Molecular Genetic Variability, Within a Population of *Mycosphaerella graminicola*, Cause of Septoria tritici Leaf blotch of Wheat

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Keywords: Mycosphaerella graminicola, genotypic diversity, RAPDs, molecular phenotypes, non- race specific resistance

Abstract

The pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*), causes speckled leaf blotch of wheat and has worldwide distribution. Information about genetic variability of the pathogen population is useful for development of disease management strategies. In this study 90 isolates of *M. graminicola* were collected using a hierarchical sampling procedure from a single wheat field and the genomic DNA of isolates were extracted. RAPD analysis was conducted using 15 random primers, according to standard protocols. Forty molecular phenotypes were detected which phenotype 1 had the highest frequency within the population. The total genotypic diversity was estimated as 0.94.When it was partitioned into within and among location components, 91% of the genetic diversity occurred within locations and only 9% occurred among locations. Low degree of variability among locations and high degree of variability within locations, points that, most likely the source of primary inoculum was air-borne ascospores, which were dispersed evenly across the field. This finding suggests that, the sexual stage of *M. graminicola,* occurs in Saskatchewan, although it has not yet been reported from Canada. High degree of genetic variability implies that most likely the pathogen will adapt rapidly to single gene resistance sources, therefore, in breeding for disease resistance, emphasis should be given to non- race specific resistance.

Introduction

M. graminicola is one of the important diseases of wheat and occurs worldwide. In some countries under favorable conditions up to 60% yield loss has been reported (Zamus *et al.,* 1970). In the northern cropping area of Saskatchewan up to 15% yield loss may be caused by the leaf spotting disease complex, of which, *M. graminicola* is an important component (G. R. Hughes personal communication). Information about the population structure of the pathogen is useful for development of disease management strategies. McDonald and Martinez (1990) using RFLP markers showed that there was high level of genetic variability within a population of this fungus. However, no attempt has been made to relate this variability at the molecular level to variability for pathogenicity.

The objective of this project was to study a specific population of the pathogen for both variability at molecular level and variability for pathogenicity, and to find possible relationships

between these two types of variability. In this paper only variability at molecular level will be discussed.

Materials and Methods

Ninety isolates of *M. graminicola* were collected using a hierarchical sampling procedure from a single wheat field in Saskatoon. Within this field, 10 different locations, about 100 m^2 apart from each other, were randomly selected. At each location from an area about 1 m^2, three different flag leaves were chosen and for each flag leaf, one pycnidium from each of three distinct lesions was isolated. The isolates were multiplied in liquid media (yeast extract 4 g/l; malt extract 0.5 g/l) for about one week, and then the genomic DNA of isolates was extracted using protocol of Raeder and Broda (1985). The PCR protocol was optimized and 300 primers were screened. Primer sets UBC 601-800 were purchased from the University of British Columbia and the primer sets OPF, OPG, OPI, and OPJ were purchased from Operon Technology Alameda, California. Fifteen random primers, which gave reproducible and clear banding patterns, were selected. The selected primers were used to screen all of the 90 isolates for DNA polymorphisms. The PCR products were resolved on 1.5 % agarose gel and stained with ethidium bromide (10mg/ml) and photographed. The DNA banding pattern was scored as presence or absence of bands.

Analysis of data

Genotypic diversity was estimated to determine how much variability existed within the population and how it was distributed at the different sampling levels. Molecular phenotypes were designated for each isolate as described by Kolmer *et al.,* (1995) and the frequency of each molecular phenotype within the population was determined. Genotypic diversity was estimated using Nie's (1973) formula: $H = 1 - \Sigma x_i^2$ in which, H is the genotypic diversity and x_i is the frequency of the i^{th} molecular phenotype in the population.

To find the amount of genetic variability at each different sampling levels, total genotypic diversity was partitioned into among- and within-location components as suggested by Goodwin *et al.,* (1992): $H_T = H_w + H_{AL}$ in which, H_T is the total genotypic diversity which is estimated from the frequency of each molecular phenotype in the population. H_w is the average of genotypic diversity within locations and H_{AL} is the amount of genotypic diversity among locations.

