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Mating Type Sequences in Asexually Reproducing *Fusarium* Species

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To assess the potential for mating in several *Fusarium* species with no known sexual stage, we developed degenerate and semidegenerate oligonucleotide primers to identify conserved mating type (*MAT*) sequences in these fungi. The putative α and high-mobility-group (HMG) box sequences from *Fusarium avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum* were compared to similar sequences that were described previously for other members of the genus. The DNA sequences of the regions flanking the amplified *MAT* regions were obtained by inverse PCR. These data were used to develop diagnostic primers suitable for the clear amplification of conserved mating type sequences from any member of the genus *Fusarium*. By using these diagnostic primers, we identified mating types of 122 strains belonging to 22 species of *Fusarium*. The α box and the HMG box from the mating type genes are transcribed in *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum*. The novelty of the PCR-based mating type identification system that we developed is that this method can be used on a wide range of *Fusarium* species, which have proven or expected teleomorphs in different ascomycetous genera, including *Calonectria*, *Gibberella*, and *Nectria*.

The genus *Fusarium* contains filamentous ascomycete fungi with a worldwide distribution. *Fusarium* species can parasitize cultivated plants (1) and/or produce mycotoxins that pose serious hazards to human and animal health (9, 18). Species of *Fusarium* can grow successfully on a variety of substrates, can tolerate diverse environmental conditions, and have high levels of intraspecific genetic and genotypic diversity (for examples, see references 8, 12, 17, and 26). Neither the origins of this diversity nor the mechanisms that maintain it are well understood.

Meiotic recombination can generate and maintain genotypic variation and result in the reassortment of genes that govern traits such as virulence or toxin production (7). The sexual spores (ascospores) produced by some *Fusarium* species also may function as infectious propagules (11, 19). Although several *Fusarium* species have a known sexual cycle, i.e., they mate in either a homothallic or heterothallic manner followed by subsequent meiosis and the production of ascospores, important pathogenic species, including *Fusarium avenaceum*, *Fusarium cerealis*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium poae*, and *Fusarium sporotrichioides*, have no known sexual stage.

Assessing the potential for mating by toxigenic strains of *Fusarium* would increase our understanding of the genetic mechanisms that maintain intraspecific diversity and biological and evolutionary species integrity. The frequency of sexual reproduction is also an important parameter for the design of strategies to control plant pathogens, since these strategies are often different for clonally and sexually reproducing organisms. High levels of race-specific resistance can be developed in plant cultivars against clonally reproducing organisms, whereas horizontal resistance could be more effective against pathogens comprising genetically diverse populations as a result of mating and meiotic recombination (16).

The known teleomorphs of *Fusarium* species belong to the genera *Calonectria*, *Gibberella*, and *Nectria* (5). In heterothallic species, e.g., *Gibberella fujikuroi*, mating type is controlled by a single locus with two idiomorphic alleles, termed *MAT-1* and *MAT-2*. These alleles contain a conserved α box domain and a high-mobility-group (HMG) box domain, respectively. Strains of *Gibberella zeae* (anamorph of *Fusarium graminearum*), a homothallic species, carry both the *MAT-1* and *MAT-2* idiomorphs, closely linked together (27). Strains of *Fusarium oxysporum*, a species complex with no known sexual stage, also contain transcribed *MAT* alleles (4, 13, 27). However, the *MAT* genes have not been studied in any other mitotic holomorph species within the genus *Fusarium*.

PCR amplification of *MAT* sequences from various *Fusarium* species belonging to the *G. fujikuroi* species complex has been utilized to standardize the mating type terminology for mating populations of this species complex (13) and to develop assays for identifying the presence of the *MAT* allele without sexual crosses (22, 25). However, the primers used in these previous studies were inadequate for the rest of the genus (13, 22), probably due to sequence divergence that may occur even in conserved *MAT* sequences of these fungi. The aims of the present study were (i) to demonstrate whether mating type sequences can be found in *Fusarium* species with no known sexual stage, (ii) to develop a PCR-based technique for the rapid identification of mating types in a wide range of *Fusarium* species with proven or expected *Calonectria*, *Gibberella*, and *Nectria* teleomorphs, and (iii) to demonstrate the transcription of mating type genes in selected "asexual" *Fusarium* species during their vegetative growth.

