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Mapping avirulence genes in the rice blast fungus *Magnaporthe grisea*

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Abstract. The fungal pathogen *Magnaporthe grisea* is involved in specific interactions with rice cultivars. Fungal strains with avirulence genes are non-pathogenic to rice cultivars with the corresponding race-specific resistance genes. Unravelling the molecular basis of such interactions requires the cloning of both avirulence and resistance genes. We have identified three genetically independent avirulence genes in M. grisea (*AvrMedNoï*-1, *Avr*Irat7-1, *Avr*Ku86-1). Analysis of the segregation of avirulence towards a collection of rice cultivars suggests the occurrence of the same resistance gene in unrelated cultivars. In order to clone avirulence genes by chromosome walking, we constructed a partial genetic map using 77 random progeny from the backcross between isolates Guy11 and 2/0/3. This map includes 75 RFLP markers corresponding to either repeated, single copy or telomeric sequences and 25 RAPD markers. Two avirulence genes were mapped to chromosome tips (*AvrMedNoï*-1 and *Avr*Ku86-1). We also identified five RAPD markers tightly linked (1 to 4 cm) to these avirulence genes using bulk segregant analysis. Most of these linked RAPD markers were composed of repeated sequences adjacent to single copy sequences, which could be used to initiate chromosome walks.

Major progress has been made in recent years in the area of genetics and molecular biology of *Magnaporthe grisea*, the main fungal pathogen of rice (Zeigler et al., 1994; Talbot, 1995). Since the best method for rice blast disease control involves rice resistance and its management, we have to understand the mechanisms involved in cultivar race specificity and its variability. Breeding for resistance has shown that a large number of rice cultivars have major genes conferring complete resistance towards specific races of the rice blast fungus (Ou 1985, Kiyosawa et al., 1986). This cultivar specificity is controlled on the pathogen side by avirulence genes corresponding to specific resistance genes in rice, in a gene-for-gene relationship that was first described in the pathosystem flax / *Melampsora lini* (Flor, 1971). While significant progress has been made on the genetic basis of disease resistance in rice and avirulence in *M. grisea*, the study of these interactions at a molecular level requires cloning of both resistance and avirulence genes. Up to now, five plant resistance genes have been isolated either by positional cloning or by transposon tagging (Staskawicz et al., 1995). The sequencing of these resistance genes showed that they encoded for proteins involved in signal transduction pathways (protein kinases or putative membrane receptors with leucine-rich repeats). We still do not know if the resistance gene products recognises directly or indirectly the avirulence gene product. Two mains strategies were used to clone avirulence genes in fungi. First, map-based cloning which implies isolation of a linked marker followed by identification of overlapping cosmids spanning the region, the marker and the avirulence gene. This strategy allowed the cloning of two avirulence genes in *M. grisea*, *Avr*2-YAMO and *Pwl*2 (Valent and Chumley, 1994), which functions are unknown up to now. The second strategy requires the isolation of an elicitor specific for the resistant cultivar, which biosynthesis should be under the control of the avirulence gene. This strategy was successfully used in isolating peptide elicitors encoded by avirulence genes of the tomato pathogen *Cladosporium fulvum* (*avr*4 and *avr*9, De Witt 1992) and the barley pathogen *Rhynchosporium secalis* (*nip1*, Knogge 1994). In order to clone new avirulence genes in *M. grisea*, we used a map-based cloning strategy. We performed a genetic analysis of avirulence using *M. grisea* field isolates pathogenic to rice. Such crosses were used to identify molecular markers closely linked to avirulence genes. These markers will be the starting point of chromosome walks towards avirulence genes.