Results

The 15 random primers, produced 132 reproducible fragments of which about 96% were polymorphic in the population (Table 1). Five fragments were fixed across all of the isolates of *M. graminicola*, but were not present in the *Stagonospora nodorum* and *Septoria triceti* isolates, which had been included as outliners. These fragments have potential to be used as species-specific markers to identify *M. graminicola* from other related species.

Table 1.Sequence of 15 random primers and number of fragments produced by each primer

Primer no.	Sequence	No of polymorphic bands	No of monomorphic bands	Total number of bands
UBC 648	GCACGCGAGA	4	0	4
UBC 726	GGTGTGGGTG	9	1	10
UBC 736	GAGGGAGGAG	8	0	8
UBC 737	GGTGGGTGTG	12	0	12
UBC 757	GGAAGGGAGG	13	0	13
UBC 758	GGTTGGGTGG	1	1	2
UBC 763	CACACCACCC	6	1	7
UBC 767	ACCCACCACC	11	0	11
UBC 772	CCCACCACCC	10	1	11
OP F6	GGGAATTCGG	11	1	12
OP G12	CAGCTCACGA	12	0	12
OP G13	CTCTCCGCCA	7	0	7
OP H8	GAAACACCCC	5	0	5
OP H13	GACGCCACAC	7	0	7
OP I10	ACAACGCGAG	11	0	11
		96%	4%	132

Forty molecular phenotypes were detected of which molecular phenotype 1 had the highest frequency in the population (Table 2). The molecular phenotypes were randomly distributed among different locations and no clonal structure was detected in the population.

Total genotypic diversity was estimated at 0.94, which indicates a high level of diversity within the population. When total genotypic diversity was partitioned into within- and among-location components, 91% occurred within locations and only 9% occurred among locations. This finding indicates that the majority of variability was distributed at lower sampling levels. Similar results have been reported for *M. graminicola* (McDonald and Martinez 1990), *Rhynchosporium secalis* (Goodwin *et al.,* 1992; McDonald *et al.,* 1999; Salamati *et al.,* 2000), *Stagonospora nodorum* (McDonald *et al.,*1994) and *Eutypa lata* (Peros *et al.,* 1997).

Discussion

The results of this study indicated that there was a high level of genetic variability within the population of *M. graminicola* and that the population was composed of many different genotypes. No clonal structure was detected within the population. Most of the genotypic diversity occurred at the lowest sampling level, covering an area about 1 m^2, a finding which, agreed with McDonald and Martinez (1990).

Low variability among locations and a high degree of variability within locations would result if the primary source of inoculum was air-borne ascospores, which would be dispersed evenly across the field. This finding suggests that the sexual stage of *M. graminicola,* occurs in Saskatchewan, although it has not yet been reported in Canada.

Table 2. Designated molecular phenotype and their distribution within 10 locations of a single field based on 15 random primers.

