MPMI Vol. 10, No. 9, 1997, pp. 1102–1105. Publication no. M-1997-1014-01N. © 1997 The American Phytopathological Society

Research Note

Physical Mapping of the *Magnaporthe grisea* *AVR1-MARA* Locus Reveals the Virulent Allele Contains Two Deletions

M. Alejandra Mandel, V. Wayne Crouch, Uvini P. Gunawardena, Travis M. Harper, and Marc J. Orbach

Department of Plant Pathology, University of Arizona, Tucson 85721-0036, U.S.A.
Accepted 30 July 1997.

The avirulence genes that have been identified in *Magnaporthe grisea* exhibit varying degrees of stability in infection assays. *AVR1-MARA* is considered one of the stable avirulence genes. In an effort to understand this stability, we analyzed the *AVR1-MARA* locus by physical mapping and chromosome walking. By walking toward *AVR1-MARA* from restriction fragment length polymorphism markers on both sides of the locus, we isolated sequences that are inseparable from *AVR1-MARA,* but we were unable to clone the complete locus. In contrast, the virulent locus *avr1-MARA* was isolated easily. A detailed comparative map of the two loci was constructed that identifies two deletions in the virulent locus, suggesting that virulence may be due to partial or complete deletion of the gene. Physical mapping also revealed that one progeny strain from a cross between avirulent and virulent parents appears to have spontaneously mutated to virulence.

The fungus *Magnaporthe grisea* is the causal agent of blast disease on rice and other gramineous species (Ou 1985). Genetic analyses of *M. grisea* have identified several avirulence genes (Leung et al. 1988; Silué et al. 1992a, 1992b; Valent et al. 1991; Valent and Chumley 1994). Among these genes, some are unstable, as indicated by the appearance of spontaneous virulent lesions during laboratory infection assays (Valent and Chumley 1994). Others, for example those that were crossed into a rice-pathogen background from other strains, appear to be stable. We are attempting to clone the stable avirulence gene *AVR1-MARA* that is responsible for the resistance response in the rice cultivar Maratelli (Valent and Chumley 1991).

The *AVR1-MARA* locus is located on chromosome 2b, between molecular markers A12B5 and *grh*25 in the restriction fragment length polymorphism (RFLP) map constructed from cross 4360, between parental strains 4224-7-8 and 6043 (Sweigard et al. 1993). In order to clone this gene, a chromosome walk was initiated in libraries of strain 4224-7-8 with a low-copy fragment of marker *grh*25, which originally mapped 1.5 map units away from the *AVR1-MARA* locus (equivalent to one progeny with a recombination crossover between the loci). After 60, 15, and 7 genome equivalents were screened in lambda, cosmid, and BAC libraries, respectively, only one lambda clone (designated λ6.2) was obtained (see Figure 1). Like *grh*25, λ6.2 contains both low-copy and high-copy repetitive DNA. The low-copy repeated sequences linked to *AVR1-MARA* are present in the avirulent parental strain, 4224-7-8, but not in the virulent parent, 6043, indicating a possible deletion of these sequences in 6043. One end of the insert in λ6.2, a 0.7-kb fragment designated AM726 (Fig. 1), is a single-copy sequence that is present in both parental strains and identifies RFLPs for all enzymes tested. Thus, this fragment likely corresponds to the end of the deletion in strain 6043.

Due to the severe under-representation of clones from this area of the locus in our libraries, a walk was started from marker A12B5, which is located on the opposite side of *AVR1-MARA* from *grh*25 (Fig. 2A). Cosmid A12B5 identified four clones of our BAC library. The end fragment of one of the BAC clones isolated, fragment AM711.1 from BAC 18C1 (see Figure 1), crossed three recombination crossover points from A12B5 in the RFLP map cross of Sweigard et al. (1993). With AM711.1 as a probe, no new BAC clones were identified, but cosmid clone AM732, which crossed the other two recombination crossover points between A12B5 and *AVR1-MARA,* was isolated. No clones were detected in any of our 4224-7-8 libraries with the end probe of cosmid AM732 (AM737.2), thus the walk from A12B5 had reached a gap.

