Mapping of Avirulence Genes in the Rice Blast Fungus, *Magnaporthe grisea*, with RFLP and RAPD Markers

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Three genetically independent avirulence genes, *AVR1-Irat7*, *AVR1-MedNoi*, and *AVR1-Ku86*, were identified in a cross involving isolates Guy11 and 2/0/3 of the rice blast fungus, *Magnaporthe grisea*. Using 76 random progeny, we constructed a partial genetic map with restriction fragment length polymorphism (RFLP) markers revealed by probes such as the repeated sequences *MGL/MGR583* and *Pot3/MGR586*, cosmids from the *M. grisea* genetic map, and a telomere sequence oligonucleotide. Avirulence genes *AVR1-MedNoi* and *AVR1-Ku86* were closely linked to telomere RFLPs such as marker TelG (6 cM from *AVR1-MedNoi*) and TelF (4.5 cM from *AVR1-Ku86*). Avirulence gene *AVR1-Irat7* was linked to a cosmid RFLP located on chromosome 1 and mapped at 20 cM from the avirulence gene *AVR1-CO39*. Using bulked segregant analysis, we identified 11 random amplified polymorphic DNA (RAPD) markers closely linked (0 to 10 cM) to the avirulence genes segregating in this cross. Most of these RAPD markers corresponded to junction fragments between known or new transposons and a single-copy sequence. Such junctions or the whole sequences of single-copy RAPD markers were frequently absent in one parental isolate. Single-copy sequences from RAPD markers tightly linked to avirulence genes will be used for positional cloning.

The ascomycetal fungus *Magnaporthe grisea* is responsible for the most damaging fungal disease of rice, called blast (Ou 1985). The use of resistant rice cultivars is essential to control this disease. The highest levels of resistance are in general conferred by one or a few major genes that are efficient only toward avirulent *M. grisea* isolates (Ou 1985; Kiyosawa et al.

1986). Fungal avirulence is also controlled by one or a few major genes that correspond to specific rice resistance genes following a gene-for-gene relationship (Silué et al. 1992a). Significant progress has been achieved through the genetic analysis of rice resistance and *M. grisea* avirulence (Zeigler et al. 1994; Khush 1996). At least 20 major rice genes conferring resistance to blast are currently described and some have been located on rice genetic maps (Kiyosawa et al. 1986; McCouch et al. 1994; Ghesquière et al. 1997). The molecular cloning of rice resistance genes is in progress with positional cloning (*Pi-b*: Miyamoto et al. 1996 and Wang et al. 1999; *Pi-ta*²: Nakamura et al. 1997; *Pi-ta*: Valent 1998). In *M. grisea*, at least 25 avirulence genes are described (Notteghem et al. 1994) and five have been mapped (Sweigard et al. 1993; Valent and Chumley 1994; Nitta et al. 1997). Among these avirulence genes, 15 originated from a parental isolate pathogenic to rice and 10 from a parental isolate nonpathogenic to rice. To date, only a few fungal avirulence genes have been cloned (Knogge 1996; De Wit 1997). The first characterized fungal avirulence genes were isolated from the tomato pathogen *Cladosporium fulvum* (*AVR9*: van Kan et al. 1991; *AVR4*: Joosten et al. 1994). A third avirulence gene, *NIP1*, was first isolated from the barley pathogen *Rhynchosporium secalis* as a phytotoxic peptide (Rohe et al. 1995). These three avirulence genes encode peptides (*AVR9*, *NIP1*) or small proteins (*AVR4*) secreted by the fungus during the infection process that efficiently and rapidly trigger plant defense responses in cultivars with the corresponding resistance gene. In *M. grisea*, three avirulence genes (*AVR2-YAMO*, *PWL2*, and *AVR1-CO39*) were isolated by positional cloning (Valent et al. 1991; Valent and Chumley 1994; Sweigard et al. 1995; Kang et al. 1995; Farman and Leong 1998). These *M. grisea* genes encode small proteins with signal peptides for secretion that seem expressed only during the infection. The mechanism by which they trigger defense responses in resistant rice cultivars is still unknown. To characterize new avirulence genes from *M. grisea* isolates pathogenic on rice, we mapped three previously described genes (Silué et al. 1992b) relative to a set of restriction fragment length polymorphism (RFLP) markers from the *M. grisea* genetic map (Nitta et al. 1997) and to random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) identified as linked to these avirulence genes by bulked segregant analysis (BSA; Michelmore et al. 1991). RAPD markers closely linked

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to avirulence genes were characterized to determine if they were composed of single-copy or repeated sequences. The molecular basis of their polymorphisms was also analyzed. The identification and characterization of RAPD markers is the first step toward positional cloning of these avirulence genes.

RESULTS

Genetic analysis of avirulence in *M. grisea*.

Genetic analysis in *M. grisea* was for a long time restricted to hermaphroditic isolates pathogenic to grasses other than rice (Leung and Taga 1989). Only a few hermaphroditic isolates pathogenic to rice have been identified within *M. grisea* populations from rice fields (Kato and Yamaguchi 1982; Nottoghem and Silué 1992; Hayashi 1994; Hayashi et al. 1997). Such hermaphroditic rice field isolates were crossed with female, sterile, rice field isolates, allowing the genetic analysis of avirulence (Nottoghem et al. 1994). In a cross between Guy11, a hermaphroditic isolate from South-America, and ML25, a female, sterile isolate from Africa, we identified four avirulence genes by tetrad analysis with either F₁ or progeny from different full-sib or back-crosses (Silué et al. 1992a, 1992b). We focused on the more fertile back-cross (full-sib progeny 2/0/3 back-crossed to parental isolate Guy11: Silué et al. 1992b) in which four genetically independent avirulence genes segregated. We isolated an additional set of 76 random ascospores from this cross. These new progeny were tested on

previously studied rice cultivars (DJ8-341, Ku86, Irat7, MedNoi) for which avirulence was under the control of a single gene. We also studied a new cultivar, Carreon, that was differentially attacked by these parental isolates. For all the five rice cultivars tested, the avirulence/virulence segregations did not significantly deviate from a 1:1 ratio (Table 1), despite the low percentage of viable ascospores (35%) observed in this cross. These results are in agreement with those obtained with full tetrads for the rice cultivars DJ8-341, Ku86, Irat7, and MedNoi (Silué et al. 1992b). As already reported with tetrad analysis, all progeny avirulent on Irat7 were also avirulent on DJ8-341. We also observed that all progeny avirulent on Carreon were also avirulent on Irat7 and DJ8-341. Such co-segregation could be interpreted as the segregation of a single avirulence gene that interacts with the same single resistance gene present in different rice cultivars. According to this hypothesis, we identified in this cross three genetically independent avirulence genes, named *AVR1-Irat7*, *AVR1-MedNoi*, and *AVR1-Ku86*. *AVR1-Irat7* confers avirulence toward rice cultivars DJ8-341, Carreon, and Irat7. *AVR1-MedNoi* confers avirulence toward rice cultivars MedNoi and Cica8 (Silué et al. 1992b). *AVR1-Ku86* confers avirulence toward rice cultivar Ku86.

