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## Short Communication

# Assessment of *Gibberella fujikuroi* mating type by PCR

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**Mating type (*MAT*)-specific fragments of the two idiomorphs of *Gibberella fujikuroi* (anamorph, *Fusarium moniliforme*) were obtained by PCR amplification using primers to conserved regions of *MAT* homologs from other fungal species and used to assign mating type by molecular criteria rather than the arbitrary historical designation. Mating type − strains of mating populations A–E and a mating type + strain of mating population F carry an $\alpha$-box motif and should therefore be designated *MAT-1*. Mating type + strains of mating populations A–E and a mating type − strain of mating population F carry an HMG-box motif and should be designated *MAT-2*. Thus, assessment of mating type of *G. fujikuroi* strains can be easily achieved using *MAT*-specific primers.**

Key Words——*Gibberella fujikuroi*; HMG-box; *MAT*; mating type; $\alpha$-box.

The single mating type locus (*MAT*) controls sexual development in ascomycetes. The two alternate forms (idiomorphs) of this locus are dissimilar, although they occupy the same chromosomal position. Molecular analysis of the *MAT* gene sequences of several ascomycete species suggested that the proteins encoded by each *MAT* gene involve alternate DNA-binding motifs. One of these motifs is a high mobility group (HMG-box) and the other is a motif called the $\alpha$-box (Turgeon, 1998).

Using the common existence of the DNA-binding motifs on *MAT* genes, Arie et al. (1997) reported a PCR-based strategy for obtaining mating type genes in Loculoascomycetes and Pyrenomycetes which is convenient, quick, and useful for assessing mating type of strains based on molecular criteria.

*Gibberella fujikuroi* (Sawada) Wollenweber (anamorph, *Fusarium moniliforme* Sheld.) is a heterothallic member of the *Gibberella* Sacc./*Fusarium* Link complex (Kuhlman, 1982; O'Donnell and Cigelnik, 1997). As Klittich and Leslie (1992) reported, the members of different mating populations of *G. fujikuroi* are not cross-fertile, and even between strains of the same variety, such as mating populations A and F they have never observed perithecia formation. This has made accurate determination of mating type difficult in *G. fujikuroi*. As this *Gibberella*/*Fusarium* complex contains many phytopathogenic species, structural and functional analyses of *MAT* are necessary to reveal the evolutional history of species and pathogenic-strain development.

In this study, mating-specific fragments that are thought to be on both *MAT* idiomorphs of *G. fujikuroi* were amplified by PCR and used to determine the mating type of the *G. fujikuroi* strains.

**Fungal strains and media** Strains used in this study are listed in Table 1. Mating populations A and F were recognized as the biological group var. *moniliformis*, B and E as var. *subglutinans*, C as var. *fujikuroi*, and D as *intermedia* (Kuhlman, 1982; Klittich and Leslie, 1992). The strains designated prefixed with IFO in Table 1 are originated from the Institute for Fermentation, Osaka (Yodogawa, Osaka, Japan). The mating tester strains designated FGSC were obtained from the Fungal Genetic Stock Center (University of Kansas Medical Center, Kansas City, KA, USA), and strains with ATCC numbers are from the American Type Culture Collection (Manassas, VA, USA) under the import permits by Minister of Agriculture, Forestry and Fisheries, Japan. Strains were maintained on potato dextrose agar medium and were grown on potato dextrose broth medium for purification of the genomic DNA under the conditions previously reported for *F. oxysporum* (Hayashi et al., 1998).