When AM711.1 was mapped, the breakpoints of only two of the four recombinant progeny between A12B5 and *AVR1-MARA* had been crossed and, surprisingly, a new one was identified in progeny 4360-R-20. This is the same strain that contains the crossover point between *grh*25 and *AVR1-MARA,* suggesting that either there were three recombination crossover points between markers A12B5 and *grh*25 in strain 4360-R-20, or one of the markers was mis-scored. Further data (see below and Figure 2) suggest a third explanation: that the *AVR1-MARA* allele of 4360-R-20 had mutated to virulence. A detailed analysis of the *AVR1-MARA* locus of strain 4360-R-20 was performed in order to determine the apparent discrepancy between the RFLP data and our results. Both probes, AM711.1 and AM737.2, detect restriction digestion patterns that correspond to the avirulent parent 4224-7-8, as do all markers including and beyond *grh*25 on chromosome

Corresponding author: Marc J. Orbach; Fax: (520) 621-9290; E-mail: orbachmj@ag.arizona.edu

2b (Sweigard et al. 1993, and shown in Figures 2 and 3). Infection assays with strain 4360-R-20 indicated that it is much less virulent (type 2 lesions; Valent et al. 1991) than the parental strain 6043 (type 5 lesions) on cv. Maratelli. Strain 4360-R-20 remains highly virulent on the susceptible check cv. Sariceltik (data not shown), indicating it does not have a general pathogenicity defect. Since the *AVR1-MARA* locus of progeny strain 4360-R-20 has the same restriction patterns as the avirulent parent 4224-7-8, but is phenotypically virulent, we therefore conclude that progeny strain 4360-R-20 likely represents a spontaneous virulent mutant of *AVR1-MARA*. Thus, there is no recombination crossover point between *grh*25 and *AVR1-MARA,* and *grh*25 and λ6.2 are inseparable from *AVR1-MARA*. AM737.2 is also inseparable from *AVR1-MARA* (Figs. 1 and 2B).

To try to bridge the gap in the *AVR1-MARA* locus between AM737.2 and λ6.2, a cosmid library made from a strain containing a virulent allele of this gene (4392-1-6) was screened with AM737.2. Six cosmid clones were isolated and a detailed map of one of them, 31G8, spanning the *AVR1-MARA* locus, is shown in Figure 1. The ends of the cosmid 31G8 insert, probes AM749.R.5 (a fragment of end clone AM749.N.1) and AM749.N.2, map on opposite sides of *AVR1-MARA*, each 3 map units away. Thus, the locus of strain 4392-1-6 has been isolated and is contained within a 43-kb cosmid insert. An additional marker inseparable from *AVR1-MARA,* AM750.N.1, was identified as one of the insert ends of cosmid 38G12 that hybridized to AM737.2 (see Figure 1). Although the origin of *avr1-MARA* in 4392-1-6 may be different from that of 6043, the loci are indistinguishable in our analyses (Fig. 3A–C). The only difference detected was with probe AM737.2 in genomic DNAs digested with *Bam*HI (Fig. 3C), and those bands, 14 kb

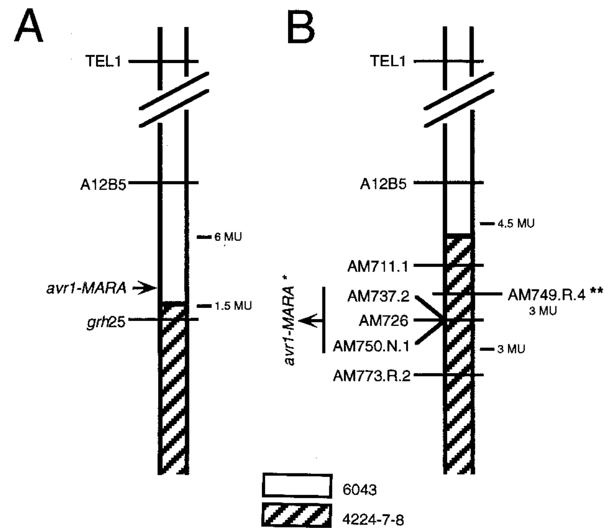

Fig. 2. Chromosome 2b from progeny 4360-R-20. **A,** Schematic representation of the region where the recombination crossover point was considered to be before the chromosome walk toward *AVR1-MARA*. This progeny showed restriction fragment length polymorphism (RFLP) segregation that corresponded to the virulent parent 6043 from the telomere marker TEL1 (upper part of the chromosome) to marker A12B5, and to the avirulent parent 4224-7-8 from marker *grh*25 and beyond. **B,** After several RFLP markers were added, the recombination crossover point is located between markers A12B5 and AM711.1. The reduced virulence of this strain is likely due to a spontaneous mutation of *AVR1-MARA,* indicated by an asterisk. Marker AM749.R.4 is a 1.2-kb *Eco*RI fragment from end probe AM749.N.2, and detects recombination crossover points in two other progeny strains (indicated by ∗∗).