Identification of RFLP and RAPD markers linked to avirulence genes.

We constructed a genetic map that includes the three segregating avirulence genes, the mating type gene, and 73 RFLP markers obtained either with repeated sequences such as MGL/MGR583 and Pot3/MGR586 (Hamer et al. 1989) or with 11 cosmids already located on the *M. grisea* genetic map (Sweigard et al. 1993). An oligonucleotide corresponding to conserved fungal telomere sequences (Farman and Leong 1995) was used to map telomere RFLPs. These markers defined 10 linkage groups. As *M. grisea* has only seven large chromosomes (Sweigard et al. 1993; Skinner et al. 1993; Orbach et al. 1996), some of these 10 linkage groups likely belong to the same chromosome. Only one RFLP marker, revealed by cosmid A11D9 from chromosome 1 (Sweigard et al. 1993; Nitta et al. 1997), was closely linked to avirulence gene *AVR1-Irat7* (4.5 cM, Fig. 1). Mapping of telomere RFLPs

Table 1. Avirulence gene segregations in cross between *Magnaporthe grisea* isolates Guy11 and 2/0/3

Rice cultivars	Guy11	2/0/3	Progeny	Chi ² test (1:1 segregation)
MedNoi	Virulent	Avirulent	37 : 39 ^a	(0.1) NS ^b
DJ8-341	Avirulent	Virulent	44 : 32	(2.2) NS
Irat7	Avirulent	Virulent	44 : 32	(2.2) NS
Carreon	Avirulent	Virulent	44 : 32	(2.2) NS
Ku86	Virulent	Avirulent	35 : 35	(0.0) NS

^a Avirulent : virulent.

^b Not significant at 5%.

Fig. 1. Genetic maps of chromosomal regions with avirulence genes *AVR1-Irat7*, *AVR1-Ku86*, and *AVR1-MedNoi*. Segregation data were obtained with 76 progeny from cross between parental isolates Guy11 and 2/0/3 and analyzed with Mapmaker (Lander et al. 1987) Version 2 for Macintosh (S. Tingey, E. I. Dupont de Nemours). Parameters for genetic linkage analysis were a minimum lod score of 3.0 and a maximum recombination fraction of 0.2. Three point tests were performed to order linked markers relative to each other.

(Fig. 1) showed that *AVR1-MedNoi* was located 6 cM from RFLP marker TelG, while *AVR1-Ku86* was located 4.5 cM from RFLP marker TelF. RAPD markers linked to these three avirulence genes were identified by BSA (Michelmore et al. 1991). Using 520 Operon oligonucleotides (10-mer), we amplified 4160 DNA fragments (0.3 to 2.5 kb) and detected 140 reproducible amplification products that were polymorphic between the two parents Guy11 and 2/0/3 (3.3% of amplification products). BSA was performed with DNA pools corresponding to 8 to 17 progeny either virulent or avirulent toward a resistant rice cultivar like Irat7, MedNoi, or Ku86. RAPD markers were identified as linked to an avirulence gene by BSA, with the full progeny (Fig. 1, Table 2). We identified 11 RAPD markers closely linked to these avirulence genes (8% of the 140 RAPD markers). Such a large number of markers linked to avirulence genes is likely in this cross (back-cross of a sib progeny), as it maintains polymorphisms at loci linked to segregating avirulence genes while homogenizing other genomic regions.

Four RAPD markers were linked to *AVR1-Irat7* (Fig. 1). Two markers, OPE-J16 and OPE-Y13, were inseparable from *AVR1-Irat7*. OPE-J16 was amplified from parent 2/0/3 that is virulent on Irat7 and OPE-Y13 from parent Guy 11 avirulent on Irat7. The RAPD markers OPE-G18 and OPE-K16 mapped at 4.5 and 7 cM, respectively, from *AVR1-Irat7*. OPE-G18 was amplified from parent 2/0/3 that is virulent on Irat7 and OPE-K16 from parent Guy 11 avirulent on Irat7. Four RAPD markers were closely linked to *AVR1-Ku86*. The RAPD marker OPE-S9 amplified from parent Guy11 that is virulent on Ku86, was inseparable from *AVR1-Ku86*. The RAPD marker OPE-E10 (Fig. 2A), amplified from parent Guy11 that is virulent on Ku86, mapped at 1.5 cM from *AVR1-Ku86*. The RAPD marker OPE-G16, amplified from parent 2/0/3 avirulent on Ku86, mapped at 1.5 cM from *AVR1-Ku86* between the avirulence gene and the telomere marker TelF. RAPD marker OPE-E12 mapped at 10 cM from *AVR1-Ku86*. Finally, we identified three RAPD markers linked to *AVR1-MedNoi*. The two RAPD markers OPE-M18 and OPE-Y16, both amplified from parent 2/0/3, which is avirulent on MedNoi, mapped to the same locus at 1.5 cM from *AVR1-MedNoi*. The RAPD marker OPE-D16 (Fig 2B), amplified from parent 2/0/3 avirulent on MedNoi, mapped at 3 cM from *AVR1-MedNoi* between the avirulence gene and the telomere marker TelG.

Characterization of RAPD markers linked to avirulence genes.

RAPD markers linked to avirulence genes were used as probes for hybridization experiments with either *M. grisea*

genomic DNA or cloned *M. grisea* repeat (MGR) sequences and some were cloned and sequenced. RFLPs revealed by single-copy RAPD markers were mapped. The genomic hybridization pattern of RAPD marker OPE-G16 was characteristic of the retroposon MGL/MGR583 with two, strong, hy-

Fig. 2. Segregation of random amplified polymorphic DNA (RAPD) markers OPE-E10 (2A) and OPE-D16 (2B). RAPD amplifications were performed with genomic DNA from progeny either avirulent (A) or virulent (V) toward rice cultivars Ku86 (2A) or MedNoi (2B). Amplification products were separated in a 1% agarose gel. **A**, RAPD amplification with primer OPE-E10. Arrow shows RAPD marker OPE-E10 (1.7 kb) linked to avirulence gene *AVR1-Ku86*. **B**, RAPD amplification with primer OPE-D16. Arrow shows RAPD marker OPE-D16 (0.3 kb) linked to avirulence gene *AVR1-MedNoi*. R: recombinants; M: molecular weight markers.

