Molecular Ecology (1998) **7**, 1611–1612

TECHNICAL NOTE

Isolation and characterization of microsatellite loci in the wheat pathogen *Mycosphaerella graminicola*

PHILIPPA G. OWEN, MING PEI, ANGELA KARP, DAVID J. ROYLE and KEITH J. EDWARDS

IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS41 9AF, UK

Keywords: microsatellite, *Mycosphaerella*, pathogen, PCR, *Septoria*

Received 22 May 1998; revision accepted 22 June 1998

Correspondence: M. Pei. Fax: +44 1275394007; E-mail: ming.pei@bbsrc.ac.uk

Microsatellites are becoming increasingly important in the study of population structure. To date, however, very few single-locus microsatellite markers have been isolated in fungi (Groppe *et al*. 1995). In this paper we report the isolation of nine single-locus microsatellite markers from the fungal pathogen *Mycosphaerella graminicola*. *M. graminicola* (Fuckel) J. Shoert. (anamorph *Septoria tritici* Rob. ex Desm.), is one of the most important foliar diseases of wheat in the northern hemisphere. The pathogen colonizes plant tissue as haploid hyphae and then reproduces both asexually and sexually. Field infections by *M. graminicola* are initiated by windborne, sexual ascospores early in the season and the disease then progresses as a consequence of repeated infections by asexual conidia (Shaw & Royle 1989). The use of molecular technology has provided insights into the population biology of *M. graminicola*. Using restriction fragment length polymorphism (RFLP), McDonald & Martinez (1990) found that a high level of genetic variability exists within North American *S. tritici* populations. Interestingly, these studies have also indicated that sexual reproduction probably plays a much more important role than was previously thought.

Population studies involving RFLPs are time-consuming to perform. Alternatively, microsatellites have been shown to be simple to use, and can be highly polymorphic between individuals within a population. The nine primer sets described here will be used to study the population dynamics of *S. tritici*, in order to develop new pathogen-management strategies, and to answer questions concerning the extent to which sexual and asexual reproduction affect population structure.

A microsatellite-enriched library was constructed from *S. tritici* genomic DNA as described by Edwards *et al*. (1996) using the following microsatellite motifs: CA, CT, CG, CAA and CGG. Seventy-six clones from a subset of 172 clones (42%), chosen at random from the library, were shown to contain microsatellite motifs by ABI^{TM} Prism Dye terminator cycle sequencing (Perkin-Elmer) using the universal forward primer. Primers were designed for 31 of the sequences using the computer program PRIMER version 0.5 (Whitehead Institute, Cambridge, MA, USA). Nine of these primer sets were found to be suitable for screening purposes.

Allelic variability of the microsatellites was determined using genomic DNA isolated from haploid mycelium using the procedure of Steiner *et al*. (1995), with the addition of a single phenol–chloroform extraction, before ethanol precipitation. A total of 12 individual isolates, derived from single ascospores produced on senescent leaves of winter wheat cv. Longbow and collected from an unsprayed field plot (12 × 8 m) at Long Ashton Research Station in June 1995, was used in this preliminary study. For all loci the forward primer from each primer pair was 5′ end-labelled with [γ^{33}P]-ATP (Amersham) and T4 polynucleotide kinase (Pharmacia). The PCR was carried out in a 25 μL reaction containing 200 μM each dNTP (Pharmacia), 30 pmol reverse primer, 30 pmol forward labelled primer, 1× PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.5), 1 unit of *Taq* DNA polymerase (BRL) and 10 ng of template genomic DNA. After an initial denaturation at 95 °C for 60 s, PCR cycle conditions were as follows: 95 °C 40 s, 58 °C for 60 s, 72 °C for 60 s, for 30 cycles, followed by a final extension step at 72 °C for 10 min. PCR products were resolved on urea–polyacrylamide (6%) sequencing gels. Gels were dried and exposed to Kodak Biomax MR film for 24 h.

The number of alleles at each locus and the size range are shown in Table 1. The number of alleles ranged from two to four with a genetic diversity value of between 0.278 and 0.736 (see footnote to Table 1). For four of the primer sets, from one to four of the isolates failed to amplify a band and were recorded as having a null allele. For three of the primer sets (ST1B3, ST1G7 and ST1D7), the size range of the alleles observed would allow for several more alleles to exist within the *Septoria* population. Of the nine primer pairs, seven contain GC-rich trinucleotide repeats. This ratio was similar to the proportion of clones containing GC-rich trinucleotide repeats within the original 76 clones; however, the number of GC-rich repeats was significantly greater from that seen in enriched libraries produced for other species (Edwards *et al*. 1996). This unusually high frequency of CG-rich trinucleotide repeats probably reflects the high GC content of the *Septoria* genome as revealed by our random sequencing studies. Thus, our study suggests that GC-rich repeats should not be overlooked when isolating microsatellites from GC-rich species such as fungi.