**DNA manipulation and PCR amplification** Genomic DNA from the strains was obtained according to Wirsel et al. (1996). To amplify the homolog of *Neurospora crassa* Shear et Dodge *mat a1* (Glass et al., 1990; Staben and Yanofsky, 1990; Glass and Staben, 1997), *Podospora anserina* (Cesati) Niessl *FPR1* (Debuchy et al., 1993; Debuchy and Coppin, 1992), *Cochliobolus heterostrophus* Drechsler *MAT-2* (Turgeon et al., 1993), and *Nectria haematococca* Berk. et Br. *MAT-2* (Arie et al.,

Table 1. List of *Gibberella fujikuroi* strains and determination of the mating type by PCR.

| Strain[a] | Mating population | Conventional mating type | Falpha 1+2 PCR product[b] | NcHMG1+2 PCR product[c] | Mating type[d] |
|---|---|---|---|---|---|
| FGSC 7603 | A | + | − | + | *MAT-2* |
| FGSC 7605 | A | − | + | − | *MAT-1* |
| FGSC 7610 | B | + | − | + | *MAT-2* |
| FGSC 7611 | B | − | + | − | *MAT-1* |
| ATCC 38940 | C | + | − | + | *MAT-2* |
| ATCC 38941 | C | + | − | + | *MAT-2* |
| ATCC 38938 | C | − | + | − | *MAT-1* |
| ATCC 38939 | C | − | + | − | *MAT-1* |
| FGSC 7612 | D | + | − | + | *MAT-2* |
| FGSC 7613 | D | − | + | − | *MAT-1* |
| FGSC 7617 | E | + | − | + | *MAT-2* |
| FGSC 7616 | E | − | + | − | *MAT-1* |
| FGSC 7619 | F | + | + | − | *MAT-1* |
| FGSC 7618 | F | − | − | + | *MAT-2* |
| IFO 31251 | Unknown | Unknown | − | + | *MAT-2* |
| IFO 9977 | Unknown | Unknown | − | + | *MAT-2* |

a) FGSC strains were obtained from the Fungal Genetic Stock Center; ATCC strains from the American Type Culture Collection; IFO strains from the Institute for Fermentation, Osaka.
b) +: ~380 bp product; −: no ~380 bp product.
c) +: ~260 bp product; −: no ~260 bp product.
d) Following the proposed mating type gene designation (Arie et al., 1997).

1997), PCR primers NcHMG1 and NcHMG2 corresponding to the highly conserved HMG-box domain (Arie et al., 1997) were used. To amplify the homolog of *N. crassa mat A1* and *P. anserina FMR1*, primers Falpha1 (5′-CGGTCAYGAGTATCTTCCTG-3′) and Falpha2 (5′-GATGTAGATGGAGGGTTCAA-3′) corresponding to the $\alpha$-box of *MAT-1* of *F. oxysporum* Schlechtend.: Fr. (Yoshida et al., 1998; Arie et al., 1998) were used. PCR was carried out by using 20 ng of genomic DNA in 50 $\mu$l of reaction buffer [1×PCR Buffer II (PE Applied Biosystems), 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2 $\mu$M of each primer, and 0.025 U AmpliTaq Gold™ (PE Applied Biosystems)]. PCR conditions were: 95°C for 15 min; 35 cycles of 94°C for 1 min/55°C for 30 s/72°C for 1 min; followed by 72°C for 10 min on GeneAmp® PCR System 9600 (PE Applied Biosystems).

**Cloning, sequencing, and analysis of PCR product** PCR products were cloned into vector pCR2.1 (Invitrogen) following the manufacturer's protocol. Plasmid DNA was purified from several clones using RPM Kit (BIO 101) following the manufacturer's recommendation. The insert DNA was sequenced using primers M13 Reverse or M13 (−40) Forward and Cy5 AutoRead Sequencing Kit (Pharmacia Biotech) by use of an ALFexpress DNA sequencer (Pharmacia Biotech) at the RIKEN DNA Sequencing Facility. Sequences were assembled and analyzed using the GeneWorks program (IntelliGenetics).

Each strain showed a single band with either of the primer sets (Falpha1+Falpha2 or NcHMG1+NcHMG2) when the PCR reaction mixture was applied on agarose gel electrophoresis (Fig. 1). No strain failed to show a band with both sets of primers.