Table 2. Characteristics of random amplified polymorphic DNA (RAPD) markers linked to avirulence genes

Marker	Avirulence gene	Distance	Guy11	2/0/3	Type of sequence	Location
OPE-Y13 (1.3 kb)	<i>AVR1-Irat7</i>	<1 cM	+Avirulent ^a	-Virulent	Single copy	Chromosome 1
OPE-J16 (0.65 kb)	<i>AVR1-Irat7</i>	<1 cM	-Avirulent	+Virulent	MGL/MGR 583 + unknown repeated	Chromosome 1
OPE-G18 (0.7 kb)	<i>AVR1-Irat7</i>	4.5 cM	-Avirulent	+Virulent	MGL/MGR 583	Chromosome 1
OPE-K16 (0.7 kb)	<i>AVR1-Irat7</i>	7.0 cM	+Avirulent	-Virulent	Pot2	Chromosome 1
OPE-S9 (0.8 kb)	<i>AVR1-Ku86</i>	<1 cM	+Virulent	-Avirulent	Unknown repeated	Telomere F
OPE-E10 (1.7 kb)	<i>AVR1-Ku86</i>	1.5 cM	+Virulent	-Avirulent	Pot2-like	Telomere F
OPE-G16 (1.6 kb) ^b	<i>AVR1-Ku86</i>	1.5 cM	-Virulent	+Avirulent	MGL/MGR 583	Telomere F
OPE-M18 (1.2kb) ^b	<i>AVR1-MedNoi</i>	1.5 cM	-Virulent	+Avirulent	Unknown repeated	Telomere G
OPE-Y16 (0.4 kb)	<i>AVR1-MedNoi</i>	1.5 cM	-Virulent	+Avirulent	Single copy	Telomere G
OPE-D16 (0.3 kb)	<i>AVR1-MedNoi</i>	3.0 cM	-Virulent	+Avirulent	Single copy	Telomere G

^a + : Parental isolate amplifying the RAPD marker, - : Parental isolate not amplifying the RAPD marker.

^b RAPD markers OPE-M18 and OPE-G16 were not sequenced.

bridizing *Hind*III fragments of 1.5 and 5.5 kb corresponding to internal conserved fragments of the element (Hamer et al. 1989; GenBank accession no. AF018033). OPE-G16 hybridized to plasmids pCB583 and pCB682, which contain MGL/MGR583 sequences (Hamer et al. 1989; GenBank accession no. AF018033), but its cloning was unsuccessful. OPE-G16 likely contains the 5' end of MGL/MGR583 element (Fig. 3) because it did not hybridize with pCB684, which contains a 4.0-kb *Hind*III fragment representing the 3' half of the MGL/MGR583 element (GenBank accession no. AF018033), nor did it hybridize to a conserved, 4-kb *Hind*III fragment from a genomic digest. The genomic hybridization pattern of RAPD marker OPE-G18 was identical with the OPE-G16 pattern and hybridized strongly with plasmids pCB583 and pCB682. Analysis of the OPE-G18 sequence showed that it has the 5' end of MGL/MGR583 (data not shown) linked to a 50-bp sequence without any homology to sequences in data bases. These data suggest that OPE-G18 is a junction fragment of an MGL/MGR583 insertion (Fig. 3). In genomic hybridization, the RAPD marker OPE-J16 revealed two strong signals corresponding to *Hind*III fragments of 4.0 and 5.5 kb, characteristic of MGL/MGR583 internal conserved fragments. OPE-J16 also hybridized with plasmids pCB682 and pCB684 containing MGL/MGR583 sequences (Hamer et al. 1989; GenBank accession no. AF018033). Hybridization of genomic digests with a 0.6-kb *Hind*III subclone from the OPE-J16 marker fragment revealed the same pattern as that of the entire marker. The sequence of this subclone was identical with the 3' end of the MGL/MGR583 retroposon (data not shown). The other OPE-J16 *Hind*III fragment (0.1 kb) corresponds to a new repeated sequence, as its genomic hybridization pattern was different from those of known MGRs and it did not hybridize with plasmids containing known MGRs such as MGL/MGR583, Pot3/MGR586, Pot2/pCB768, pCB608, pCB619, Grasshopper, STR, and Fosbury/Maggy. It was not possible to relate this new MGR to known transposons as it lacks homology to sequences from data bases. These results suggest that OPE-J16 is a junction fragment between an uncharacterized repeated sequence and an MGL/MGR583 element (Fig. 3). Using different pairs of 20-mer oligonucleotides specific to the RAPD marker OPE-J16, we were able to am-

plify a fragment from the virulent parent 2/0/3 but not from the avirulent parent Guy11. The lack of amplification of RAPD marker OPE-J16 in Guy11 could result from the lack of one or both MGR sequences at this locus in Guy11.

