barley) and its ability to overcome currently-used sources of resistance on the Canadian prairies

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Key Words: *Pyrenophora teres* f. *maculata,* spot form net blotch, barley, pathotype, resistance

Abstract

Eighty two isolates of the fungus *Pyrenophora teres* f. *maculata*, causal agent of spot form net blotch (SFNB) of barley, were collected across the Canadian prairies. Following genetic analysis using 13 microsatellite DNA markers, 27 isolates were selected to assess the degree of pathogenic variation in the fungus. Pathogenic variability was evaluated by inoculating isolates onto 11 barley genotypes as differential hosts at the seedling stage. One week following inoculation, the second and third leaves of each plant were rated on to a 1–9 disease severity scale, and plants with scores of 1–3 and >3 were scored as resistant and susceptible, respectively. The entire experiment was repeated. Cluster analysis revealed 13 distinct pathotype groups (virulence patterns) among the 27 representative isolates. Disease severity ratings ranged from 2.2 to 6.1 with a mean of 4.6. To assess the risk of resistance breakdown, the resistance of four barley cultivars ('AAC Synergy', 'CDC Meredith', 'Cerveza' and 'Major'), which had been previously identified as resistant to SFNB on the Canadian prairies, was evaluated at the seedling stage against six isolates of *P. teres* f. *maculata* representing four different pathotypes. The resistance in 'CDC Meredith' was clearly overcome by all isolates tested, while 'AAC Synergy', 'Cerveza' and 'Major' still exhibited resistance against all of the pathotypes. The identification of fungal isolates virulent on 'CDC Meredith' suggests that producers should avoid growing the same resistant barley variety in short rotation, and avoid relying on resistance as the sole approach to disease management. Judicious use of fungicides, coupled with rotations of at least two years between barley crops and diversity in the barley varieties grown, will promote effective and sustainable management of spot form net blotch.

Introduction

Net blotch, caused by the fungus *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoem.) (Smedegard-Petersen 1978) is an important foliar disease of barley (*Hordeum vulgare*) on the Canadian prairies. The fungus consists of two morphologically similar but genetically distinct forms: *P. teres* f. *teres* (Ptt) and *P. teres* f. *maculata* (Ptm), which cause the

net form of net blotch (NFNB) and spot form of net blotch (SFNB), respectively (Tekauz 1990, Rau *et al.* 2007, Ellwood *et al.* 2012). Yield losses of 10% to 40% are typical for net blotch in most cases, but *P. teres* has the potential to cause a total loss in susceptible cultivars under conditions that favour pathogen development (Mathre 1997, Murray and Brennan 2010). Both forms can reproduce both sexually and asexually, with the genetic structure of the population dependent on the relative importance of these two types of reproduction in the pathogen life cycle (Liu *et al.* 2011).

Significant genetic diversity in pathogen populations helps them to adapt to environmental changes (Peltonen *et al.* 1996) and may affect fungicide sensitivity and the stability of resistance in hosts. The genetic diversity and population structure of *P. teres* populations have been explored in several parts of the world. In most of these investigations, a significant level of variability within the pathogen populations has been demonstrated (McLean *et al.* 2010, Lehmensiek *et al.* 2010, Liu *et al.* 2012). Simple sequence repeats (SSRs) or microsatellites have been proven to be a powerful tool for taxonomic, phylogenetic and population genetic studies (Singh *et al.* 2011, Garoia *et al.* 2007). In recent years, an abundance of sequence data has been generated, enabling the use of computational tools to identify SSR loci (Robinson *et al.* 2004, Singh *et al.* 2011). Keiper *et al.* (2007) identified 25 sequence tagged microsatellite sites (STMS), which revealed 26 polymorphic loci. Bogacki *et al.* (2010) found that out of 20 SSR loci tested, 17 (85 %) were polymorphic within the Ptt and Ptm populations. Ellwood *et al.* (2010) also identified 68 polymorphic SSRs, 20 from STMS markers (Keiper et al 2007), 44 from the genome assembly sequence of Ptt, and four from expressed sequence tags (ESTs) (Ellwood *et al.* 2010).