I – Genetics of avirulence in *M. grisea*

Genetic analysis in *M. grisea* was for a long time limited to hermaphroditic isolates pathogenic to grasses other than rice (Leung and Taga, 1989), since most of the isolates pathogenic to rice are female sterile (Notteghem et al., 1992). Two strategies were developed to obtain fertile *M. grisea* isolates pathogenic to rice (Notteghem et al., 1994). First, crosses between hermaphroditic isolates pathogenic to hosts other than rice, followed by crosses with isolates pathogenic to rice, led to laboratory strains with improved fertility (Valent and Chumley, 1991). This strategy allowed the identification of avirulence genes either from the non-rice parental isolate or from the rice pathogen (Ellingboe and Chao, 1994; Leung et al 1988; Leong et al., 1994, Valent and Chumley 1994). Second, a few hermaphroditic isolates pathogenic to rice

were found in world wide survey of *M. grisea* populations (Kato et al., 1982; Notteghem et al., 1992, Hayashi 1994). Such fertile rice isolates were crossed with rice field isolates leading to the identification of new avirulence genes (Notteghem et al., 1994). This second strategy is expected to be the best to study avirulence genes from *M. grisea* populations pathogenic to rice. Using this strategy, we crossed an hermaphroditic isolate from South America, Guy11, with a female sterile isolate from Africa, ML25. From that first cross 4 avirulence genes were identified in the F1 progeny, which was confirmed by subsequent crosses (Silue et al., 1992a; Silue et al., 1992b). To generate a large number of progeny including few complete tetrads, we focused on the more fertile backcross (2/0/3 by Guy11), in which three genetically independent avirulence genes were characterized: *Avr*MedNoï-1, *Avr*Irat7-1, *Avr*Ku86-1. All progeny avirulent to rice cultivar MedNoï were also avirulent to cultivar Cica8. Likewise, progeny avirulent to rice cultivar Irat7 were also avirulent to cultivar DJ8-341 and Carreon. Such a cosegregation could be due to a strong genetic linkage between two avirulence genes. Alternatively, the same gene could be responsible for avirulence towards two different cultivars. This situation can be explained by a gene-for-gene relationship, already demonstrated in *M. grisea* for one pair of avirulence and resistance genes (Silue et al., 1992a). That is, one particular avirulence gene interacts specifically with its corresponding resistance gene present in different cultivars. Our results suggest the presence of the same resistance gene, interacting with *Avr*MedNoï-1, in the unrelated rice cultivars MedNoï and Cica8. Another resistance gene interacting with *Avr*Irat7-1, should be present in the 3 cultivars DJ8-341, Irat7 and Carreon. Either one of the two independent avirulence genes present in isolate 2/0/3 (*Avr*MedNoï-1 and *Avr*Ku86-1) was responsible for avirulence towards rice cultivar Cica6, suggesting the presence of two different resistance genes in this cultivar. These experiments clearly show that a set of progeny segregating for avirulence is efficient for the identification of race-specific resistance genes in various rice cultivars. As rice cultivars frequently have more than one resistance gene (Ou, 1985), a set of progeny is more reliable than natural isolates with an unknown number of avirulence genes. It particularly allowed direct observation of the contribution of each segregating avirulence gene to the avirulent phenotype of parental isolates.

II – Map-based cloning of avirulence genes in *M. grisea*

1. Mapping avirulence genes

In order to clone avirulence genes by chromosome walking, we constructed a partial genetic map using 77 random ascospores from the cross between isolates Guy11 and 2/0/3. This map included 75 RFLP markers obtained with probes such as the repeated and dispersed sequences MGR 583 and MGR 586, (Hamer et al. 1989), some cosmids already mapped (Sweigard et al., 1993) and a fungal telomere oligonucleotide (Farman and Leong, 1995). We also mapped RAPD markers (Williams et al., 1990), three segregating avirulence genes and the mating type locus *Mat*1. Among 80 RAPDs generated by 280 Operon primers, we identified five RAPDs linked to avirulence genes using bulked segregant analysis (Michelmore et al., 1991). We also found one RFLP marker linked to avirulence gene *Avr*Irat7-1 which corresponded to cosmid A11D9 already mapped on chromosome one (Sweigard et al., 1993). Overall, each avirulence gene is closely linked to at least one molecular marker (Fig. 1). Mapping telomeric RFLPs showed that two avirulence genes, *Avr*MedNoï-1 and *Avr*Ku86-1, were located near the chromosome tips (3 to 5 cM). Two other *M. grisea* avirulence genes mapped near chromosome tips (*Avr*2-YAMO and *Avr*1-TSUY, Sweigard et al., 1993). Overall, four of the eight mapped avirulence genes are sub-telomeric. Such a chromosomal location might not be due to chance. In yeast these regions are subject to frequent rearrangements or gene silencing effects (Gottschling, 1992; Louis et al., 1994). If such phenomena exist in *M. grisea*, sub-telomeric location of avirulence genes could give rise to virulent isolates at high frequency by deletion or silencing. This situation occurred at the subtelomeric *Avr*2-YAMO locus, where 70% of the spontaneous virulence mutations are due to deletions of the chromosome tip (Valent and Chumley, 1994).