The genomic hybridization pattern of RAPD marker OPE-E10 was characteristic of a repeated sequence (Fig. 4A2). OPE-E10 hybridized with plasmid pCB768 containing MGR sequences detected near avirulence genes *PWL2* and *PWL3* (Sweigard et al. 1995; Kang et al. 1995) and to a polymerase chain reaction (PCR) product obtained with oligonucleotides deduced from Pot2 inverse terminal repeat (ITR) sequences (Kachroo et al. 1994). Digestion of OPE-E10 with *Hind*III produced a 1.1-kb fragment that revealed the same genomic hybridization pattern as that of the entire marker. Sequence analysis of this 1.1-kb *Hind*III fragment showed that this marker contained the 5' end of a transposon related to Pot2 (83% sequence identity at the nucleotide level; Fig. 5). This new Pot2-like element is more related to Pot2 than to other Pot1-like transposons (Daboussi et al. 1992; Daboussi and Langin 1994), as it clustered significantly with Pot2 in the transposon's phylogenetic tree obtained by maximum parsimony (Fig. 6). The genomic hybridization pattern revealed by the 0.6-kb *Hind*III fragment of OPE-E10 was typical of a single-copy sequence (Fig. 4A1). Two hybridizing fragments were observed in parent Guy11 for all restriction enzymes tested. In each restriction digest performed on parent 2/0/3, we observed one hybridizing fragment of a size similar to one of the two fragments observed in Guy11. The hybridizing fragments unique to Guy11 mapped near *AVR1-Ku86* at the OPE-E10 locus (data not shown). The single-copy sequence of RAPD marker OPE-E10 is duplicated in Guy11 with one copy present at the same position in Guy11 and 2/0/3 (same RFLPs) and the other copy unique to Guy11 located near *AVR1-Ku86*. Using a pair of 20-mer oligonucleotides specific to the sequences of the 5' and 3' ends of RAPD marker OPE-E10, we amplified a fragment from Guy11 but not from 2/0/3. This result suggests that the RAPD marker OPE-E10 is an insertion of a Pot2-like transposon in a single-copy sequence specific to parent Guy11. The genomic hybridization pattern of RAPD marker OPE-K16 was also indicative of a repeated sequence. It hybridized to the PCR product obtained with oligonucleotides deduced from Pot2 ITR sequences (Kachroo et al. 1994). These results show that this RAPD marker contains sequences related to the Pot2 transposable element, like OPE-E10.

The genomic hybridization pattern of RAPD marker OPE-S9 was indicative of a repeated and dispersed sequence. RAPD marker OPE-S9 did not hybridize to plasmids containing known MGRs and did not reveal hybridization patterns typical of known MGRs. It defines a new family of repeated sequence in *M. grisea*. The digestion of OPE-S9 by *Eco*RI produced two fragments, of 0.55 and 0.25 kb. The 0.55-kb *Eco*RI fragment gave the same genomic pattern as that of the whole marker. The 0.25-kb *Eco*RI fragment gave a hybridization pattern typical of a single-copy sequence and it was only detected in parent Guy11. These results showed that RAPD marker OPE-S9 is a junction fragment between a single-copy sequence and an uncharacterized, repeated sequence specific to parent Guy11. The RAPD marker OPE-M18 gave a genomic hybridization pattern typical of a repeated sequence without any conserved fragments characteristic of known MGRs, therefore defining another family of *M. grisea* repeated sequences.

Fig. 3. Comparison of random amplified polymorphic DNA (RAPD) markers containing retroposon MGL/MRG583. These RAPD markers corresponded to junction fragments between the 5' or 3' end of a MGL/MGR583 retroposon and an uncharacterized genomic sequence (OPE-G18, OPE-G16) or another repeated sequence (OPE-J16). MGL/MGR583 sequences are shown as black-filled rectangles; other genomic sequences as slash-filled rectangles. Hd: *Hind*III restriction site.

Fig. 4. Genomic hybridization patterns revealed by random amplified polymorphic DNA (RAPD) markers OPE-E10 (4A) and OPE-D16 (4B). Genomic DNA from progeny either avirulent (A) or virulent (V) toward rice cultivars Ku86 (4A) or MedNoi (4B) digested by various restriction enzymes was separated in a 0.7% agarose gel. Blots were hybridized with 32 P-radiolabeled probes derived from RAPD markers OPE-E10 or OPE-D16. **A1**, *HindIII* subclone (0.6 kb) from OPE-E10 RAPD marker. **A2**, *HindIII* subclone (1.1 kb) from OPE-E10 RAPD marker. **B**, Whole RAPD marker OPE-D16.

Three RAPD markers were composed only of single-copy sequences. One of them, the RAPD marker OPE-D16, linked to *AVR1-MedNoi*, hybridized only to parent 2/0/3, which is avirulent on MedNoi (Fig. 4B). These RFLPs mapped at the OPE-D16 locus near *AVR1-MedNoi* (data not shown). The RAPD marker OPE-Y16, linked to *AVR1-MedNoi*, detected hybridizing fragments of the same size in Guy11 and 2/0/3 genomic digests, and an additional fragment only in parent 2/0/3. This suggests that sequences homologous to OPE-Y16 are present at two loci in 2/0/3, while present at only one locus in Guy11. RAPD marker OPE-Y13, linked to *AVR1-Irat7*, revealed a genomic hybridization pattern typical of a single-