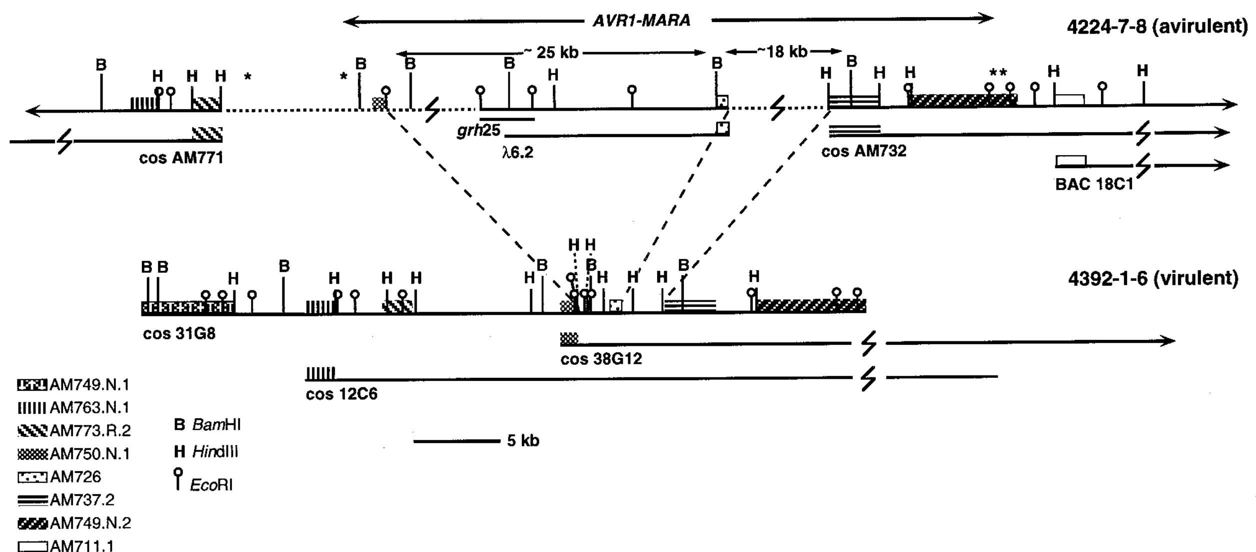

Fig. 1. *AVR1-MARA* loci from *Magnaporthe grisea*. Diagram of the avirulent locus *AVR1-MARA* from strain 4224-7-8 and virulent locus *avr1-MARA* from strain 4392-1-6. Boxes represent end probes from different steps in the chromosome walk. Probes AM749.N.1, AM763.N.1, AM750.N.1, and AM749.N.2 have DNA sequences from 4392-1-6, and probes AM773.R.2, AM726, AM737.2, and AM711.1 are from strain 4224-7-8. Dashed lines in 4224-7-8 represent uncloned DNA fragments. Asterisks (∗) represent recombination crossover points (the genetic distance between two markers separated by one recombination crossover is 1.5 map units). A 1.2-kb *Eco*RI fragment from probe AM749.N.2 detects recombination crossover points in two progeny, as *Eco*RV restriction fragment length polymorphisms. Lambda and cosmid libraries were constructed in λ-GEM11 (Promega, Madison, WI) and pMOcosX (Orbach 1994), respectively, by the partial fill-in method (Zabarovsky and Allikmets 1986). Construction of the BAC library has been described elsewhere (Diaz-Perez et al. 1996).

for 6043 versus 18 kb for 4392-1-6, correspond to sequences outside of the locus (i.e., to the right of AM737.2 in Figure 1).

A detailed physical comparison of the avirulent and virulent loci was carried out by probing genomic and cloned DNAs with several of the markers located at, or tightly linked to, *AVR1-MARA* (Figs. 1 and 3). Between AM726 and AM737.2, a deletion of approximately 13.5 kb was detected in the *avr1-MARA* locus, as seen by these markers being 4.5 kb apart in 6043 and approximately 18 kb apart in 4224-7-8. (Fig. 3B and C, *Bam*HI digestions). A second deletion of approximately 25 kb, which includes most of the *M. grisea* sequences of λ6.2, was detected in the *avr1-MARA* locus between markers AM726 and AM750.N.1.

To isolate sequences on the other side of the *AVR1-MARA* locus from strain 4224-7-8, the end fragment AM763.N.1 of another of the 4392-1-6 cosmid clone inserts (12C6) was used to probe the 4224-7-8 cosmid library. Clone AM771 was isolated and, like AM763.N.1, its end nearest to *AVR1-MARA* mapped 3 map units away from the locus, at a physical distance of approximately 9 kb from marker AM750.N.1 (see Figure 1).