2. Characterisation of molecular markers linked to avirulence genes

The RAPD fragments linked to avirulence genes (Fig. 1) were cloned and characterized by their genomic hybridisation patterns. OPG18 (0.7 Kb), mapping 4 cM from *Avr*Irat7-1, gave a pattern typical of a repeated sequence. OPJ16 (0.7 Kb) was completely linked to *Avr*Irat7-1 and also gave a pattern typical of a repeated sequence with some similarity to the OPG18 pattern. Sub-cloning of OPJ16 produced a 0.6 Kb

*Hind*III fragment giving the same pattern as the whole marker, while the 0.1 Kb *Hind*III fragment revealed another repeated sequence. These results showed that OPJ16 was composed of two different repeated sequences. The avirulence gene *Avr*Ku86-1 was located between the two RAPD fragments OPE10 (1.7 Kb) and OPG16 (1.6 Kb). OPE10 and OPG16 both corresponded to distinct repeated sequences, since their genomic hybridisation patterns differed. When subcloning the 1.7 Kb OPE10 fragment with *Hind*III, we found that the largest fragment (1.1 Kb) gave the same pattern as the whole marker, while the smallest one (0.6 Kb) corresponded to a single copy sequence in parent 2/0/3, and to a duplicated sequence in parent Guy11. Therefore, this marker might be a junction fragment between a repeated sequence and single copy sequence. The single copy OPE10 subclone can be used to initiate a chromosome walk towards *Avr*Ku86-1. Finally, the RAPD fragment OPD16 (0.3 Kb), mapping 3 cM from avirulence gene *Avr*MedNoï-1, gave a pattern typical of a single copy sequence. As this sequence was only detected in parent 2/0/3 (avirulent on cultivar MedNoï), we assume that it was deleted in parent Guy11 (virulent on cultivar MedNoï). OPD16 can be used to initiate a chromosome walk towards *Avr*MedNoï-1. Overall, the majority (4/5) of the RAPD fragments linked to avirulence genes corresponded to repeated sequences. This situation could reflect that most of the polymorphisms between the two parental isolates involved repeated and dispersed sequences, as we found that only 40% the single copy cosmids revealed RFLPs while 60% of MGR 586 markers were polymorphic. It could also reflect that avirulence genes could be located in chromosomal regions rich in repeated sequences, as already showed for *Pwl*2 and *Avr*2-YAMO loci (Valent and Chumley, 1994).

III – Conclusions

M. grisea field isolates pathogenic to rice and fertile in crosses are now available for genetic studies of avirulence. We have identified 3 avirulence genes in a backcross between the two isolates pathogenic to rice, Guy11 and 2/0/3. Progeny of such cross were useful to detect resistance genes in rice cultivar using avirulence cosegregation analysis. These progeny were also needed for isolating avirulence genes by a map-based cloning strategy. Two avirulence genes were located near the chromosome tips which may be highly variable regions. We found five RAPDs and three RFLPs linked to the three avirulence genes in segregating in our cross. Most of these markers corresponded to repeated sequences. However, sub-cloning single copy junction fragments was possible for several linked RAPDs. Such single copy fragments are the best candidates to initiate chromosome walks towards mapped avirulence genes.