1	TAACGTTGGG	CTCCACATCT	CGACCCCC	TAAAGTCGGG	CCCTAAAAA	OPE-E10
	...C	TA...C.C.G	T...G.A...	CCCT...T...	...A.C...	Pot2
51	TATCTACTCA	GCCAGTCAA	CCTTTGTITT	TATTTTATAA	ATATCTAGAC	OPE-E10
	...A.A.A.T	TTT.TT.TT	T...CCAAC	...C.A...	...C.T...	Pot2
101	GAATTTTICA	CTTTAGAACT	TGATTTTTCG	AGATTCTTGC	TCGAAACCTT	OPE-E10
	...C.G...	...T.G.T	...AC...TC	T...T.AAT	...TCC.TT...	Pot2
151	CCAATGA AAC	AGTACACTGA	AAATCAGCTT	CTTGCTGCCA	TTTCTGACAT	OPE-E10
	...G	...A...T...	...A...	A.AT...A	...AAC...G	Pot2
201	AAGAAATGGC	AAATCAGTTC	ACAAAACTC	GCAAAAATGG	GGTATTCCTC	OPE-E10
	C.AT...	...TC...A.G	CA...	C.G...	...A...A	Pot2
251	GGAGTAACCT	TCACGATCGT	TTAAAGGGGG	CCGAGTCAAT	TCGAAACGCG	OPE-E10
	...TC...A	...AAG...A	C.T...A.TT	...T.AG.TTA	...A...A	Pot2
301	AAAAGATTTT	GTCAGAGGCT	TTCAACGAGG	CAGGAAACCT	ATTTGGCAGA	OPE-E10
	C...CCC	T...	...CAG...A	...AGC	...T...	Pot2
351	TTGGGTACTT	GCCAGGGGCG	CGTTAGGCGT	TCCGCCAACG	CATCAAGAAC	OPE-E10
	...	A.C...AA.A	...T...G...	...	...T	Pot2
401	TACGCTATTT	TGCGGAACGA	ATTCTTCAGG	CGCCCGGAGA	AAGAAAAAGC	OPE-E10
	...T...	...C...	...A	...	G.C...G...	Pot2
451	CTTGGGAAAC	ATTGGGTTAG	CGTTTATTATA	GCCGATATC	CAATTTTGAA	OPE-E10
	...A...	G...A.A.C	T...T.G...	...T...T...	...CC.T...	Pot2
501	AACCAAAAGA	CCCGGGCGAA	TAGAAAATGC	CCGGTTAAT	GGGGCTACAA	OPE-E10
	...G	...T...	...T.C...	...C...	...T...	Pot2
551	CCGAGTAAAT	TAAATCTTGG	TGGTCTATT	TGMAAATCC	CGTTGTGAT	OPE-E10
	...G	...	...CTT...A	T.OG...C...	G...A.TA.C	Pot2
601	ACTATAAAC	CGCCCAACCG	TTGGAATATG	GACGAAACAG	GTATAATGGA	OPE-E10
	G...T...	...AA...	...	...C	...	Pot2
651	AGGCAAAACGA	TCTAATGGCC	TGGTGTAGG	GCGTAATAAA	ATCCGGCCAT	OPE-E10
	...	...	...A...A...	...T...GGG	...G...	Pot2
701	TACAGCGAAA	AGACCTGGA	AACCGGGTTT	GACGAGCAT	AATCGAATGT	OPE-E10
	...G.A...	...C...	...T...CT...	...	...	Pot2
751	GTATCAGCTA	CGGGGGCTGT	TATACCTGCC	CTGTTATAT	TTAAGGGGAA	OPE-E10
	A...G...	...C.T...C	...C...	...	...A...	Pot2
801	AAACGTACAG	CAACAATGGT	TTCCAGCTGA	TTTAAGTCTC	TTGATACTCT	OPE-E10
	...A	...	...CA.G...	...C...	...AT...	Pot2
851	GGCAATTTCA	TGCAACCGAA	AACGGGTGGA	CAATAATGA	AACAGGTATC	OPE-E10
	...	...	...	...CC	...G.C...	Pot2
901	GAATGGTTAA	AAAAGGTGTT	TATTCGGTAT	AACCAACCTT	TAACCCCTGA	OPE-E10
	...	...	...	...	...	Pot2
951	AAAGCGGTTA	TTAGTTCTGG	ATGGCCATGG	ATCAGATATA	ACGGAOGAAT	OPE-E10
	...	...	...	...	...	Pot2
1001	TTATGCTTCT	TTGCTTGCAA	AACAATATTC	AACTCCTATA	TTTACCCCTT	OPE-E10
	...	...	...T...	...	...	Pot2

Fig. 5. Comparison of the nucleotide sequences from random amplified polymorphic DNA (RAPD) marker OPE-E10 (1.1-kb *HindIII* subclone) and transposon *Pot2*. OPE-E10 1.1-kb *HindIII* subclone and *Pot2* transposon sequences (Farman et al. 1996) were aligned with Clustal-W software (EMBL, Heidelberg, Germany). Dots represent identity between *Pot2* and OPE-E10 sequences. Inverse terminal repeat (ITR) sequence homology (2 to 42 bp) was weak, but strong identity was found between sequences corresponding to transposases (153 bp to the end).

copy sequence. Hybridization signals were detected only in the parent Guy11, which is avirulent on *Irat7* and mapped at a OPE-Y13 locus inseparable from *AVR1-Irat7* (data not shown). RAPD marker OPE-E12 was not characterized as it was too far from avirulence gene *AVR1-Ku86*.

Localization of *AVR1-MedNoi*, *AVR1-Irat7*, and *AVR1-Ku86* on the *M. grisea* genetic map.

The RFLP marker revealed by cosmid A11D9 is located at 4.5 cM from avirulence gene *AVR1-Irat7*. This marker was mapped on chromosome 1 at 16 cM from the avirulence gene *AVR1-CO39* (Valent and Chumley 1994; Smith and Leong 1994; Nitta et al. 1997; Farman and Leong 1998). Other RFLP markers (A15H5, A14D7, and A12D8) from chromosome 1 were mapped with our progeny. The results showed that the relative order of these markers and the genetic distances between them were similar to those observed for the two other *M. grisea* genetic maps (Fig. 7). Colinearity among markers in these genomic regions is sufficiently reliable to map *AVR1-Irat7* at 20 cM from *AVR1-CO39*.

To assign *AVR1-MedNoi* and *AVR1-Ku86* to known *M. grisea* linkage groups, RAPD markers OPE-S9 and OPE-D16 were mapped with progeny from the cross between isolates 4224-7-8 and 6043 (Sweigard et al. 1993). RAPD marker OPE-D16 linked to *AVR1-MedNoi* was closely linked to markers Tel3(js), A11D6, and MGR2, which were not clearly assigned to a chromosome (Sweigard et al. 1993; M. Orbach, unpublished results). A single-copy subclone from RAPD marker OPE-S9 revealed two hybridizing fragments, whatever the restriction enzyme used. One OPE-S9 RFLP marker co-segregated with Tel50(js) and *AVR1-TSUY* (Sweigard et al. 1993) located at the tip of chromosome 1

according to the integrated map chromosome numbering (Nitta et al. 1997). The other OPE-S9 RFLP marker co-segregated with Tel38(js) corresponding to the tip of chromosome 4 (Nitta et al. 1997). By contour-clamped homogeneous electric field (CHEF) analysis, we detected five high-molecular-weight chromosomal bands that are similar in size in both parental strains. As *M. grisea* has seven chromosomes (Orbach et al. 1996; Nitta et al. 1997), results from our CHEF analysis suggest that two pairs of high-molecular-weight chromosomes co-migrated. The 0.6-kb *HindIII* single-copy subclone of RAPD marker OPE-E10 that is linked to *AVR1-Ku86* hybridized to the largest chromosomal band, migrating at approximately 10 Mb (data not shown). This chromosomal band corresponds to the migration of *M. grisea* chromosomes 1 and 2 (Orbach et al. 1996; Nitta et al. 1997). Therefore, *AVR1-Ku86* is likely to be located near the tip of chromosome 1 as defined by the hybridization of its linked marker OPE-E10 to large *M. grisea* chromosomes separated by CHEF and the co-segregation of one OPE-S9 RFLP marker with Tel50(js) from the chromosome 1 tip. Other RFLP markers located near the chromosome 1 tip should be mapped with our progeny to confirm this hypothesis. In summary, *AVR1-Irat7* was located on chromosome 1 of *M. grisea* near avirulence gene *AVR1-CO39*. Indirect arguments support the location of *AVR1-Ku86* at the tip of chromosome 1 near *AVR1-Tsuy*. *AVR1-*

Fig. 6. Phylogenetic tree of fungal transposable elements related to *Pot1*. Transposases protein sequences from transposons related to *Pot2* (Daboussi and Langin 1994) such as *Flipper* (Levis et al. 1997), *Pot3* (Farman et al. 1996), *Nht1* (Enkerli et al. 1997) and *Fot1* (Daboussi et al. 1992) were aligned with Clustal-W (EMBL, Heidelberg, Germany). Alignments were analyzed by maximum parsimony (PAUP, version 3.0; Swofford 1991). The phylogram presented was obtained after bootstrapping with a heuristic search with *Pot3* as an outgroup. Bootstrap values shown on branches are expressed as percentages of 100 repetitions and are estimates of the significance of the clustering between two transposon sequences according to their sequence homology.