To test whether the cloned portions of the *AVR1-MARA* locus contain the gene, clones λ6.2 and cosmid 732 were separately transformed into the virulent strain 6043. Several transformed strains containing these DNAs were screened on Maratelli and Sariceltik seedlings for their virulence phenotypes (data not shown). Neither clone transformed 6043 to avirulence on Maratelli. The alternative hypothesis, that virulence is dominant, was tested by transforming the avirulent strain 4224-7-8 with cosmid 31G8, which contains the virulent allele *avr1-MARA*. The resultant transformants remained avirulent on Maratelli, thus demonstrating that avirulence is indeed dominant over virulence.

In conclusion, we have cloned the virulent *avr1-MARA* locus from strain 4392-1-6 and have demonstrated that the avirulent locus contains two segments of about 13.5 and 25 kb that are absent in the virulent locus. We have also determined that progeny strain 4360-R-20 likely has a spontaneous mutation in the *AVR1-MARA* gene that has rendered it virulent. This would be the first spontaneous mutation of this stable locus to be identified. The low virulence of this strain suggests that it may be a partial loss-of-function mutation, but verification of this awaits cloning of this virulent allele.

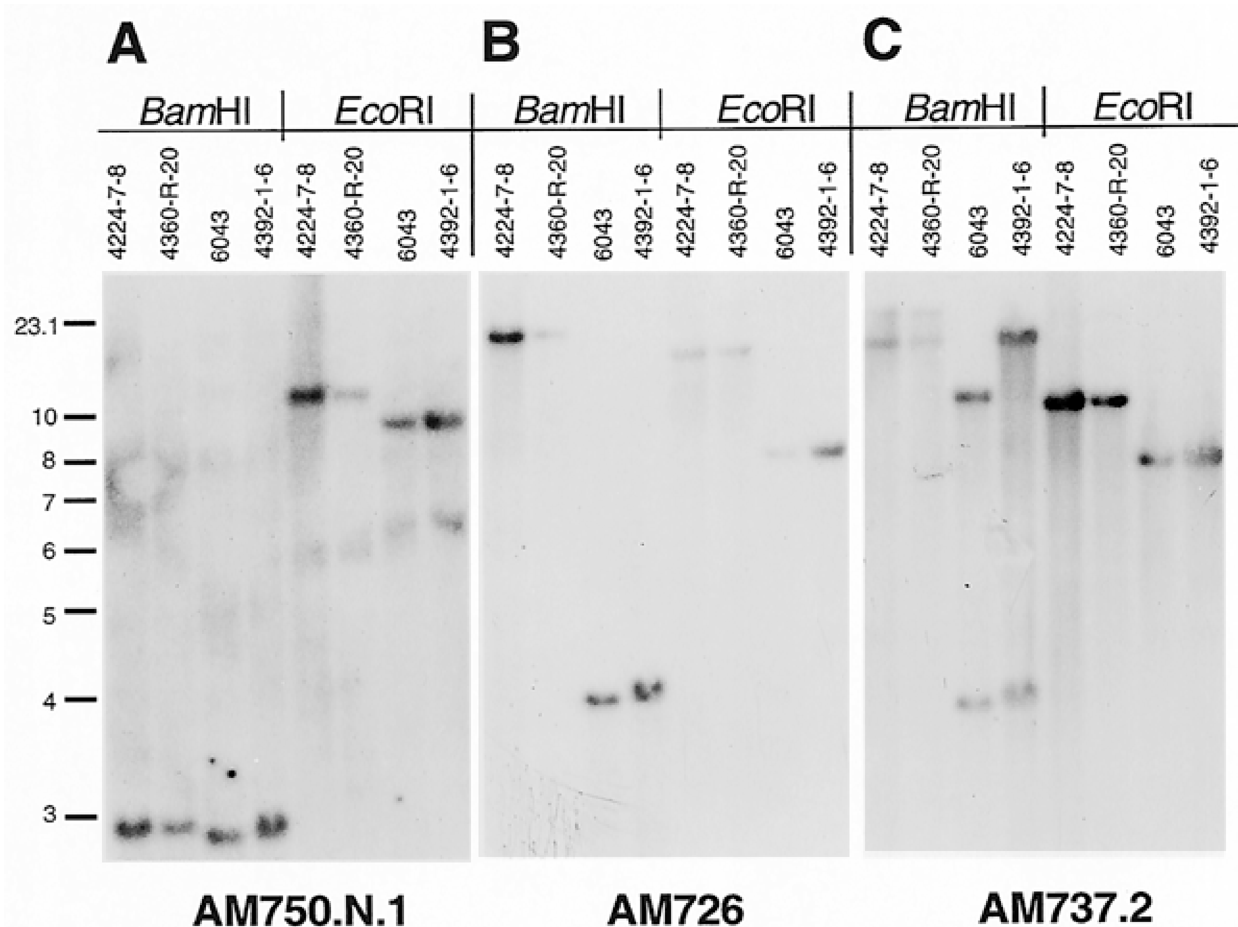

Fig. 3. DNA gel blot analyses of the avirulent and virulent *AVR1-MARA* loci. Genomic DNAs were digested with *Bam*HI and *Eco*RI, electrophoresed on 0.7% agarose, and transferred onto a nylon membrane. The same blot was used for consecutive hybridizations with the following probes: **A,** AM750.N.1; **B,** AM726; and **C,** AM737.2. The restriction patterns of 4224-7-8 and 4360-R-20 DNAs are identical within the locus. The virulent *avr1-MARA* locus from strains 6043 and 4392-1-6 shows similar restriction patterns, with the exception of a polymorphism detected in *Bam*HI with probe AM737.2. These sequences are located beyond the locus, as shown in Figure 1. 4224-7-8 and 6043 are the parental strains used for the crosses in the restriction fragment length polymorphism map, 4360-R-20 is the progeny shown in Figure 2, and 4392-1-6 is the strain used to prepare the cosmid library from which *avr1-MARA* was isolated. Molecular markers are indicated in kilobases.