The virulence of *P. teres* isolates varies on differential host lines and has been examined in many studies (Tekauz 1990, McLean *et al.* 2010, Liu *et al.* 2012, Bouajila *et al.* 2012). Different virulence phenotypes have been observed in both the spot and net forms of the fungus, increasing the risk that phenotypes may emerge that are able to infect previously resistant cultivars. In the last comprehensive assessment of pathogenic diversity among Ptm isolates collected from western Canada, Tekauz (1990) classified 42 isolates into 7 main pathotype groups (P-V) with 1 - 5 subgroups in each group (20 distinct virulence profiles in total). The development of resistant cultivars is an important strategy in net blotch management, and is a high priority for all western Canadian barley-breeding programs. It has been shown that some cultivars resistant to Ptt are not resistant to Ptm isolates (Bockelman *et al.* 1983). These results suggest that there are distinct host–pathogen interactions associated with each form of *P. teres,* and that resistance breeding efforts should focus on each form individually (Liu *et al.* 2011); this is currently the case on the prairies, where candidate cultivars are evaluated for each of the two forms independently.

There is limited knowledge of the virulence and genetic structure of the current spot form net blotch pathogen populations from the Canadian prairies. In addition, the nature and diversity of these populations have not been studied using molecular genetics techniques. Therefore, this study was conducted to determine the genetic structure and pathogenic variation of SFNB pathogen populations from this region, and also to assess the ability of representative Ptm pathotypes to overcome the currently-used sources of resistance.

Materials and Methods

Genetic structure of fungal populations

Collection of isolates**,** genomic DNA extraction, species-, form-, mating type-, and mating type form-specific PCR analysis were conducted as described by Akhavan *et al.* (2015). Simple

sequence repeat (SSR) polymorphisms were detected with polymorphic SSR primers developed from the genome assembly of *P. teres* (Ellwood *et al.*, 2010; Liu *et al.*, 2012). A total of 82 *P. teres* f. *maculata* isolates were examined. The PCR analysis was conducted as described by Liu *et al.* (2012), and the SSR-PCR products were separated by capillary electrophoresis on a 3730 DNA analyzer (Applied Biosystems, Foster City, CA). For each locus, microsatellite allele sizes were determined by comparing the amplicons with a LIZ 500 internal size standard (Applied Biosystems) using GeneMapper software v3.7 (Applied Biosystems) (Bogacki *et al.*, 2010). Chi-square tests were conducted to determine if the observed mating type ratio for each of the populations of *P. teres* from each province and over all of the Canadian prairies departed significantly from the null hypothesis of a 1:1 MAT1:MAT2 sex ratio (Bogacki *et al.*, 2010). Similarity matrices representing all possible pairwise comparisons of the tested isolates were constructed based on the presence/absence data for each marker type. The similarity matrix was then used to perform cluster analysis by the unweighted pair-group method using the arithmetic means (UPGMA) procedure. The software NTSYSpc version 2.2 (Exeter Software, New York, NY) was used to construct the similarity matrix and perform the UPGMA analysis (McLean *et al.*, 2010). Analysis of molecular variance was calculated using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012). Gene flow was calculated using the formula Nm = 0.5 (1-Gst)/Gst by PopGene 1.32 (Yeh *et al.* 1999).

Pathogenic diversity and resistance screening

A subset of 27 isolates was evaluated for virulence on seedlings of 11 barley differentials (two-rowed 'Betzes', 'Herta', 'Norbert', 'TR 473', 'CI 5791', and 'CI 9820'; and six-rowed 'Bonanza', 'Heartland', 'OAC 21', 'Steptoe', and 'CI 9214') with known reactions to western Canadian Ptm isolates (Tekauz, 1990). These isolates originated from Alberta (8 isolates), Saskatchewan (10), and Manitoba (9); while a reference isolate (WRS 857) also was included. One week following inoculation, the second and third leaves of each seedling were rated on a 1–9 disease severity scale developed by Tekauz (1985). Plants rated as 1–3 and >3 were scored as resistant and susceptible, respectively. The entire experiment was repeated. When the results were inconsistent between the two experiments, inoculations were repeated as necessary to clarify the host reaction. Data were then transformed into a 0-1 matrix with 0 as resistant and 1 as susceptible. For ease of comparison, the isolates were grouped based on the relative number of resistant (r) and susceptible (s) reactions on the 11 differentials (Tekauz, 1990). These groups ranged from 11r/0s, designated as group L, to 0r/11s, designated as group W. Each isolate was further designated with a number (1-6) based on the specific reaction of each of the 11 barley genotypes. To assess the risk of resistance breakdown, the resistance of four barley cultivars ('AAC Synergy', 'CDC Meredith', 'Cerveza' and 'Major'), previously identified as resistant to SFNB on the Canadian prairies, was evaluated at the seedling stage against six isolates representing four pathotypes, using the same inoculation and rating methods described above.