Fig. 7. Comparison of genetic maps from chromosome 1 with avirulence genes *AVR1-Irat7* and *AVR1-CO39*. Genetic maps from regions of chromosome 1 (numbering as in Nitta et al. 1997) containing known avirulence genes were compared with locations of common markers corresponding to restriction fragment length polymorphisms (RFLPs) revealed by cosmids such as A11D9, A12D9, A15H5, A14D7, and cos231 (Sweigard et al. 1993). The map from cross 4224-7-8 by 6043 includes *AVR1-TSUY*, OPE-SO6 restriction fragment length polymorphism (RFLP), and telomere RFLP Tel50(js) (Sweigard et al. 1993). The map from cross Guy11 by 2539 includes *AVR1-CO39* and telomere RFLP tel2(nn) (Nitta et al. 1997).

MedNoi was closely linked to telomere RFLP markers that cannot be assigned yet to a chromosome.

DISCUSSION

Genetic control of avirulence by a single gene is frequent in plant-fungal interactions (Knogge 1996; De Wit 1997). In *M. grisea*, most of the avirulence genes identified determine specificity toward a single rice cultivar. The avirulence to the six cultivars we studied is also under monogenic control but only three loci are involved. Such co-segregations of avirulence toward different cultivars of the same plant species were also described for the fungal plant pathogen *Phytophthora sojae* (Gijzen et al. 1996; Whisson et al. 1995; Judelson 1996). To fit with the gene-for-gene relationship, two hypotheses can be proposed. The first one is based on the existence of only three specific resistance genes. Here, cultivars Carreon, DJ 8-341, and Irat7 share the same resistance gene that interacts with *AVR1-Irat7*. Similarly, rice cultivars Cica 8 and *MedNoi* would have a common resistance gene that interacts with *AVR1-Ku86*. According to their breeding genealogy, none of these six rice cultivars have common ancestors. Genetic analysis of resistance including test crosses between these rice cultivars will test the validity of this hypothesis. The second hypothesis is that more than three avirulence genes segregated but that some of these genes are closely linked. According to the number of progeny analyzed, the avirulence genes toward Carreon, DJ 8-341, and Irat 7 or Cica 8 and *MedNoi* would be separated by less than 1 cM. Without recombinant progeny, only three avirulence genes will be described. Linkages between avirulence genes have been observed in *M. grisea* (Leung et al. 1988; Valent et al. 1991), but distances between such genes were at least 20 cM. Clusters of avirulence genes have not yet been observed in fungi, but mapping of more avirulence genes is needed for this to be conclusive.

AVR1-Ku86 and *AVR1-MedNoi* were linked to telomere RFLPs (4.5 and 6 cM, respectively), suggesting their localization near chromosome tips. *M. grisea* avirulence genes *AVR2-YAMO* and *AVR1-TSUY* (Sweigard et al. 1993) also mapped near telomeres, but at a closer distance (less than 1 cM). Four of the eight avirulence genes mapped in *M. grisea* to date are located near chromosome tips. In yeast, sub-telomere regions are subject to frequent rearrangements or gene silencing (Gottschling 1992; Louis et al. 1994). Such instabilities were already reported in *M. grisea* at the sub-telomere locus *AVR2-YAMO*, for which a high frequency of mutations to virulence was observed (Valent and Chumley 1994). At this locus, 60% of the spontaneous mutations from avirulence to virulence are due to deletions of the chromosome tip that do not affect the viability of the fungus nor its pathogenicity to susceptible rice cultivars (Valent and Chumley 1994). In *Cladosporium fulvum*, the avirulence gene *AVR9* is also located in a region that can be deleted without any effect on pathogenicity (van Kan et al. 1991), but its chromosomal location is unknown. Localization of avirulence genes in nonessential chromosomal regions such as sub-telomere regions could explain the frequent appearance of virulent races by deletion of small chromosomal regions without noticeable loss of viability or pathogenicity.

Most RAPD markers (7/10: 70%) we have characterized corresponded to junction fragments between either two dif-

ferent repeated sequences or one repeated sequence and a single-copy sequence. Previous reports showed that RAPD markers amplified in fungi contained repeated sequences (Kazan et al. 1993; Williams et al. 1993; Arnau et al. 1994). These RAPD markers were not further characterized and their relationships to transposable elements were not established. Recently, RAPD markers amplified in soybean (Bhattacharyya et al. 1997) and in grapevine (Böhm and Zyprian 1998) were shown to contain sequences related to plant retrotransposons. In our experiments with *M. grisea*, five of the 10 characterized RAPD markers contained sequences related to known *M. grisea* transposable elements such as the *Pot2* DNA transposon (OPE-E10 and OPE-K16) or retroposon *MGL/MGR583* (OPE-G18, OPE-J16 and OPE-G16). *MGL/MGR583* was present in 3/7 (43%) of the RAPD markers containing repeated sequences. Its copy number in the *M. grisea* genome (about 100: Shull and Hamer 1994) is close to those of other *M. grisea* transposons (*Fosbury/Maggy*: ≥ 100 ; *Pot2*: ≥ 100 ; *MGR586/Pot3*: 50; Shull and Hamer 1994; Farman et al. 1996; Nishimura et al. 1998). *MGL/MGR583* is apparently randomly distributed in the *M. grisea* genome, since *MGL/MGR583*-derived RFLP markers are distributed on all chromosomes at different locations (Sweigard et al. 1993). Other *M. grisea* transposons seemed to be clustered (*Grasshopper*: Sweigard et al. 1993; *Pot2* and *Fosbury/Maggy*: Nitta et al. 1997; Nishimura et al. 1998). *MGL/MGR583* was not significantly associated with avirulence genes, since it was only detected once near an avirulence gene among six chromosomal regions analyzed (*PWL1*, *PWL2*, *PWL3*, and *PWL4*: Kang et al. 1995; *AVR1-CO39*: Farman and Leong 1998; *AVR1-MARA*: Mandel et al. 1997). Neither *MGL/MGR583* copy number nor a nonrandom genomic distribution can explain its frequent occurrence among these RAPD markers linked to avirulence genes. The high proportion of different repeated sequences linked to avirulence genes we observed is consistent with previous results obtained at various *M. grisea* avirulence loci. Chromosome walking toward these genes revealed the occurrence of uncharacterized, repeated sequences such as pCB608/pCB619 or known transposable elements such as *Pot3/MGR586*, *Pot2/pCB768*, and *Grasshopper* (Valent and Chumley 1994; Kang et al. 1995; Farman and Leong 1998; Mandel et al. 1997). Two of the avirulence genes we studied were located near sub-telomere regions that are also known to be rich in repeated sequences in many eukaryotes (Biessmann and Mason 1992). Our study showed that the occurrence of a high density of repeated sequences near telomeres may vary from one chromosome tip to another. For example, two RAPD markers (OPE-D16 and OPE-Y16) among three located between *AVR1-MedNoi* and telomere marker TelG were composed only of single-copy sequences. On the other hand, all RAPD markers (OPE-S9 and OPE-G16) located between *AVR1-Ku86* and telomere marker TelF contained repeated sequences.

