Molecular Ecology Notes (2005) **5**, 622–624 doi: 10.1111/j.1471-8286.2005.01015.x

PRIMER NOTE

Simple sequence repeat markers for species in the *Fusarium oxysporum* complex

M. BOGALE,* B. D. WINGFIELD,* M. J. WINGFIELD† and E. T. STEENKAMP†
**Departments of Genetics, †Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa*

Abstract

We describe nine simple sequence repeat (SSR) markers developed for studying *Fusarium oxysporum*. Allelic diversity at the nine loci ranged from 0.003 to 0.895, with a total of 71 alleles among 64 isolates. These markers will facilitate studies on relationships amongst isolates of *F. oxysporum*.

Keywords: *Fusarium oxysporum*, polymorphic markers, simple sequence repeat

Received 17 February 2005; revision accepted 1 April 2005

Fusarium oxysporum is grouped into *formae speciales* and/or races based on pathogenicity. However, pathogenicity tests are tedious, inconclusive and preclude nonpathogenic strains. To circumvent these problems, vegetative compatibility and various protein- and DNA-based techniques have been used. These techniques, however, have not provided adequate solution, and the need for improved and simplified methods remains.

Simple sequence repeats (SSRs) provide a powerful tool for taxonomic and population genetic studies. There are SSR markers developed in *Fusarium circinatum* (Britz *et al*. 2002), but these were not useful in *F. oxysporum*. In this study, we describe nine SSR markers developed for the study of *F. oxysporum*.

Random amplified microsatellites (RAMS) polymerase chain reaction (PCR) with intersimple sequence repeat (ISSR) primers was used to develop SSR markers (Van der Nest *et al*. 2000) using isolates FCC3799 (from Ethiopian soil) and FCC3175 (from Centraalbureau voor Schimmelcultures [CBS], the Netherlands; CBS no. 413.90). RAMS products were cloned and sequenced with vector-specific primers. For SSRs at ends of inserts, flanking primers were designed after genome walking (Siebert *et al*. 1995).

To evaluate the SSR primers, these were used in 64 isolates (not shown). Twenty-one of these were from the CBS, and represented 21 *formae speciales*. The remaining strains were isolated from agricultural soils and plant tissues from Ethiopia. PCRs (25 μL) consisted of 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 50 mM KCl, 2.5 μM of each dNTP, 0.20 μM of each primer, 0.05 U/μL *Taq* polymerase and approximately 4 ng/μL template DNA. PCR cycles consisted of a 4-min denaturation (at 94 °C), followed by 35 cycles of denaturation, annealing and extension (at 72 °C), each for 30 s. PCRs were terminated after a final 10-min extension. Appropriate annealing temperatures were used for each primer set (Table 1). Polymorphism of amplicons was tested by agarose gel electrophoresis.

Allele sizes were determined using Applied Biosystems' ABI PRISM™ 3100 DNA sequencer, LIZ 500 size standard and GENEMAPPER version 3.0. For this reason, forward primers were labelled with fluorescent dyes (Applied Biosystems), and PCRs were done as with the unlabelled primer sets. Amplicons were then separated on the DNA sequencer.

The SSR primers amplified single bands that were polymorphic with a total of 71 alleles across the 64 isolates (Table 2). A range of allele sizes and diversities were evident at each locus. For example, locus FO13 with allele sizes of 144–500 bp had the highest allelic diversity (0.895), whereas locus FO10 with alleles of 206 and 208 bp had the least allelic diversity (0.003). The size variation among some of the alleles did not appear to be a function of their repeating units. For example, the difference in increasing order of the three alleles at locus FO14 was only 1 bp (Table 2), whereas the repeating unit included at least three nucleotides (Table 1). This suggests that insertions/deletions also play a role in the polymorphisms observed.

Correspondence: M. Bogale, Fax: 124203947;
E-mail: mesfin.bogale@ fabi.up.ac.za

Table 1 Primer sequences, SSR motifs in the respective amplicons and GenBank Accession nos for sequences of SSR PCR products from isolates FCC3175 and FCC3799