Results and Discussion

A total of 35 alleles were detected among the isolates at 13 polymorphic SSR loci, with an average of 2.7 alleles per locus and a range of 1 to 5. High levels of diversity were found among the isolates with a clonal fraction of approximately 11%. Following clone-correction, analysis of molecular variance showed no significant genetic differentiation among populations collected from Alberta, Saskatchewan and Manitoba. Ninety nine percent of the total genetic diversity

was found within populations and only 1% between populations. Cluster analysis using the UPGMA procedure and Jaccard's similarity coefficient also indicated no obvious clustering based on geographical origin of the isolates. Moreover, a high level of gene flow (Nm = 0.5(1-Gst)/Gst = 11.6) was observed among the populations. These results are consistent with recent reports (Liu *et al.* 2012, Lehmensiek *et al.* 2010, McLean *et al.* 2010) demonstrating a high level of genetic diversity among isolates within populations. The lack of a significant difference among the populations combined with a high level of gene flow between the provinces suggests the occurrence of one singular (panmictic) population of the pathogen on the prairies. Furthermore, the high number of distinct haplotypes combined with an equal ratio of both pathogen mating types indicates extensive sexual recombination in the prairie *P. teres* f. *maculata* population.

Cluster analysis using the UPGMA procedure and simple similarity coefficient revealed 13 pathotype groups among the 27 isolates tested for virulence phenotype. Based on the pathotyping procedure of Tekauz (1990), two pathotype groups (V1 and T1) comprising 52% of the isolates were found to be predominant on the prairies, while nine isolates (33%) had distinct virulence profiles. Variation in virulence ranged from that of a Manitoba isolate (MBV25) that was virulent on 10 of the 11 differentials (with an average disease rating of 6.1), to an avirulent isolate from Alberta (AB57) with an average disease rating of 2.2. Isolate SK60, from Saskatchewan, caused an average disease rating of 5.7 and was virulent on all 11 differentials. Comparison of the results from this study with those of Tekauz (1990) suggests that the virulence profile of *P. teres* f. *maculata* on the prairies has changed in the last three decades. While pathotypes Q and R (previously reported by Tekauz) were not identified in the current study, eight new pathotypes (L1, N1, O1, P3, T6, U3, U4 and W1) were found for the first time on the prairies. Among the differential genotypes tested, 'Herta', 'Betzes', 'Norbert', and 'TR473' were the most susceptible, while the differential 'CI 9214' was resistant to all but two isolates. Thus, the resistance in CI9214, which has been used extensively in western Canadian breeding programs (Tekauz, 1990), is still effective against all current pathotypes except for pathotype W1. Resistance to SFNB in 'CDC Meredith', previously identified as a Ptm resistant commercial cultivar, was overcome by all pathotypes tested, while 'AAC Synergy', 'Cerveza' and 'Major' were resistant to all of the pathotypes. Nonetheless, the identification of isolates virulent on 'CDC Meredith' suggests that producers should not rely on host resistance as the sole management strategy for spot form net blotch, but rather combine this with other strategies such as crop rotation. When the risk of disease is high, judicious fungicide application also may be a useful strategy.

Acknowledgements

The authors would like to thank Mr. Meconnen Beyene, Ms. Noryne Rauhala and Ms. Jackie Busaan (Agriculture and Agri-Food Canada) for assistance with the collection of isolates, and Ms. Ileana S. Strelkov, Ms. Kelley Dunfield, Dr. Reem Aboukhaddour and Dr. Tiesen Cao (University of Alberta) for technical advice and insight. The financial support provided by the Western Grains Research Foundation, Alberta Barley Commission, Rahr Malting Inc, Canadian Wheat Board, Agriculture and Agri-Food Canada and the University of Alberta is gratefully acknowledged.

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