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PRIMER NOTE

Development of VNTR markers for two *Fusarium graminearum* clade species

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Abstract

Using a bioinformatics approach, we developed 10 variable number of tandem repeat (VNTR) markers for *Fusarium graminearum* and *Fusarium asiaticum* useful for population genetic studies. Repeat sequences in the genome sequence of *F. graminearum* were identified by a tandem repeat finding program. Length polymorphisms at 54 loci were examined for five strains each from the United States, Italy and China. From these 54 loci, 10 were selected based on polymorphisms detected across species, ease of scoring, and their dispersed location in the genome.

Keywords: bioinformatics, *Fusarium*, population genetics, VNTR

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Fusarium head blight (FHB) or scab is a significant fungal disease of wheat and barley. The disease has become a threat to the world's food supply due to outbreaks in Canada, USA, Australia, Europe and China (Stack 2003). A major causal agent is the ascomyceteous clade species *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch]. In addition to quantitative losses, *F. graminearum* also causes a reduction in grain quality due to the production of trichothecene mycotoxins. These pose a serious hazard to human and animal health as they are potent inhibitors of eukaryotic protein biosynthesis.

Molecular phylogenetic analyses based on 11 nuclear genes at six loci revealed that *F. graminearum* consists of at least nine biogeographically structured lineages (O'Donnell *et al*. 2000; Ward *et al*. 2002), which have recently attained species status (O'Donnell *et al*. 2004). *F. graminearum* and *F. asiaticum* were suggested to be the most recently evolved among species in the *F. graminearum* clade; both species are predominant in the Northern Hemisphere (O'Donnell *et al*. 2000). Previously, AFLP and RFLP markers have been developed and used for population analysis of *F. graminearum* in the USA and of *F. asiaticum* in eastern China, respectively. High levels of gene flow were observed for both populations (Gale *et al*. 2002; Zeller *et al*. 2003). Compared to the above-mentioned markers, VNTR markers may be more desirable for population genetic analysis due to the simplicity to collect accurate polymorphic data and due to their codominance. Generally, the development of VNTR markers is costly and cumbersome and therefore few VNTR markers have been reported to date for *F. graminearum* (Giraud *et al*. 2002). However, the recent whole genome sequencing of *F. graminearum* strain PH-1 (NRRL 31084) by the Center for Genome Research, Cambridge, MA, USA, has allowed a bioinformatic approach for the development of VNTR markers in these species.

The genome sequence of *F. graminearum* was obtained from http://www.broad.mit.edu. Repeat sequences were searched for using Tandem Repeats Finder (Benson 1999). Strict conditions of alignment parameters (match, mismatch and indel: 2, 7 and 7) were used for the search. Repeat sequences with a minimum alignment score of 50- and periods shorter than 500 nucleotides were obtained. With the goal of applying the VNTR markers to studies of linkage disequilibrium, 54 loci evenly scattered in the genome were selected from the search results. Nine loci of two to five nucleotide repeat units with more than six repeats were selected from scaffold 1 and 42 loci of six to 18 nucleotides with more than three repeats were selected from scaffolds 1–7 that together cover 92% of whole genome sequence (33.2 Mbp of 36 Mbp). The remaining three loci were obtained from sequence comparisons between PH-1 and a field strain of *F. graminearum* (00–676) at loci arbitrarily chosen from an EST library of strain PH-1 (Trail *et al*. 2003).

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Table 1 Isolates of *Fusarium graminearum** and *Fusarium asiaticum*† used in this study

Isolate	Species	Origin‡
PH-1 (NRRL 31084)	F.g.*	USA, MI
00–676	F.g.	USA, MN
00–330	F.g.	USA, MN
00–355	F.g.	USA, SD
00–487	F.g.	USA, MN
01–11	F.g.	Italy, Ancona
01–14	F.g.	Italy, Emilio-Romagna
01–26	F.g.	Italy, Lombardia
01–30	F.g.	Italy, Lazio
01–33	F.g.	Italy, Emilio-Romagna
00–88	F.a.†	China, Haining
00–246	F.a.	China, Haining
00–252	F.a.	China, Haining
00–275	F.a.	China, Haining
00–278	F.a.	China, Haining

‡All strains were originally isolated from wheat, except for PH-1 (corn) and 01–30 (cyclamen).

Primer pairs for polymerase chain reaction (PCR) were designed to amplify about 250 bp in PH-1 by the primer-designing tool in the Saccharomyces Genome Database (http://www.yeastgenome.org/). PCR was carried out in a Robocycler Gradient 96 Temperature Cycler (Stratagene) as follows: total volume of the reaction mixture was 10 μl containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP, 1 μM of each primer, 0.25 unit *Taq* polymerase (Takara), and 10 ng genomic DNA. Cycling conditions were: 95 °C for 2 min; then 25 cycles of 95 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute; and a final extension at 72 °C for 10 min. Screening for VNTR loci was conducted using five *F. graminearum* isolates each from the United States (including PH-1 and 00–676) and Italy, and five *F. asiaticum* isolates from China (Table 1). PCR products were separated by 3% MetaPhor agarose (Cambrex) including 0.5 μg/mL ethidium bromide and visualized on a transilluminator. Among the 54 loci, 43, 28, and 14 loci showed polymorphisms within isolates from the U.S., Italy, and China, respectively. Nine loci were polymorphic for all three groups of isolates (Table 2). In order to determine the alleles, PCR products of these nine VNTR loci, and