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MATERIALS AND METHODS

Strains and culture conditions. Fungal strains involved in mating type identification were as follows (species and strain number [mating type identified by PCR]): *Fusarium acuminatum* subsp. *acuminatum* ITEM 791 (*MAT-1*), ITEM 1042 (*MAT-2*), ITEM 1137 (*MAT-1*), ITEM 1716 (*MAT-1*), ITEM 1892 (*MAT-2*), ITEM 1895 (*MAT-2*), ITEM 1896 (*MAT-2*), ITEM 1897 (*MAT-1*), ITEM 1899 (*MAT-1*), and ITEM 1901 (*MAT-1*); *F. acuminatum* subsp. *armeniacum* ITEM 795 (*MAT-2*), ITEM 796 (*MAT-2*), ITEM 797 (*MAT-1*), ITEM 800 (*MAT-2*), ITEM 988 (*MAT-1*), ITEM 992 (*MAT-1*), and ITEM 998 (*MAT-1*); *F. avenaceum* ABC-A2 (*MAT-2*), ABC-A3 (*MAT-2*), ABC-A10 (*MAT-2*), ITEM 158 (*MAT-1*), ITEM 858 (*MAT-2*), ITEM 859 (*MAT-1*), ITEM 1787 (*MAT-2*), ITEM 3397 (*MAT-2*), ITEM 3400 (*MAT-1*), ITEM 3406 (*MAT-1*), ITEM 3410 (*MAT-2*), and ITEM 3411 (*MAT-2*); *Fusarium camptoceras* ITEM 1116 (*MAT-2*) and ITEM 1128 (*MAT-2*); *F. cerealis* ITEM 661 (*MAT-1*), ITEM 663 (*MAT-2*), ITEM 664 (*MAT-2*), ITEM 665 (*MAT-2*), and ITEM 666 (*MAT-2*); *Fusarium chlamydosporum* ITEM 1806 (*MAT-1*), ITEM 1873 (*MAT-1*), and ITEM 2035 (*MAT-2*); *Fusarium compactum* ITEM 156 (*MAT-1*), ITEM 427 (*MAT-2*), ITEM 488 (*MAT-2*), ITEM 616 (*MAT-2*), ITEM 1288 (*MAT-2*), ITEM 1289 (*MAT-1*), ITEM 1867 (*MAT-2*), and ITEM 3695 (*MAT-1*); *F. culmorum* ABC-HUCU2 (*MAT-2*), ABC-HUCU3 (*MAT-2*), CBS 173-31 (*MAT-2*), CBS 251-52 (*MAT-1*), IPPV 72186 (*MAT-1*), IPPV 72305 (*MAT-2*), ITEM 627 (*MAT-2*), ITEM 628 (*MAT-2*), ITEM 741 (*MAT-1*), ITEM 3392 (*MAT-1*), ITEM 3393 (*MAT-2*), NRRL 23141 (*MAT-1*), NRRL 29138 (*MAT-1*), NRRL 29139 (*MAT-2*), NRRL 29140 (*MAT-1*), PRI 11F1 (*MAT-2*), PRI 19A1 (*MAT-1*), and SUF 995 (*MAT-1*); *Fusarium decemcellulare* (teleomorph, *Calonectria rigidiuscula*) PPI-F 29 (*MAT-2*); *F. equiseti* ITEM 192 (*MAT-1*), ITEM 358 (*MAT-1*), ITEM 359 (*MAT-1*), ITEM 372 (*MAT-1*), ITEM 679 (*MAT-2*), ITEM 1158 (*MAT-2*), ITEM 1736 (*MAT-2*), and ITEM 1856 (*MAT-1*); *F. graminearum* PRI 24F1 (*MAT-1*/*MAT-2*); *Fusarium longipes* ITEM 3202 (*MAT-2*); *Fusarium merismoides* CBS 798.70 (*MAT-2*); *F. poae* ABC-A15 (*MAT-1*), ABC-A18 (*MAT-2*), ABC-TAPO1 (*MAT-1*), ABC-TAPO7 (*MAT-1*), ABC-TAPO18 (*MAT-1*), ABC-TAPO21 (*MAT-1*), ABC-TAPO24 (*MAT-1*), and ABC-TAPO34 (*MAT-2*); *Fusarium scirpi* ITEM 1166 (*MAT-1*), ITEM 1717 (*MAT-2*), and ITEM 1718 (*MAT-2*); *Fusarium semitectum* ITEM 2562 (*MAT-2*), ITEM 3192 (*MAT-1*), ITEM 3193 (*MAT-2*), ITEM 3390 (*MAT-2*), ITEM 3391 (*MAT-1*), and ITEM 3394 (*MAT-2*); *Fusarium solani* (teleomorph, *Nectria haematococca*) ITEM 3320 (*MAT-2*) and PPI-9 (*MAT-1*); *F. sporotrichioides* ITEM 3592 (*MAT-2*), ITEM 3593 (*MAT-2*), ITEM 3594 (*MAT-2*), ITEM 3595 (*MAT-2*), ITEM 3596 (*MAT-1*), ITEM 3597 (*MAT-2*), ITEM 3598 (*MAT-2*), ITEM 3599 (*MAT-2*), ITEM 3600 (*MAT-2*), and ITEM 3601 (*MAT-2*); *Fusarium torulosum* ITEM 839 (*MAT-1*), ITEM 840 (*MAT-2*), ITEM 842 (*MAT-2*), ITEM 843 (*MAT-2*), ITEM 844 (*MAT-1*), and ITEM 845 (*MAT-1*); *Fusarium tricinctum* ABC-HF 011 (*MAT-2*), ITEM 2054 (*MAT-2*), ITEM 3405 (*MAT-1*), PPI-F 27 (*MAT-2*), and PPI-F 28 (*MAT-2*); *Fusarium tumidum* ITEM 2118 (*MAT-2*), ITEM 2119 (*MAT-2*), and NRRL 13394 (*MAT-2*); and *Fusarium verticillioides* (teleomorph, *G. fujikuroi* mating population A; synonymous organism, *Gibberella moniliformis*) FGSC 7600 (*MAT-1*) and FGSC 7603 (*MAT-2*). The strains were obtained from the following organizations: ABC, Agricultural Biotechnology Center, Gödöllő, Hungary; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; FGSC, Fungal Genetic Stock Center, University of Kansas Medical Center, Kansas City; IPPV, Agricultural Research Centre, Institute of Plant Pathology, Vantaa, Finland; ITEM, Institute of Sciences of Food Production, CNR, Bari, Italy; NRRL, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.; PPI, Plant Protection Institute, HAS, Budapest, Hungary; PRI, Plant Research International, Wageningen, The Netherlands; SUF, Shinshu University, Ueda, Nagano-ken, Japan. All *Fusarium* strains used in this study were maintained as conidial suspensions in 16% glycerol at −70°C. For genomic DNA extractions, about 10^6 conidia were inoculated into 100 ml of complete medium (6) and grown for 2 days at 25°C with shaking (120 rpm). After that, the mycelium was filtered, washed with sterile water, and lyophilized. For reverse transcription-PCR (RT-PCR) experiments, plugs of mycelium were transferred to carrot agar, which is widely used to induce perithecia formation in crossing experiments (14), and incubated for 1 week at 25°C under a diurnal cycle of 12 h of light and 12 h of darkness.

