Characterization of polymorphic microsatellite loci of *Blumeria graminis* f. sp. *tritici*, the cause of powdery mildew of wheat.

Ryan Parks[1], Warren Booth[2], and Christina Cowger[1].

[1]USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA.

[2]Department of Entomology and W.M. Keck Centre for Behavioral Biology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613, USA.

Keywords: microsatellite, primers, *Blumeria graminis*, powdery mildew

Corresponding author: Ryan Parks, 3408 Gardner Hall, North Carolina State University, Plant Pathology, Campus Box 7616, Raleigh, NC 27695. Fax: 919-856-4816. Email: Ryan.Parks@ars.usda.gov

Abstract

In many wheat-growing regions of the world, powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is a major disease that results in significant yield losses. Using a microsatellite enrichment protocol, we developed primers for nine polymorphic microsatellite DNA loci to aid in studies of *B. g. tritici* population genetics and biology. When tested against 45 individuals from the eastern U.S., 2 to 20 alleles per locus were detected with an average allelic diversity of 0.543.

Powdery mildew, caused by *Blumeria graminis* (DC.) Speer f. sp. *tritici* (Em. Marchal), is a major pathogen of wheat (*Triticum aestivum*), causing significant economic losses in wheat-growing regions of the world with maritime climates (Lipps & Madden, 1989). *B. g. tritici* is a filamentous ascomycete with a heterothallic mating system. Haploid conidiospores (conidia) drive numerous asexual reproductive cycles during a single wheat growing season, while cleistothecia (sexual fruiting bodies) may form toward the end of the season. Subsequently, haploid meiotic ascospores may form within the *B. g. tritici* cleistothecia and infect new host material. Both conidia and ascospores are wind-dispersed (Wolfe & Schwarzbach, 1978) and, at least for conidia, long-distance dispersal (over 800 km) has been demonstrated in the closely related barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) pathosystem (Hermansen *et al.*, 1978). A previous study of *B. g. tritici* population genetics, using single nucleotide polymorphisms (SNPs), pointed to a recent (<500-yr-old) introduction of the organism into the United States, as well as significant population subdivision on both a continental and inter-continental scale (Parks *et al.*, 2009). The presence of extensive homoplasy, combined with the limited number of polymorphic sites in previous studies, made population inferences difficult and indicated a need for more markers with a higher mutation rate. Microsatellite DNA loci provide another tool for population inferences and should have a higher mutation rate than SNPs.

Libraries enriched for di-, tri-, and tetra-nucleotide repeats were developed from pooled genomic DNA extracted from haploid conidia of four *B. g. tritici* isolates (two collected from Salisbury, NC and two from Kinston, NC). Microsatellite enrichment followed established protocols (Dopman *et al.*, 2004) with modifications (Booth *et al.*, 2008). DNA fragments from the enriched library were cloned via the TOPO TA cloning kit (Invitrogen) with inactivity of β-galactosidase used to selectively identify recombinant DNA colonies. In total, 1,710 colonies

were individually selected and grown in 96-well tissue culture plates containing Luria broth for 24 hours at 37ºC. After 24 hours, 1.5 µl of recombinant bacteria in Luria broth were used for colony PCR (Glenn & Schable, 2005). PCR amplicons were sequenced at the Genome Sciences Lab at North Carolina State University and at the DNA Facility of the Iowa State University Office of Biotechnology. Sequences were screened for repetitive regions using MSATCOMMANDER (Faircloth, 2008). Primers were developed from sequences containing putative microsatellite regions using Primer3 (Rozen & Skaletsky, 2000). In total, sufficient flanking region was available to design primers for 58 unique microsatellite regions.

Primer sets were tested under various conditions, and a set of 10 primer pairs was identified that reproducibly generated products consistent with amplification of a single-copy nuclear region in a haploid organism. Primers were either 5' directly labeled or M13(-21) labeled (Schuelke, 2000) using FAM, PET, NED, or VIC (Table 1). Standard PCR reactions contained 1x PCR buffer, 207.5µM dNTP, 1.4-2.0 mM MgCl2, 0.3 µM reverse primer, 0.3 µM forward primer, 1U Biolase *Taq* polymerase and 20-30 ng DNA. Amplifications using the M13(-21) labeled primer contained 0.08µM m13 tagged forward primer and 0.3 µM of the labeled primer. Cycling conditions for primers *Bgt*-1 through *Bgt*-9 were 96°C for 4 min followed by 33 cycles of 96°C for 25 s, Ta (see Table 1) for 25 s, 72° for 45 s, and a final extension step of 72° for 3 min. Bgt-*10* followed a modified Touchdown PCR protocol (Don *et al.*, 1991) with cycle conditions of 96°C for 4 min followed by 10 cycles of 96°C for 25 s, 65°C for 25 s, 72°C for 45 s with a 1°C reduction in the annealing temperature per cycle, followed by 24 cycles of 96°C for 25 s, 55°C for 25 s, 72° for 45 s, and a final extension step of 72° for 3 min.