Table 2 Characterization of 10 SSRs in *Fusarium graminearum* and *Fusarium asiaticum*

Locus*	Primer sequences†	Repeat motif	Size (bp)‡	No. of alleles			
				USA	Italy	China	Total
HK1043/Sc1/ Ct1.52/41 839	F: ACAGGCATCCAAGGACATTT R: GTTTGATGGCGCATTCAAAG	$(TCGAAGAGCCAGCTG)_6$	286	5 (0.80)§	2 (0.32)	3 (0.64)	7 (0.73)
HK913/Sc1/ Ct1.73/664	F: GCAGGACCTGGATGATGAA R: ATGTGTGCAGCCATGAGATT	$(GAA)_{10}(GAG)_9$	234	4 (0.72)	4 (0.72)	4¶(0.75)	10 (0.89)
HK917/Sc1/ Ct1.82/2471	F: ATCTCCCAAGCTGGCTAATT R: AGAACCGGCAAAGTTCGATT	$(CA)_{16}$	234	3 (0.56)	2 (0.48)	2 (0.32)	3 (0.55)
HK957/Sc1/ Ct1.91/16 055	F: TCCGAAGGTAGAAGCGTTGT R: TCAAGCCCATCTATGCTGTT	$(GGGAGTCAAT/C)_{16}$	298	3 (0.56)	5 (0.80)	5 (0.80)	10 (0.87)
HK965/Sc2/ Ct1.154/51 671	F: GAGATGGCAACATTATTTGCA R: ATTGGCAGCAGGGCTTGATT	$(CTTATC)_8$	252	5 (0.80)	4 (0.72)	4 (0.72)	8 (0.85)
HK967/Sc2/ Ct1.154/53 868	F: AAGAGGGCGTGTCTCTGTTTT R: CGCTTCCTTCCTTTCAATTC	$(CAGTGA)_5$	215	2 (0.48)	2 (0.48)	2 (0.32)	3 (0.65)
HK1059/Sc3/ Ct1.196/164 228	F: AAGACTGGTCAGCAGTAGGGA R: TGAGAGCGAGACTGAGCATGA	$(GCACTG/AGTCTCA/GGCA)_5$	248	3 (0.64)	3 (0.56)	2 (0.32)	4 (0.64)
HK977/Sc3/ Ct1.208/47 696	F: AAACGTAAACGGATCAACGG R: AGATTCGCAACTTTGTGCTG	$(CACAGG/A)_6$	210	2 (0.32)	1 (0.00)	3 (0.56)	4 (0.68)
HK630/Sc6/ Ct1.371/112 325	F: TGGATATGGTTCCCCAGCT R: TACTGACCTTGAGGAGCACCATAC	$(TATGGTGCTCCC CAGGGCCAG)_2$	239	2 (0.32)	2 (0.48)	2 (0.32)	2 (0.39)
HK1073/Sc6/ Ct1.398/70 812	F: TATGATGCAGCGAATGCAAC R: TAGAGACCTGGCCCATACCA	$(CAGGCA/GCAA/GG/CC/AG/A)_6(CAG)_8$	221	4 (0.72)	3 (0.56)	5 (0.80)	10 (0.86)

*Locus name/Scaffold No./Contig No./Position in contig. Information based on the *Fusarium graminearum* genome sequencing project.
†F, forward primer; R, reverse primer.
‡Based on the genome sequence of isolate PH-1 (NRRL 31084).
§The value of Nei's gene diversity was shown in parenthesis.
¶Excluding strain 00–88 with no amplification.

one VNTR locus (HK977) that was polymorphic for U.S. and Chinese isolates were analysed by 6% denaturing polyacrylamide gels using Sequi-Gen GT (Bio-Rad) according to manufacture's instructions (Table 2). After electrophoresis, bands were visualized by silver staining (Schumacher *et al*. 1986). These VNTR markers were successfully applied to a preliminary population study of Japanese isolates.

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References

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research*, **27**, 573–580.

Gale LR, Chen L-F, Hernick CA, Takamura K, Kistler HC (2002) Population analysis of *Fusarium graminearum* from wheat fields in eastern China. *Phytopathology*, **92**, 1315–1322.

Giraud T, Fournier E, Vautrin D *et al*. (2002) Isolation of eight polymorphic microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Fusarium culmorum*. *Molecular Ecology Notes*, **2**, 121–123.

O'Donnell K, Kistler HC, Tacke BK, Casper HH (2000) Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences of the USA*, **97**, 7905–7910.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics Biology*, in press.

Schumacher J, Meyer N, Riesner D, Weidemann HL (1986) Diagnostic procedure for detection of viroids and viruses with circular RNAs by 'return'-gel electrophoresis. *Journal of Phytopathology*, **115**, 332–343.

Stack RW (2003) History of Fusarium head blight with emphasis on North America. In: *Fusarium Head Blight of Wheat and Barley* (eds KJ Leonard, WR Bushnell), pp. 1–34. The American Phytopathological Society Press.

Trail F, Xu JR, Miguel PS, Halgren RG, Kistler HC (2003) Analysis of expressed sequence tags from *Gibberella zeae* (anamorph *Fusarium graminearum*). *Fungal Genetics Biology*, **38**, 187–197.

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K (2002) Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. *Proceedings of the National Academy of Sciences of the USA*, **99**, 9278–9283.

Zeller KA, Bowden RL, Leslie JF (2003) Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. *Phytopathology*, **93**, 874–880.