Molecular techniques. Common DNA and RNA manipulation techniques were performed as described by Sambrook et al. (21). PCRs were performed with reaction mixtures containing 1× PCR buffer (MBI Fermentas, Vilnius, Lithuania), 1.5 mM $MgCl_2$, a 0.5 mM concentration of each deoxynucleoside triphosphate, a 0.25 μM concentration of each primer, 1 U of *Taq* polymerase (MBI Fermentas), and about 20 ng of fungal DNA. Initial denaturation was done at 95°C for 2 min, followed by 30 cycles consisting of 30 s at 94°C, 30 s at 55 to 60°C (depending on the melting temperature of the primers), and 0.5 to 5 min at 72°C (depending on the expected length of the amplicon), and a final elongation step at 72°C for 10 min. The amplification products were separated by electrophoresis in agarose gels, stained with ethidium bromide, and visualized with UV light. Amplicons were cloned into the pBluescript II KS (Stratagene, La Jolla, Calif.) or pGEM-T Easy (Promega, Madison, Wis.) plasmid vector, transformed into *Escherichia coli* DH5α cells (Clontech, Palo Alto, Calif.), and sequenced by the Sequencing Service of the Agricultural Biotechnology Center. DNA sequences were analyzed with the Lasergene (DNAStar Inc., Madison, Wis.) and Wisconsin (10) software packages, and BLAST searches were done with the GenBank database (2).

PCR amplification of conserved *MAT* boxes. Conserved portions of the α or HMG box of the *MAT-1* or *MAT-2* idiomorph were amplified from different *Fusarium* species by using the degenerate Fα1 (CGNCCNCTNAAYGSNTTY ATG) and Fα2 (ACYTTNGCNATNAGNGCCCAYTT) primers or the previously described NcHMG1 and NcHMG2 primers (3). Primers Fα1 and Fα2 were designed based on deduced amino acid sequence data for *MAT-1* idiomorphs known from *G. moniliformis* (synonym, *G. fujikuroi* mating population A) (accession no. AF100925), *G. zeae* (accession no. AF318048), and *F. oxysporum* (accession no. AB011379) (27). The putative *MAT*-specific amplicons, identified on the basis of the expected lengths of the fragments, were cloned and sequenced. BLAST comparisons confirmed that these amplicons were indeed *MAT*-box homologs, i.e., α and HMG box homologues. Amplified fragments of the expected sizes were isolated from the gel, cloned, and sequenced.

Cloning entire *MAT* genes. Inverse PCR was performed as previously described (23) by using Herculase *Taq* polymerase (Stratagene) according to the manufacturer's instructions. Amplicons were cloned and sequenced, and the appropriate contigs were assembled by using the SeqMan computer program (DNAStar). To clone intact *MAT-1-1* and *MAT-2* genes, we designed specific primer pairs (Table 1) based on the sequence information derived from inverse PCR. Using these primers, we amplified, cloned, and sequenced the entire *MAT-1-1* and *MAT-2* genes from *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum*.

***MAT* box-specific PCR.** To identify the mating type of different *Fusarium* species, we designed diagnostic PCR primers (see Results). For amplification, we used the general PCR protocol described above, except for some minor modifications as follows: the concentration of $MgCl_2$ was elevated to 2 to 2.5 mM, the number of cycles was increased to 35, and the elongation time was expanded to 30 s.

RT-PCR experiments. Total RNAs were extracted from the mycelia grown on carrot agar plates by use of the TRI reagent (Sigma, St. Louis, Mo.) according to the manufacturer's instructions. The first-strand cDNA reaction (40 μl) contained 1× avian myeloblastosis virus buffer (Promega), a 0.5 mM concentration of each deoxynucleoside triphosphate, 1.7 μM oligo$(dT)_{15}$ primer, 20 U of RNasin (Promega), 5 U of avian myeloblastosis virus reverse transcriptase (Promega), and about 5 μg of total RNA and was followed by an RQ1 DNase (Promega) treatment. The mixture was incubated at 42°C for 50 min and at 65°C for 10 min to inactivate the reverse transcriptase. Five microliters of the first-strand cDNA reaction was used as a template in a 25-μl standard PCR mixture. Amplifications were done with the same program as described above, except that the number of cycles was increased from 30 to 40.