The RAPD markers closely linked to avirulence genes that were characterized in this study are now being used for positional cloning. The screening of genomic libraries by hybridization is achieved with the single-copy sequences from these RAPD markers. Alternatively, an RAPD-based screening strategy (Dioh et al. 1997) is used to identify cosmid clones for RAPD markers composed of repeated sequences.

MATERIALS AND METHODS

Magnaporthe grisea isolates, mating, and genetic analysis of avirulence.

Isolate Guy11 is a hermaphroditic, female, fertile isolate pathogenic to rice (Notteghem et al. 1992). 2/0/3 is a sib-progeny from the cross (Guy11 $\times$ ML25). Virulence and mating type of isolates Guy11 and 2/0/3 are already described (Silué et al. 1992b). Isolates were grown at 26°C on a rice flour agar medium (1 liter of distilled water with 15 g of agar and 20 g of paddy rice flour, 1.5 g of yeast extract and 500,000 U.I. of specilline G added after autoclaving at 120°C for 20 min. Crosses and cultures were performed as previously described (Silué et al. 1992a) in petri dishes containing rice flour agar medium. Four mycelial plugs, two from each isolate, were placed in a petri dish a few centimeters apart. Plates were incubated for 3 days at 26°C and transferred to 20°C for 14 days under fluorescent light. Mature perithecia (14 to 21 days old) were transferred to 4% water agar (1 liter of distilled water with 40 g of Bacto agar) and opened with a scalpel, releasing the asci that were separated with a thin glass needle. After 3 min of incubation at room temperature, each ascus was gently crushed with a glass needle to release the ascospores. After overnight incubation at 26°C, germinated ascospores were individually transferred to fresh medium and designated by a numeric code with three numbers. The first number refers to the cross, the second to the ascus number (0 for random ascospores), and the third to the ascospore number. Strains were stored on filter paper disks as previously described (Silué et al. 1992b). For inoculation, isolates were grown for 7 to 10 days on rice medium at 26°C under light. The mycelium was scraped and flooded with water. This suspension was filtered with a cheesecloth and adjusted to 2.5×10^4 spores per ml. Virulence assays were performed by injection with a syringe of the spore suspension through the last leaf sheaths of 3- to 4-week-old plants (four- to five-leaf stage). Rice cultivars Maratelli, Irat7, Ku86, MedNoi, DJ8-341, and Carreon were grown in the greenhouse at temperatures kept between 20°C (night) and 30°C (day). Cultivar Maratelli has no known resistance gene toward isolates pathogenic to rice and was used to detect nonpathogenic progeny. Disease assessment was performed with a six-class scale (Silué et al. 1992b). Class 1 lesions corresponded to resistance with “no visible symptom or brown pin point” and class 6 lesions to maximum susceptibility with “purple-gray lesions without a brown margin.” Lesions of class 3 are clearly visible brown spots with no sporulation (avirulent). Lesions of class 4 have a clear sporulating zone surrounded by brown margins (virulent). Isolates inducing class 1–3 symptoms were considered avirulent whereas isolates inducing class 4–6 symptoms were considered virulent. Inoculations were conducted twice with no difference in classification of the progeny as virulent or avirulent.

DNA extraction, RAPD analysis, BSA, and genetic linkage.

Mycelia grown on rice flour medium petri dishes for 7 to 14 days were harvested and ground in a mixer with 10 ml of sterile water. Five milliliters of this mycelium suspension was used to inoculate Roux flasks with 100 ml of Tanaka minimal medium (Ou 1985) with 2 g/liter yeast extract. The liquid culture was maintained at 26°C for 4 days. Mycelia were har-

vested by filtration through a piece of sterile Miracloth (Calbiochem-Novabiochem, La Jolla, CA), quickly frozen in liquid nitrogen, and stored at -80°C until used or lyophilized. DNA was extracted by either a CTAB protocol (Murray and Thompson 1990) from lyophilized mycelia or a Sarkosyl-cesium chloride protocol (Daboussi et al. 1989) from frozen mycelia. DNA from 15 progeny avirulent toward cultivar MedNoi and virulent toward Ku86 was pooled. Likewise, DNA from 13 progeny virulent toward MedNoi and avirulent toward Ku86 were pooled. DNA from 17 progeny avirulent toward cultivar Irat7 were pooled and DNA from 8 progeny virulent toward Irat7 were pooled. The final concentration of each DNA in the pool was adjusted to 10 ng/ml. For BSA, RAPD amplifications were performed on each pool and on parental strains. For genetic linkage analysis, RAPD amplifications were performed with genomic DNA from each of the 76 random ascospores. Operon primers used for amplification of RAPD markers linked to avirulence genes were as follows:

OPE-S9 (CCTGGTCCCC), OPE-M18 (CACCATCCGT), OPE-D16 (AGGGCGTAAG), OPE-Y13 (GGGTCTCGGT), OPE-E10 (CACCAGGTGA), OPE-Y16 (GGGCAATGT), OPE-K16 (GAGCGTCGAA), OPE-J16 (CTGCTTAGGG), OPE-E12 (TTATCGCCCC), OPE-G16 (AGCGTCCTCC), OPE-G18 (GGCTCATGTG).