Using the primer pairs to screen the 12 isolates, we were able to distinguish all 12 as individual genotypes. Even within the relatively small geographical area sampled, all the isolates could be distinguished by using the five microsatellites: ST1A2, ST1A4, ST1B3, ST1E7 and ST1D7. In the near future, we hope to use the microsatellites described here to examine more geographically distinct material.

Acknowledgements

The Institute of Arable Crop Research-Long Ashton Research Station receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK. The nucleotide

sequence data will appear in EMBL with the Accession nos AJ007031–AJ007039.

References

Groppe K, Sanders I, Wiemken A, Boller T (1995) A microsatellite marker for studying the ecology and diversity of fungal endophytes (*Epichloe* spp.) in grasses. *Applied and Environmental Microbiology*, **61**, 3943–3949.

Shaw MW, Royle DJ (1989) Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola)* infections in winter wheat crops in the UK. *Plant Pathology*, **38**, 35–43.

McDonald BA, Martinez JP (1990) DNA restriction fragment length polymorphism among *Mycosphaerella graminicola* (anamorph *Septoria tritici)* isolates collected from a single wheat field. *Phytopathology*, **80**, 1368–1373.

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. *Biotechniques*, **20**, 758–760.

Steiner JJ, Poklemba CJ, Fjellstrom RG, Elliot LF (1995) A rapid one-tube genomic DNA extraction process for PCR and RAPD analysis. *Nucleic Acid Research*, **23**, 2569–2570.

Table 1 Primer sequences and characteristics of *Septoria tritici* microsatellite loci. The number of alleles was calculated for a population sample of 12 individual isolates collected during 1995

Locus	Repeat motif	Primer sequence	Size range (bp)	No. of alleles	No. of samples with null alleles	Genetic diversity*
ST1A2	$(GGC)_7/(GGT)_2$	F: 5'TTTTCCTCTCTCCCGTGC3' R: 5'CTCTCGTCAACCCCAGAC3'	90–99	3	0	0.486
ST1A4	$(CGG)_7$	F: 5'GGTTCGATGGAGAGATTT3' R: 5'TCACCTCCTCATCGCAGA3'	98–101	2	0	0.278
ST1B3	$(CCG)_8$	F: 5'ACGTTCACATCTCATACC3' R: 5'CTCTAGCTCTACCTTAAT3'	180–191	2	4	0.542
ST2E4	$(GGC)_5$	F: 5'GAAGATCAACAGCATGGGCGG3' R: 5'CTCCAGAGGGATCACAAAGGC3'	77–80	2	1	0.375
ST1E3	$(CGG)_5$	F: 5'GTTCCGCCGGTCGAAGTCG3' R: 5'GCCAAGGCACTGCTGCTCC3'	61–63	2	0	0.375
ST2C10	$(AGCGG)_4$	F: 5'AGGCGAGAAACTTGCTTGCAG3' R: 5'AATGAACGTCCCATGGACGTG3'	70–72	2	4	0.542
ST1G7	$(TG)_9$	F: 5'ATGCTGAGAAGTTCGGTGAGG3' R: 5'CGTTCTTCCACCTCCAACACT3'	100–112	2	4	0.653
ST1E7	$(CGG)_5$	F: 5'GATCTCGAGCAGGGCGGAAGT3' R: 5'TCACACGCTGGTCTGTGAATC3'	140–143	2	0	0.444
ST1D7	$(AC)_{22}$	F: 5'ATCCTCCATTCACTACTGCAT3' R: 5'TGTGGAACAGGAATAGGCTTG3'	70–85	4	0	0.736

*Genetic diversity was calculated for each locus by including 'nulls' as a separate allele. The formula for calculating genetic diversity is the same as for expected heterozygosity in diploid species [$(1 - \Sigma p_i^2)$, where p_i is the frequency of allele i]. In the case of haploids this represents the probability of two individuals, sampled at random from a population, having different alleles at that locus.