Nucleotide sequence accession numbers. The sequences of the amplified regions of *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum* were deposited in the EMBL database under accession numbers AJ535625 to AJ535632.

RESULTS

Cloning of *MAT* genes from *Fusarium* species with no known sexual stage. Regions flanking the α or HMG boxes were amplified by inverse PCR from *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum*, and the resulting fragments were cloned and sequenced. Based on these sequences, new PCR primer pairs were designed for border regions of the *MAT-1-1* and *MAT-2* genes, and the DNA fragments generated by these primers were cloned and sequenced.

Putative *MAT*-specific fragments, *MAT-1* and *MAT-2* genes, and *MAT-1*-specific α box and *MAT-2*-specific HMG box sequences (Table 2) were identified by comparison with se-

TABLE 1. Primers used for amplification of entire *MAT-1-1* and *MAT-2* genes from *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum*

Primer	Nucleotide sequence (5′-3′)	Description
AVE-1-F	TCTTTAAATCCTCTCTCTCTGCCCA	*F. avenaceum MAT-1-1* forward
AVE-1-R	ACCTTCTGACCAACCAGAAGCCT	*F. avenaceum MAT-1-1* reverse
AVE-2-F	CACCCCAACAACCATTCGGAGTTT	*F. avenaceum MAT-2* forward
AVE-2-R	CAATGGGATGGCAGGCTGTCCA	*F. avenaceum MAT-2* reverse
CUL-1-F	AATTCATCTCTCCTGGCCTTTTGC	*F. culmorum MAT-1-1* forward
CUL-1-R	ATTTCTCAGCCCTAGATCTCATTGC	*F. culmorum MAT-1-1* reverse
CUL-2-F	TTCAGAACGCCAGCAGGACCAG	*F. culmorum MAT-2* forward
CUL-2-R	GAGCGGGACGTTTGTGCCTACTTA	*F. culmorum MAT-2* reverse
POA-1-F	GCCTCACACTTTTTTCCTTCTTC	*F. poae MAT-1-1* forward
POA-1-R	CAGTAAACCGGAATCATCAACG	*F. poae MAT-1-1* reverse
POA-2-F	ACGTACCATCTGACACTTGCTCG	*F. poae MAT-2* forward
POA-2-R	AGTCGAGGAGGTCGTCAATCAAT	*F. poae MAT-2* reverse
SEM-1-F	TCTCTTCTCTCATCTCAGGCTTTCA	*F. semitectum MAT-1-1* forward
SEM-1-R	TCGCGTGCTACCCTAAACTTTT	*F. semitectum MAT-1-1* reverse
SEM-2-F	CTCACAAAACGCCAACAGAACCAT	*F. semitectum MAT-2* forward
SEM-2-R	TCCAGTCGATAACAACGCTCAAGA	*F. semitectum MAT-2* reverse

quences available for the *MAT* idiomorphs of *F. oxysporum*, *G. fujikuroi*, and *G. zeae* (27). The *MAT-1-1* gene identified in *F. avenaceum* ITEM 859 was 1,218 bp long and encoded a putative protein with an α-box motif. The sequences of *MAT-1-1* genes from *F. culmorum* strain 19A1, *F. poae* TAPO21, and *F. semitectum* ITEM 3192 were 1,085, 1,203, and 1,129 bp long, respectively, and encoded putative proteins with conserved α-box domains. All of these *MAT-1-1* gene sequences contained introns at conserved positions (20). No in-frame stop codons were found in these sequences.