Primers were used at a concentration of 1 μM , with 30 ng of DNA from parental strains or from pools, 2.5 μl of $10\times$ buffer (Appligene, Illkirch, France), 0.2 mM dNTP (Pharmacia, Lyon, France), 1 U of *Taq* DNA polymerase (Appligene) and sterilized water up to 25 μl . A standard program for RAPD was used with a PTC-100 MJ-Research PCR apparatus: 1 cycle of 2 min 30 s at 95°C and 45 cycles of 1 min at 95°C , 1 min 30 s at 37°C , 1 min 30 s at 72°C , followed by 1 cycle of 15 min at 72°C . Amplification products were separated by gel electrophoresis in TAE (10 mM Tris, pH 7.8, 5 mM sodium acetate, and 0.5 mM EDTA) with 1% agarose at 3 V/cm with the 1-kb ladder (Gibco BRL, Gaithersburg, MD) as marker. Segregation data obtained with 76 progeny were analyzed with the software Mapmaker (Lander et al. 1987) version 2 for Macintosh (S. Tingey, E. I. Dupont de Nemours, Wilmington, DE). Parameters for map construction were a minimum lod score of 3.0 and a maximum recombination fraction of 0.2. The Haldane function was used to compute recombination distances in centimorgans.

Molecular characterization of RAPD markers.

RAPD markers of interest were separated by agarose electrophoresis from other amplified fragments, extracted from the gel by glassmilk purification (Jetsorb; Bioprobe, Les Ulis, France), and cloned into pGEM-T vector (Promega, Lyon, France). Transformation of plasmids into DH5 α -competent cells was performed following the protocol from Inoue et al. (1990). Plasmid inserts from transformants were amplified with forward and reverse primers in PCRs with a small amount of white bacterial cells as template. PCR conditions were as follows: 1 min at 95°C , 1 min at 60°C , 1 min at 72°C during 25 cycles, and 1 cycle of 15 min at 72°C . Positive transformants amplified a PCR product of a size corresponding to the RAPD marker with an additional 200 bp from the vector. Cloned RAPD markers were sequenced by dideoxy terminator cycle sequencing (Applied Biosystems, Warring-

ton, UK) according to the manufacturer's instructions and an ABI373 automated DNA sequencer (Applied Biosystems). Sequences were submitted to EMBL under the accession numbers AJ249918 for OPE-G18, AJ249919 for OPE-J16, and AJ249920 for OPE-E10.

SCAR oligonucleotides deduced from RAPD markers sequences were used for amplification of the following:

OPE-E10, CTGACGCCAAAAGCAAGT and
TGTGACGAATGAGGGGGTAA;
OPE-J16, TAGGGCGACAGGTTGAAGAGT and
TAGGGGGTGTAGTTCACGTACG.

Primer pairs were used at the concentration of 0.2 µM, with 30 ng of DNA from parental strains, 10 µl of 10× buffer (Appligene), 0.2 mM dNTP (Pharmacia), 1 U of *Taq* DNA polymerase (Appligene), and sterilized water up to 100 µl, with standard PCR protocols with a PTC-100 MJ-Research PCR apparatus (30 cycles of 1 min at 95°C, 1 min 30 s at 55°C, 1 min 30 s at 72°C, followed by 1 cycle of 15 min at 72°C). Amplification products were separated by gel electrophoresis in TAE with 0.7% agarose at 3 V/cm. A 1-kb ladder (Gibco BRL) was used as a size marker.

RAPD and RFLP probes for Southern hybridization.

Genomic DNA (5 µg) from parental strains Guy11 and 2/0/3 or from progeny were digested by 30 I.U. of restriction enzymes *Bam*HI, *Eco*RI, *Hind*III, and *Eco*RV (Boehringer Mannheim, Meylan, France), separated by agarose gel electrophoresis, and transferred to a Hybond N membrane (Amersham, Saclay, France). Membranes were probed with RAPD markers. For hybridization, RAPD markers of interest were separated by agarose gel electrophoresis from other PCR products, extracted from the gel by glassmilk purification (Jetsorb; Bioprobe) and labeled with α³²P (Amersham) by random priming (Pharmacia). Hybridization was performed at 65°C overnight in buffer 1 (6× SSC, [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.5 % SDS [sodium dodecyl sulfate], 5× Denhardt's solution), with two washes at 65°C with buffer 2 (0.1× SSC, 0.2% SDS) for 1 h. Membranes were wrapped in plastic films and exposed to an autoradiographic film (Hyperfilm; Amersham) between intensifying screens for several hours to 4 days of storage at -80°C. Zoo blots of plasmids containing cloned repeated sequences from *M. grisea* were provided by S. Kang (The Pennsylvania State University, University Park) and M. Orbach (University of Arizona, Tucson). These blots contained cloned sequences from retroelement MGL/MGR583 (pCB583; Hamer et al. 1989; pCB684; GenBank accession no. AF018033), *Fot1*-like element *Pot3*/MGR586 (pCB586; Hamer et al. 1989; Farman et al. 1996), *Fot1*-like elements from *Pot2* family (pCB768; Kang et al. 1995), *Pot2* PCR product obtained by amplification with an oligonucleotide deduced from *Pot2* ITR sequences: Kachroo et al. 1994), MGR pCB608 (Hamer et al. 1989; Kang et al. 1995), MGR pCB619 (Hamer et al. 1989; Kang et al. 1995), gypsy-like retroelement *Grasshopper* (Dobinson et al. 1992), long terminal repeat (LTR) retroelement STR (S. Kang, *personal communication*), gypsy-like retroelement *Fosbury*/ *Maggy* (Shull and Hamer 1996; Farman et al. 1996).

Forty-five RFLP markers were obtained with either the retroelement MGL/MGR583 (pCB583; Hamer et al. 1989) or the *Fot1*-like element *Pot3*/MGR586 (pCB586; Hamer et al.

1989) as probes. Eleven cosmids already located on the *M. grisea* genetic map (cos256, cos229, cos243, A11D9, A2E8, A15H5, A12D9, A12B5, A12G1, A14D7, and A14C4; Sweigard et al. 1993) were used to map 18 RFLPs. An oligonucleotide corresponding to conserved fungal telomere sequences (Farman and Leong 1995) was used as a probe to map 10 telomere RFLPs.

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