No.	Molecular phenotype (haplotype)	Number within population	Locations
HP 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	18	1(4), 3(2), 4(3), 5(1), 6(3), 7(2), 8(1), 9(1), 10(1)
HP 2	1 1 2 1 1 1 1 1 1 1 1 1 1 1 1	1	2(1)
HP 3	1 1 1 2 1 1 1 1 1 1 1 1 1 1 1	2	7(1), 9(1)
HP 4	1 1 1 1 1 1 1 2 1 1 1 1 1 1 1	1	7(1)
HP 5	1 1 1 1 1 1 1 1 1 1 2 1 1 1 1	5	2(1), 3(1), 4(1), 6(1), 7(1)
HP 6	1 1 1 1 1 1 1 1 1 1 1 2 1 1 1	6	1(3), 3(1), 4(1), 10(1)
HP 7	1 1 1 1 1 1 1 1 1 1 1 1 2 1 1	6	2(1), 5(1), 7(2), 8(2)
HP 8	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1	1	1(1)
HP 9	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2	3	3(1), 5(1), 9(1)
HP 10	1 1 3 1 1 1 1 1 1 1 1 1 1 1 1	1	5(1)
HP 11	1 1 1 3 1 1 1 1 1 1 1 1 1 1 1	1	6(1)
HP 12	1 1 1 1 3 1 1 1 1 1 1 1 1 1 1	1	9(1)
HP 13	1 1 1 1 1 1 1 1 1 1 3 1 1 1 1	4	4(1), 6(1), 8(1), 9(1)
HP 14	1 1 1 1 1 1 1 1 1 1 1 3 1 1 1	2	5(1), 8(1)
HP 15	1 1 1 1 1 1 1 1 1 1 1 4 1 1 1	1	2(1)
HP 16	2 1 1 1 1 1 1 1 1 1 1 1 2 1 1	1	3(1)
HP 17	1 1 2 1 1 1 1 2 1 1 1 1 1 1 1	1	1(1)
HP 18	1 1 2 1 1 1 1 1 1 1 1 1 2 1 1	2	3(1), 10(1)
HP 19	1 1 2 1 1 1 1 1 1 1 2 1 1 1 1	1	7(1)
HP 20	1 1 1 2 1 1 1 1 1 1 1 1 2 1 1	1	4(1)
HP 21	1 1 1 1 1 1 1 1 1 1 1 2 2 1 1	5	2(1), 4(1), 5(1), 8(1), 9(1)
HP 22	1 1 1 1 1 1 1 1 1 1 1 1 2 1 2	2	5(1), 8(1)
HP 23	1 1 2 1 1 1 1 1 1 1 1 3 1 1 1	1	10(1)
HP 24	1 1 1 2 1 1 1 1 1 1 1 3 1 1 1	1	7(1)
HP 25	1 1 1 1 1 1 1 1 1 1 2 1 3 1 1	1	6(1)
HP 26	1 1 1 1 1 1 1 1 1 1 1 2 3 1 1	1	8(1)
HP 27	1 1 2 1 1 1 1 1 1 1 1 4 1 1 1	1	9(1)
HP 28	1 1 1 1 1 1 1 1 1 1 1 2 4 1 1	3	5(1), 6(2)
HP 29	1 1 1 1 1 1 1 1 1 1 1 3 2 1 1	1	2(1)
HP 30	1 1 1 1 1 1 1 1 1 1 1 3 1 1 2	1	10(1)
HP 31	1 1 1 1 1 1 1 1 1 1 1 4 2 1 1	2	10(2)
HP 32	1 1 1 1 1 1 1 1 1 1 1 4 3 1 1	1	2(1)
HP 33	1 1 1 5 1 1 1 1 1 1 2 1 1 1 1	1	2(1)
HP 34	1 1 2 1 1 1 1 1 1 1 2 2 1 1 1	1	9(1)
HP 35	2 1 2 1 1 1 1 1 1 1 1 1 1 3 1	1	10(1)
HP 36	1 1 3 1 1 1 1 1 1 1 1 3 2 1 1	1	5(1)
HP 37	1 1 2 2 1 1 1 1 1 1 1 2 1 1 2	1	3(1)
HP 38	2 1 1 1 4 1 1 1 1 1 1 4 3 1 1	1	3(1)
HP 39	4 1 2 1 1 1 1 1 1 1 1 4 2 1 1	1	2(1)
HP 40	1 1 1 1 2 1 1 1 1 1 1 4 5 1 2	1	8(1)

Numbers within brackets shows number of molecular phenotype within that location

Sexual stage of *M. graminicola* has been reported from New Zealand, Australia, Chile, the United Kingdom and the United States (Wiese 1987).

The high level of genetic variability at the small-scale level implies that most likely the pathogen will adapt rapidly to single gene resistance sources (Goodwin *et al.,* 1992). Therefore, in breeding for resistance to *M. graminicola* emphasis should be given to developing cultivars with non- race specific resistance or a combination of major resistance genes.

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