That the virulent loci of strains 6043 and 4392-1-6 differ from the avirulent locus by two large deletions leads to speculation that *AVR1-MARA* might have been either partially or completely deleted in these strains. The ease with which the *avr1-MARA* locus was isolated, in comparison to the *AVR1-MARA* locus, suggests that sequences represented by those deletions may be lethal, or unclonable in *Escherichia coli*. We are testing this prediction and attempting to circumvent cloning obstacles through direct physical isolation of the sequences represented in the deletions and by cloning the *AVR1-MARA* locus in yeast.

ACKNOWLEDGMENTS

We are grateful to Hans VanEtten, Sandy Pierson, Ken Feldmann, and Tony Meyn for helpful discussions and critical reading of this manuscript. This work was supported by the U.S. Department of Agriculture N.R.I.C.G.P. (Grant 92-370303-7794). T. M. H. is the recipient of a fellowship from the ARCS Foundation.

LITERATURE CITED

Diaz-Perez, S. V., Crouch, V. W., and Orbach, M. J. 1996. Construction and characterization of a *Magnaporthe grisea* bacterial artificial chromosome library. Fungal Genet. Biol. 20:280-288.

Leung, H., Borromeo, E. S., Bernardo, M. A., and Notteghem, J. L. 1988. Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. Phytopathology 78:1227-1233.

Orbach, M. J. 1994. A cosmid with a Hy^R marker for fungal library construction and screening. Gene 150:159-162.

Ou, S. H. 1985. Blast. Pages 109-201 in: Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, UK.

Silué, D., Notteghem, J. L., and Tharreau, D. 1992a. Evidence for a gene-for-gene relationship in the *Oryza sativa-Magnaporthe grisea* pathosystem. Phytopathology 82:577-580.

Silué, D, Tharreau, D., and Notteghem, J. L. 1992b. Identification of *Magnaporthe grisea* avirulence genes to seven rice cultivars. Phytopathology 82:1462-1467.

Sweigard, J. A., Valent, B., Orbach, M. J., Walter, A. M., Rafalski, A., and Chumley, F. G. 1993. A genetic map of the rice blast fungus *Magnaporthe grisea*. Pages 3.112-3.115 in: Genetic Maps, 6th ed. S. J. O'Brien, ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus *Magnaporthe grisea*. Annu. Rev. Phytopathol. 29: 443-467.

Valent, B., and Chumley, F. G. 1994. Avirulence genes and mechanisms of genetic instability in the rice blast fungus. Pages 111-134 in: Rice Blast Disease. R. S. Zeigler, S. Leong, and P. S. Teng, eds. CAB International, Wallingford, UK.

Valent, B., Farrall, L., and Chumley, F. G. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of back crosses. Genetics 127:87-101.

Zabarovsky, E. R., and Allikmets, R. L. 1986. An improved technique for the efficient construction of gene libraries by partial filling-in of cohesive ends. Gene 42:119-123.