The *MAT-2* gene from *F. avenaceum* ITEM 858 was 860 bp long and encoded a putative protein with an HMG box domain. Similar sequences from *F. culmorum* 11F1, *F. poae* TAPO34, and *F. semitectum* ITEM 3390 were 865, 859, and 856 bp long, respectively, and encoded proteins with conserved HMG domains. These *MAT-2* gene sequences also contained introns at conserved positions (20). In-frame stop codons were not found in these sequences. Sequence similarities ranged from 49 to 99%, but the percentages of similarity between the *MAT*-specific box sequences were always higher than the values obtained from comparisons of entire *MAT* gene sequences. The Treebase database accession number for these comparisons is SN 1779.

TABLE 2. Similarity of *MAT* sequences of *Fusarium* species with no known sexual stage to *MAT* sequences described previously for other members of the genera *Fusarium* and *Gibberella*

MAT sequence	Accession no.	% Similarity for entire gene/% similarity for conserved boxes		
		F. oxysporum[a]	*G. fujikuroi*[b]	*G. zeae*[c]
F. avenaceum MAT-1-1	AJ535625	63.1/64.9	51.1/75.7	55.2/75.5
F. culmorum MAT-1-1	AJ535626	48.7/58.9	52.7/75.7	97/97.3
F. poae MAT-1-1	AJ535627	52.5/66.7	55.3/76.6	81.3/85.3
F. semitectum MAT-1-1	AJ535628	49.8/61.3	60.5/75.2	70.7/78.8
F. avenaceum MAT-2	AJ535629	61.7/73.8	67.6/71.6	63.8/71.2
F. culmorum MAT-2	AJ535630	52.8/71.6	58.8/68.4	97.8/98.5
F. poae MAT-2	AJ535631	57.7/73.8	59.7/69.5	80.8/89.7
F. semitectum MAT-2	AJ535632	61.4/74.2	62.2/69.5	75.2/82.4

[a] *F. oxysporum MAT-1-1* gene (AB011379) and *MAT-2* gene (AB011378).
[b] *G. fujikuroi MAT-1-1* gene (AF100925) and *MAT-2* gene (AF100926).
[c] *G. zeae MAT-1-1* and *MAT-2* genes (AF318048).

Diagnostic PCR for mating type in *Fusarium* species. We designed new degenerate oligonucleotide primers, namely fusALPHAfor (CGCCCTCTKAAYGSCTTCATG), fusALPHArev (GGARTARACYTTAGCAATYAGGGC), fusHMGfor (CGACCTCCCAAY GCYTACAT), and fusHMGrev (TGGGCGGTACTGG TARTCRGG), and defined appropriate PCR conditions. The positions of these primers in the α and HMG box sequences of *F. avenaceum* (AJ535625 and AJ535629) are nucleotides 282 to 302, 456 to 479, 540 to 559, and 775 to 795, respectively. The sizes of the *MAT-1*- and *MAT-2*-specific fragments amplified from different species of *Fusarium* were 200 and 260 bp, respectively (Fig. 1).

The usability of the diagnostic PCR method for mating type identification was tested on 122 *Fusarium* strains representing