Primer	Primer sequence (5′–3′)	T_a*	SSR motif	Locus†	Isolate‡	GenBank Accession no.§
MB2	F: TGCTGTGTATGGATGGATGG R¶: CATGGTCGATAGCTTGTCTCAG	57	$(GT)_{11}(GA)_6$	FO2	FCC3175	AY931024
MB5	F¶: ACTTGGAGGAAATGGGCTTC R: GGATGGCGTTTAATAAATCTGG	54	$(TG)_9$	FO5	FCC3799	AY931030
MB9	F: TGGCTGGGATACTGTGTAATTG R¶: TTAGCTTCAGAGCCCTTTGG	51	$(CA)_9$	FO9	FCC3175	AY931029
MB10	F¶: TATCGAGTCCGGCTTCCAGAAC R: TTGCAATTACCTCCGATACCAC	48	$(AAC)_6$	FO10	FCC3175	AY931028
MB11	F: GTGGACGAACACCTGCATC R: AGATCCTCCACCTCCACCTC	68	$(GGC)_7$	FO11	FCC3799	AY931025
MB13	F: GGAGGATGAGCTCGATGAAG R: CTAAGCCTGCTACACCCTCG	68	$(CTTGGAAGTGGTAGCGG)_{14}$	FO13	FCC3799	AY931026
MB14	F: CGTCTCTGAACCACCTTCATC R: TTCCTCCGTCCATCCTGAC	57	$(CCA)_5$	FO14	FCC3175	AY931027
MB17	F: ACTGATTCACCGATCCTTGG R¶: GCTGGCCTGACTTGTTATCG	57	$(CA)_{21}$	FO17	FCC3799	AY931023
MB18	F¶: GGTAGGAAATGACGAAGCTGAC R: TGAGCACTCTAGCACTCCAAAC	57	$(CAACA)_6$	FO18	FCC3175	AY931031

*, PCR annealing temperature; †, locus indicated by an FO-number (FO = *Fusarium oxysporum*); ‡, isolate in which primers were designed; §, GenBank Accession no. for sequence of SSR PCR product; ¶, primer developed after genome walking.

Table 2 SSR allele sizes, number of alleles and allelic diversities based on 43 *Fusarium oxysporum* strains from Ethiopia and 21 *formae speciales* from CBS (numbers of isolates tested are indicated in parentheses)

	Sizes of SSR alleles (bp)				
Locus	Ethiopian isolates from soil (27)	Ethiopian isolates from plant tissues (16)	CBS isolates (21)	Total number of alleles	Allelic diversity*
FO2	237, 238, 250, 252, 254, 260, 264, 271, 275	246, 248, 254, 257, 260, 264	234, 237, 240, 242, 246, 248, 252, 254, 257, 264	15	0.891
FO5	252, 254, 267, 269	254, 256, 267	252, 254, 256, 267, 274, 344	7	0.768
FO9	126, 238	105, 126	126, 130, 141, 234, 237, 240, 254	9	0.693
FO10	206	206	206, 208	2	0.003
FO11	172, 175, 180, 182	175, 177, 180	172, 175, 180, 182, 186	6	0.707
FO13	264, 296, 382, 345, 395, 400, 422, 476, 483, 492, 500	296, 376, 395, 400, 476, 483, 492, 500	144, 264, 296, 345, 376, 395, 422, 476	13	0.895
FO14	183, 184	184	184, 186	3	0.351
FO17	303, 312, 317, 319, 320, 339	299, 312, 317, 319	299, 301, 308, 312, 317, 321, 331, 334, 337	13	0.719
FO18	284, 289	284, 289, 293	284, 289, 293	3	0.327

*Allelic diversity ($1 - \sum p_i^2$), where p_i is frequency of the ith allele (Nei 1973).

SSR amplicon sequences (Table 1) were compared with the *Gibberella zea* and *Neurospora crassa* sequences in the National Center for Biotechnology Information database (www.ncbi.nih.gov) to determine linkage between the markers. This is pertinent because *F. oxysporum* is an asexual fungus and linkage cannot be determined by mating tests. However, none of the sequences, except that of locus FO2, were significantly homologous to those in the database. It was consequently not possible to assess linkage between the markers.

The polymorphism revealed with these primers should be sufficient for studies of genetic diversity in *F. oxysporum*. These primers should be particularly useful because the fungus is one of the most common *Fusarium* spp. residing in the soil environment.

Acknowledgements

We acknowledge financial support from the Tree Pathology Co-operative Programme, the National Research Foundation, the THRIP initiative of the Department of Trade and Industry, South Africa and the Agricultural Research and Training Project, Ethiopia.

References

Britz H, Coutinho TA, Wingfield BD, Wingfield MJ (2002) Sequence characterized amplified polymorphic markers for the pitch canker pathogen, *Fusarium circinatum*. *Molecular Ecology Notes*, **3**, 577–580.

Nei M (1973) Analysis of genetic diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA*, **70**, 3321–3323.

Siebert PD, Chenchik A, Kellogg DE, Lukyanov KA, Lukyanov SA (1995) An improved PCR method for walking in uncloned genomic DNA. *Nucleic Acids Research*, **23**, 1087–1088.

Van der Nest MA, Steenkamp ET, Wingfield BD, Wingfield MJ (2000) Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR). *Plant Breeding*, **119**, 433–436.