PCR using Falpha1+Falpha2 primers yielded a 375 bp fragment in approximately the half of the strains (Table 1 and Fig. 1). The fragment of ATCC38939 was cloned in pCR2.1. Plasmid DNA carrying ~380 bp insert was purified from several clones and the insert DNA was sequenced. Homology search against the NCBI/GenBank database revealed high similarity to the $\alpha$-box of *N. crassa mat A1* (in amino acid level, 40.7%), *P. anserina FMR1* (36.8%), and *F. oxysporum MAT-1* (85.2%). There is a putative 47 bp intron in the same position on these genes (Fig. 2). PCR using primers NcHMG1+NcHMG2 yielded a 262 bp fragment in the strains which showed no band with Falpha1+Falpha2 primers (Table 1 and Fig. 1). The fragment of IFO 31251 was also cloned and sequenced. Sequencing of one clone revealed homology to the HMG-box of *N. crassa mat a1* (in amino acid level, 47.8%), *P. anserina FPR1* (52.2%), *C. heterostrophus MAT-2* (31.0%), *Nectria haematococca MAT-2* (66.2%), and *F. oxysporum MAT-2* (83.3%) in the database. The sequence contains a putative 47 bp intron in the same position on these genes (Fig. 2). The DNA sequence data have been deposited in the DDBJ, EMBL, and GenBank Nucleotide Sequence Database with the accession number AB015641 [ATCC 38939 (*MAT-1*)] and AB005041 [IFO 31251 (*MAT-2*)], respectively. The amino acid alignments of the both fragments are shown in Fig. 2. Sequencing of the same region in the other strains is under way.

Following the proposed mating type gene designations by Arie et al. (1997) and Turgeon (1998), the frag-