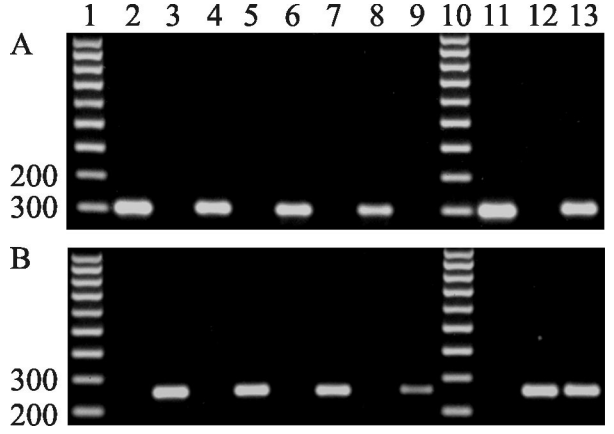

FIG. 1. PCR amplification of mating type-specific boxes from *Fusarium* species with no known sexual stage. (A) Amplification of *MAT-1*-specific α box by using the fusALPHAfor and fusALPHArev primers. (B) Amplification of *MAT-2*-specific HMG box by using the fusHMGfor and fusHMGrev primers. Lanes 1 and 10, 100-bp DNA ladder; lanes 2 to 9, *F. avenaceum* ITEM 859 (*MAT-1*) and ITEM 858 (*MAT-2*), *F. culmorum* 19A1 (*MAT-1*) and 11F1 (*MAT-2*), *F. poae* TAPO21 (*MAT-1*) and TAPO34 (*MAT-2*), and *F. semitectum* ITEM 3192 (*MAT-1*) and 3390 (*MAT-2*), respectively; lanes 11 to 13, *F. verticillioides* FGSC 7600 (*MAT-1*) and FGSC 7603 (*MAT-2*) and *F. graminearum* 24F1 (*MAT1/2*), respectively (used as controls).

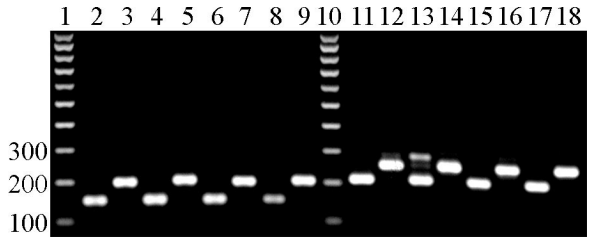

FIG. 2. Expression of mating type genes in *Fusarium* species with no known sexual stage. Lanes 1 and 10, 100-bp DNA ladder; lanes 2 to 9, PCR amplification of *MAT-1*-specific α box from first-strand cDNA (even numbers) and genomic DNA (odd numbers) from *F. avenaceum* ITEM 859 (lanes 2 and 3), *F. culmorum* 19A1 (lanes 4 and 5), *F. poae* TAPO21 (lanes 6 and 7), and *F. semitectum* ITEM 3192 (lanes 8 and 9); lanes 11 to 18, PCR amplification of *MAT-2*-specific HMG box from first-strand cDNA (odd numbers) and genomic DNA (even numbers) from *F. avenaceum* ITEM 858 (lanes 11 and 12), *F. culmorum* 11F1 (lanes 13 and 14), *F. poae* TAPO34 (lanes 15 and 16), and *F. semitectum* ITEM 3390 (lanes 17 and 18).

22 species from 9 sections. Both *MAT-1* and *MAT-2* individuals were identified among strains of *F. acuminatum* subsp. *acuminatum*, *F. acuminatum* subsp. *armeniacum*, *F. avenaceum*, *F. cerealis*, *F. chlamydosporum*, *F. compactum*, *F. culmorum*, *F. equiseti*, *F. poae*, *F. scirpi*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, *F. torulosum*, and *F. tricinctum*. Only the *MAT-2* mating type was found among strains of *F. camptoceras*, *F. decemcellulare*, *F. longipes*, *F. merismoides*, and *F. tudmidum*, but the number of isolates we could obtain for this assay was limited. The only strain for which both *MAT*-specific amplicons were identified belonged to *G. zeae*, a true homothallic member of the genus *Gibberella* that is known to harbor both *MAT-1* and *MAT-2* idiomorphs (27). The two opposing mating types of the reference strains of *F. verticillioides* were also clearly identified by using the semidegenerate primers developed in this work. The mating types of all of the strains are indicated in Materials and Methods.