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References

• **De Witt P.J.G.M.** (1992). Molecular characterization of gene systems in plant fungus interactions and the application of avirulence genes in control of plant pathogens. *Annual Review of Phytopathology,* 30: 391-402.

• **Ellingboe A.H., Chao C.C.T.** (1994). Genetic interactions in *Magnaporthe grisea* that affect cultivar specific avirulence/virulence in rice. In: Zeigler RS, Leong SA, Teng PS (eds), *Rice Blast Disease*, Wallingford: CAB International, pp. 51-64.

• **Farman M.L., Leong S.A.** (1995). Genetic and physical mapping of telomeres in the rice blast fungus, *Magnaporthe grisea*. *Genetics,* 140: 479-492.

• **Flor H.H.** (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, 9: 275-296.

• **Gottschling D.E.** (1992). Telomere-proximal DNA in Saccharomyces cerevisae is refractory to methyltransferase activity in vivo. In: *Proceedings of the National Academy of Sciences of America*, 89: 4062-4065.

• **Hayashi N.** (1994). Rice blast disease in Yunnan Province: China. Distribution and use of fertile rice blast fungus isolated from Yunnan Province in China. *JIRCAS Workshop Papers*, 1:22-33.

• **Hamer J.E., Farral L., Orbach M.J., Valent B., Chumley F.G.** (1989). A host species specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. In: *Proceedings of the National Academy of Sciences of America*, 86: 9981-9985.

• **Kato H., Yamaguchi T.** (1982). The perfect state of Pyricularia oryzae Cav. from rice plants in culture. *Annals of the Phytopathological Society of Japan*, 48: 607-612.

• **Kiyosawa S., Mackill D.J., Bonman J.M., Tanaka Y., Ling Z.Z.** (1986). An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. *Bulletin of National Institute for Agrobiological Resources,* 2: 13-23

• **Knogge W., Gierlich A., Hermann H., Wernert P., Rohe M.** (1994). Molecular identification and characterization of the NIP1 gene, an avirulence gene from the barley pathogen, *Rhynchosporium secalis*. In: Daniels M.J, Downie A.J, Osbourn A.E, *Advances in molecular genetics of plant-microbe interactions*, vol. 3. Dordrecht: Kluwer academic publishers, pp. 207-214.

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

• **Leung H., Taga M.** (1989). *Magnaporthe grisea* (Pyricularia species), the blast fungus. In: Sidhu R (ed.), *Genetics of Phytopathogenic Fungi, Advances in Plant Pathology*, vol. 6, New York: Academic Press, pp. 175-188.

• **Leong S.A., Farman M., Smith J., Budde A., Tosa Y., Nitta N.** (1994). Molecular genetic approach to the study of cultivar specificity in the rice blast fungus. In: Zeigler R.S, Leong S.A, Teng P.S (eds), *Rice Blast Disease*, Wallingford: CAB International, pp. 51-64.

• **Louis E.J., Naumova E.S., Lee A., Naumov G., Haber J.** (1994). The chromosome end in yeast: Its mosaic nature and influence on recombinational dynamics. *Genetics,* 136: 789-802.

• **Michelmore R.W., Paran I., Kessel R.V.** (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions using segregating populations. *Proceedings of the National Academy of Sciences of America*, 88: 9828-9832.

• **Notteghem J.L., Silue D., Milazzo J.** (1992). Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. *Phytopathology*, 82: 421-424.

• **Notteghem J.L., Tharreau D., Silue D., Roumen E.** (1994). Present knowledge on rice resistance genetics and strategies for analysis of *M. grisea* pathogenicity and avirulence genes. In: Zeigler RS, Leong SA, Teng PS (eds), *Rice Blast Disease*, Wallingford: CAB International, pp. 155-166.