These 10 loci were amplified in 45 isolates of *B. g. tritici* from the eastern U.S. (26 from eastern North Carolina and 19 from eastern Virginia). Fragments were analyzed by Eton

Biosciences, Inc. on an ABI 3730xl DNA analyzer. With one exception, all exhibited moderate to high levels of polymorphism with between two and 20 alleles per locus (average 7.56). Null alleles were detected in *Bgt*-4, *Bgt*-6, and *Bgt*-7 with an estimated frequency of 2.2%. Allelic diversity (Nei, 1987), calculated using FSAT version 2.9.3 (Goudet, 1995), ranged from 0.068 to 0.932, with a mean of 0.543. In preliminary screening, polymorphisms at the *Bgt*-3 locus have been found at moderate levels in another *B. g. tritici* population from Israel. However, insufficient data has been collected to determine usefulness of this locus in population studies. Linkage disequilibrium (LD) tests, conducted using GENEPOP version 4.0 (Garnier-Gere & Dillmann, 1992), found significant LD in several pairs of loci (Table 1). Probable causes include the high ratio of asexual (clonal) to sexual reproduction and/or founder effects resulting from the organism's recent introduction to the U.S. Preliminary results indicate these markers will be useful in population studies of *B. g. tritici.*

Table 1. Primer sequences, PCR amplification conditions, and molecular diversity indexes of nine microsatellite DNA loci developed and used to screen 45 isolates of powdery mildew of wheat (*Blumeria graminis* f. sp. *tritici*) from the eastern U.S.

Locus	Primer sequence	Repeat motif	Ta (°C)	mM $MgCl^2$	Allelic diversity	Number of alleles	LD test*	Genbank accession number
Bgt-1	F:TGGCTAATACTCACCAATCAGAA R:AGCAGAAGCCTTAGGACTTTAAT	$(GTT)^{53}$	50	2.0	0.932	20	A	HQ631372
Bgt-2	F:GACCCAATGTGGAAAAGATAGC R:AGCAGAAGCCTCTTGTTTGT	$(AAAC)^{19}$	54	2.0	0.763	7		HQ631373
Bgt-4±	F:TTCTGTAAACGCATTCTTGATA R:AAAAGAGGATAGGAGCACTGTA	$(GT)^{11}$	55	2.0	0.238	2	B	HQ631366
Bgt-5±	F:GGAGAATGGAGGAACTTGTAT R:CCACAGAATGAGGAAAGATAAT	$(ATC)^{11}$	59	2.0	0.627	3		HQ631367
Bgt-6	F:CGAGGGTTGGAACTGGATAA R:TTCTCTGTTTGACGCTCTCG	$(AATG)^5$	55	2.0	0.068	2	C	HQ631368
Bgt-7	F:AGAAGGCTGGACAGATAGGT R:TTACCCTTCTCCTGATGTTGC	$(CATA)^2CTTA(CATA)^9$	55	2.0	0.418	3	C	HQ631369
Bgt-8	F:GGCATTCTGCTATATTCTATCCTA R:TGCTGCCAATGTCAAGATGT	$(CTGTT)^{24}$	58	1.4	0.901	20	A	HQ631370
Bgt-9	F:AGCTCGCATAGGAGCTTTCA R:CTGACGTCGATGGTTCACTCA	$(GTCT)^5ATCT(GTCT)^6$ GTCA$(GTCT)^8$	55	2.0	0.330	4		HQ631371
Bgt-10	F:CCAATCCTCAGGGTTCGGT R:TGGATGTCACATGGTATCAGAGC	$(AGT)^{14}AGC(AGT)^4ATT$ $(AGT)^5AGC(AGT)^{12}$	65-55	1.4	0.614	7	A B	HQ631364

* Loci with significant pairwise LD ($P < 0.05$); loci with same letter were linked.

± PCR reactions performed using 3-primer method of Schuelke (2000).

Acknowledgements.

We Thank Dr. Ed Vargo for facilitating this project.

References

Booth W, Lewis VR, Taylor RL, Schal C, Vargo EL (2008) Identification and characterization of 15 polymorphic microsatellite loci in the western dry wood termite, *Incisitermes minor*. *Molecular Ecology Resources* **8**, 1102-1104.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research* **19**, 4008.

Dopman EB, Bogdanowicz SM, Harrison RG (2004) Genetic mapping of sexual isolation between E and Z pheromone strains of the European corn borer (*Ostinia nubilalis*). *Genetics* **167**, 301-309.

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 92-94.

Garnier-Gere P, Dillmann C (1992) A computer program for testing pairwise linkage disequilibria in subdivided populations. *Journal of Heredity* **83**, 239.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology* 202-222.

Goudet J (1995) FSTAT (Vers 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86**, 485-485.

Hermansen JE, Torp U, Prahm LP (1978) Studies of transport of live spores of cereal mildew and rust fungi across the North Sea. *Grana* **17**, 41-46.

Lipps PE, Madden LV (1989) Assessment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. *Phytopathology* **79**, 462-470.

Nei M (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York, NY.

Parks R, Carbone I, Murphy JP, Cowger C (2009) Population genetic analysis of an Eastern U.S. wheat powdery mildew population reveals geographic subdivision and recent common ancestry with U.K. and Israeli populations. *Phytopathology* **99**, 840-849.

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. (eds Krawetz S & Misener S), pp. 365-386. Humana Press, Totowa, NJ.

Schuelke M (2000) An economic method for fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233-234.

Wolfe MS, Schwarzbach E (1978) Patterns of race changes in powdery mildews. *Annual Review of Phytopathology*. **16**, 159-180.