Transcription of *MAT* genes in *Fusarium* species with no known sexual stage. RT-PCR experiments primed with α box- or HMG box-specific primers, respectively, were performed to examine the expression of *MAT* genes in *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. semitectum*. Electrophoretic separation of the RT-PCR products resulted in the appearance of one characteristic band at the appropriate size, i.e., an ~150-bp and an ~200-bp fragment in all samples (Fig. 2). The size differences observed between the amplicons obtained by RT-PCR and fragments generated from the genomic DNAs were due to the presence of an intron in the genomic copies of these *MAT* boxes. Northern blot analyses of these RT-PCR products, with the appropriate cloned *MAT-1-1* or *MAT-2* gene as a probe, confirmed the identities of the fragments. Thus, both the *MAT-1-1* and *MAT-2* genes were transcribed in all asexual *Fusarium* species involved in this experiment.

DISCUSSION

For the present study, we developed a robust PCR-based method suitable for the identification of mating type in several *Fusarium* species with no known sexual stage. To achieve this, we designed PCR primers on the basis of known mating type sequences as well as conserved α and HMG box sequences of four asexual *Fusarium* species. All *Fusarium* species involved in this work were found to contain one or the other mating type idiomorph, with the exception of *F. graminearum*, which was used as a control. The *MAT-1*- and *MAT-2*-specific fragments that were amplified from these fungi showed substantial sequence similarities to conserved motifs of the *MAT-1-1* and *MAT-2* genes from *F. oxysporum*, *G. fujikuroi*, and *G. zeae*, suggesting that these partial sequences represent the mating type idiomorphs in these fungi. Degenerate *MAT*-specific primers designed by Arie et al. (3) or the *G. fujikuroi*-specific *MAT* primers developed in previous studies (13, 22) were unsuitable for generating unambiguous PCR fragments in such diverse *Fusarium* species (representatives of nine sections) due to sequence differences within the conserved *MAT* regions of these fungi.

Our findings clearly show that conserved *MAT*-specific sequences are present and expressed in *Fusarium* species with no known sexual stage. Since the strains of *F. acuminatum*, *F. avenaceum*, *F. camptoceras*, *F. cerealis*, *F. chlamydosporum*, *F. compactum*, *F. culmorum*, *F. equiseti*, *F. longipes*, *F. merismoides*, *F. poae*, *F. semitectum*, *F. scirpi*, *F. sporotrichioides*, *F. torulosum*, *F. tricinctum*, and *F. tumidum* all contained only a single *MAT* allele, presumably they are capable of heterothallic, but not homothallic, mating. These results are consistent with the hypothesis (24) that these fungi may have a cryptic sexual cycle, even though sexual structures have not been identified in field collections and there are no reports of successful forced parings among them in laboratory experiments.

Leslie and Klein (15) explained the absence of sexual reproduction in local populations of the *G. fujikuroi* species complex by the presence of mutations that concomitantly resulted in female sterility with an increased vegetative propagation capability. Selection for an increased number of asexual propagules can result in a selective accumulation of female sterile strains, which could become prevalent even in large geographic areas. Under such conditions, mating is limited by the absence of normal female fertile partners. The *Fusarium* species that we examined seem to have functional mating type genes, are aggressive pathogens, and can colonize a wide range of decaying substrates. Populations of these fungi could easily be dominated by successful female sterile clonal lineages that produce more asexual propagules and are therefore not under significant immediate selection pressure to participate in sexual reproduction. The female fertile strains could be such a small minority (<10% in some natural populations [15]) that they are likely to be infrequent, especially under epidemic conditions. Thus, their sexual structures may not be observed in nature simply because of their rarity. Alternatively, the purportedly asexual species also may require environmental conditions for sexual reproduction that are uncommon when disease epidemics occur or that are difficult or unusual conditions to mimic in the laboratory.

The PCR method that we developed for the mating type assessment of these *Fusarium* species facilitates the recognition of potentially compatible strains that could be used in crossing experiments to obtain teleomorphic structures. This approach could increase our knowledge of reproductive strategies in these fungi and allow a realistic evaluation of the potential for generating strains with new pathotypes and/or

altered mycotoxin-producing abilities and could be used to assess disease control strategies that presume that limited genotypic variation and rearrangement occur within the pathogen population.

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