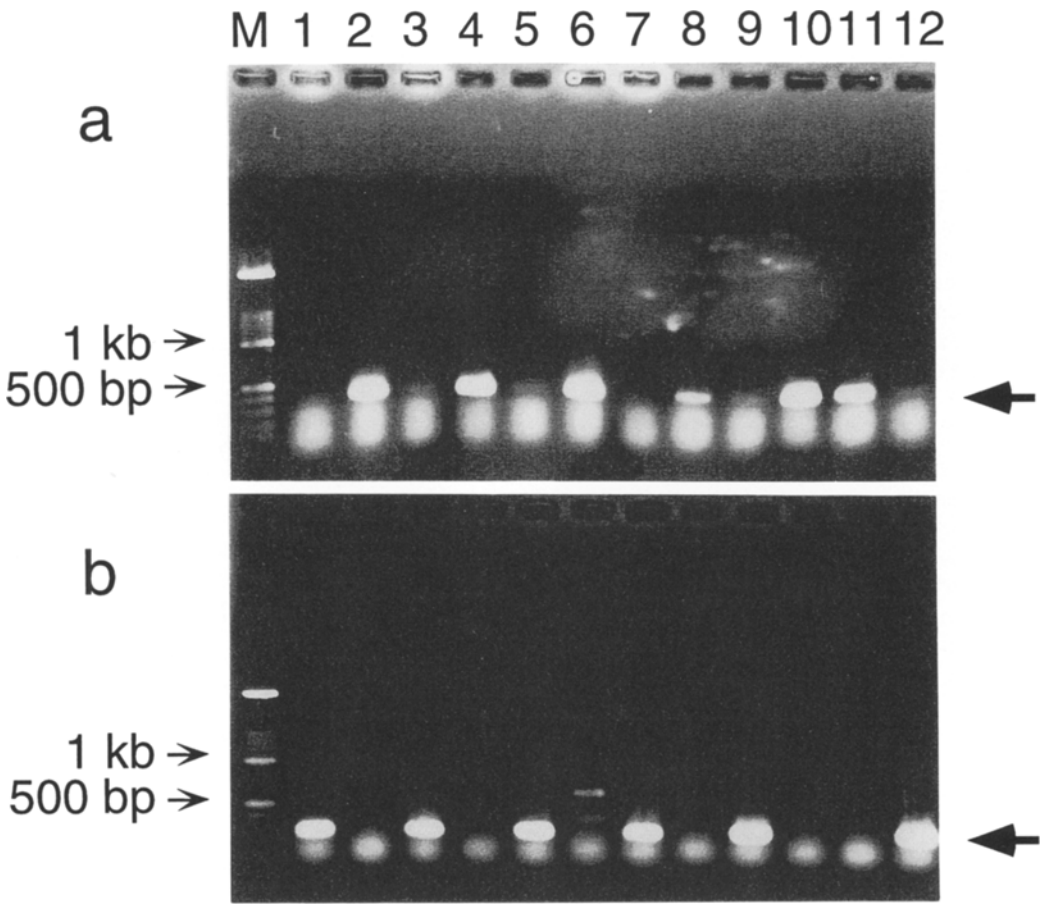


Fig. 1. Mating type determination by PCR.
a: Products generated with primers Falpha1+Falpha2.
b: Products generated with primers NcHMG1+NcHMG2.
Lanes M: 100 base pair ladder markers; 1: FGSC 7603 (A, +); 2: FGSC 7605 (A, −); 3: FGSC 7610 (B, +); 4: FGSC 7611 (B, −); 5: ATCC 38940 (C, +); 6: ATCC 38939 (C, −); 7: FGSC 7612 (D, +); 8: FGSC 7613 (D, −); 9: FGSC 7617 (E, +); 10: FGSC 7616 (E, −); 11 FGSC 7619 (F, +); 12 FGSC 7618 (F, −).

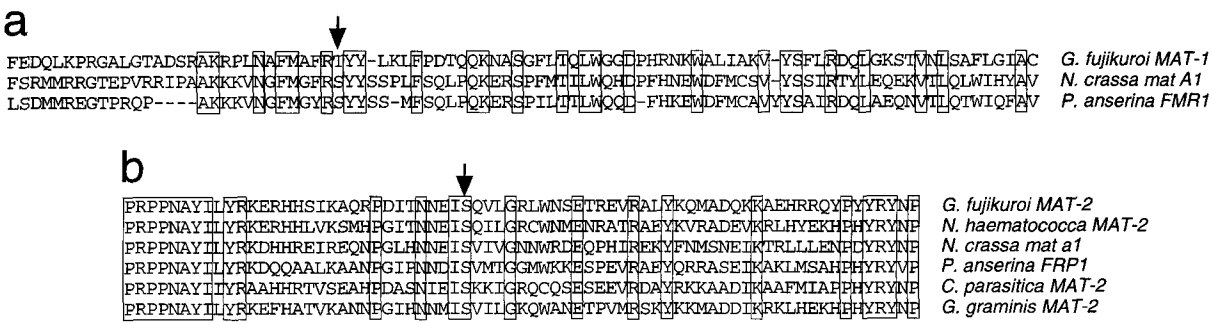


Fig. 2. Amino acid alignment for *MAT* $\alpha$-box and HMG-box.
a: $\alpha$-box; b: HMG-box. All the species listed belong to Pyrenomycetes. Sequences for *Neurospora crassa* and *Podospora anserina* were obtained from GenBank. HMG-box data for *Nectria haematococca*, *Cryphonectria parasitica*, and *Gaeumannomyces graminis* were obtained from Arie et al. (1997). Amino acids corresponding to the primers used for PCR are included at the 5′ and 3′ ends of the sequences. Boxed residues are identical among the sequences, and the dashes show gaps. The arrow indicates the conserved position of an intron.

ment containing the $\alpha$-box should be recognized as a part of *MAT-1* corresponding to *mat A1* of *N. crassa* as well as *MAT−* (*FMR1*) of *P. anserina*, *MAT-1* of *C. heterostrophus*, *MAT1-1* of *M. grisea* (Kang et al., 1994), and *MAT-1* of *F. oxysporum*. The *G. fujikuroi* strains carrying this fragment were previously designated as mating

type − in mating populations A, B, C, D and E, and mating type + in mating population F (Table 1). The fragment containing the HMG-box should be a part of *MAT-2*. *G. fujikuroi* strains carrying this fragment were previously designated mating type + in mating populations A–E and mating type − in mating population F.

Our data show that conventionally designated mating types + and − in the mating population F were different from those of mating populations A–E. This might be due to the difficulty in comparing mating types of different mating populations by conventional method. These results suggested that the PCR method is reliable for determining mating type in species-complex such as *G. fujikuroi*.

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