• **Ou S.H.** (1985). *Rice Diseases*. CAB International, 380 p.

• **Silue D., Notteghem J.L., Tharreau D.** (1992a). Evidence for a gene for gene relationship in the *Oryza sativa-Magnaporthe grisea* pathosystem. *Phytopathology*, 82: 577-582.

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

• **Staskawicz B.J., Ausubel F.M., Baker B.J., Ellis J.G., Jones J.D.G.** (1995). Molecular genetics of plant disease resistance. *Science*, 268: 661-667.

• **Sweigard J.A., Valent B., Orbach M.J., Walter A.M., Rafalski A., Chumley F.G.**, 1993. Genetic map of the rice blast fungus *Magnaporthe grisea*. In: O'Brien SJ (ed.), *Genetic Maps 1993*, New-York: Cold Spring Harbor Press, pp. 3.112-3.117.

• **Talbot N.J.** (1995). Having a blast: exploring the pathogenicity of *Magnaporthe grisea*. *Trends in Microbiology*, 3: 9-15.

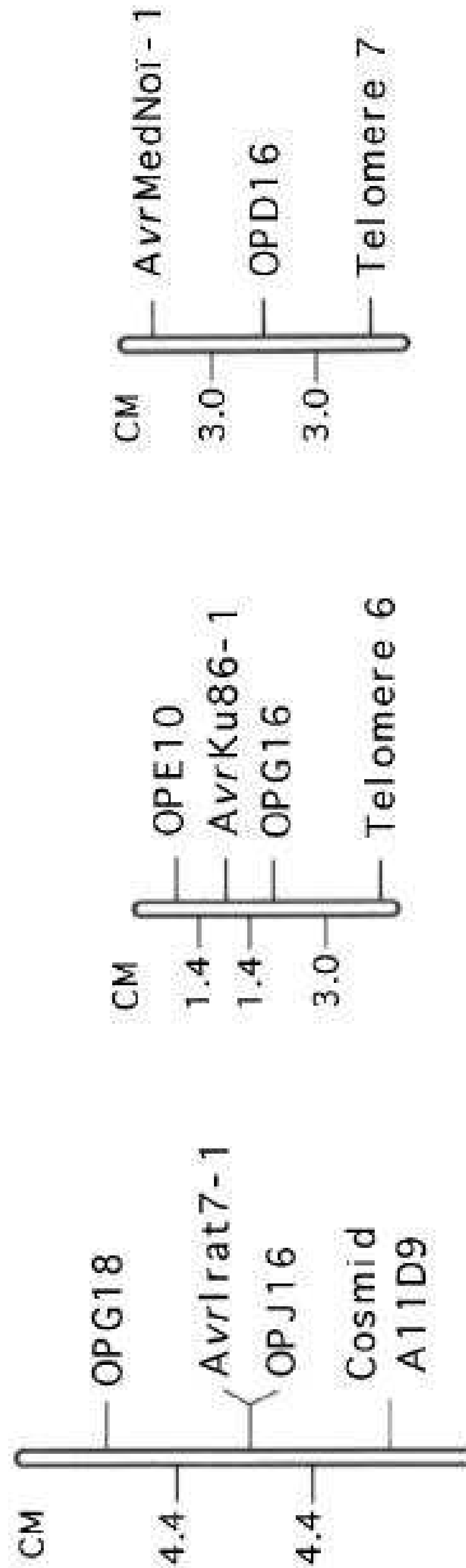
• **Valent B., Chumley F.G.** (1991). Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. *Annual Review of Phytopatholoy,* 29: 443-463.

• **Valent B., Chumley F.G.** (1994). Avirulence genes and mechanisms.of genetic instabilities in the rice blast fungus. In: Zeigler RS, Leong SA, Teng PS (eds), *Rice Blast Disease*, Wallingford: CAB International, pp. 111-134.

• **Williams J.G.K., Kubelik A., Livak K., Rafalski J.A., Tingey S.V.** (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research*., 18: 6531-6536.

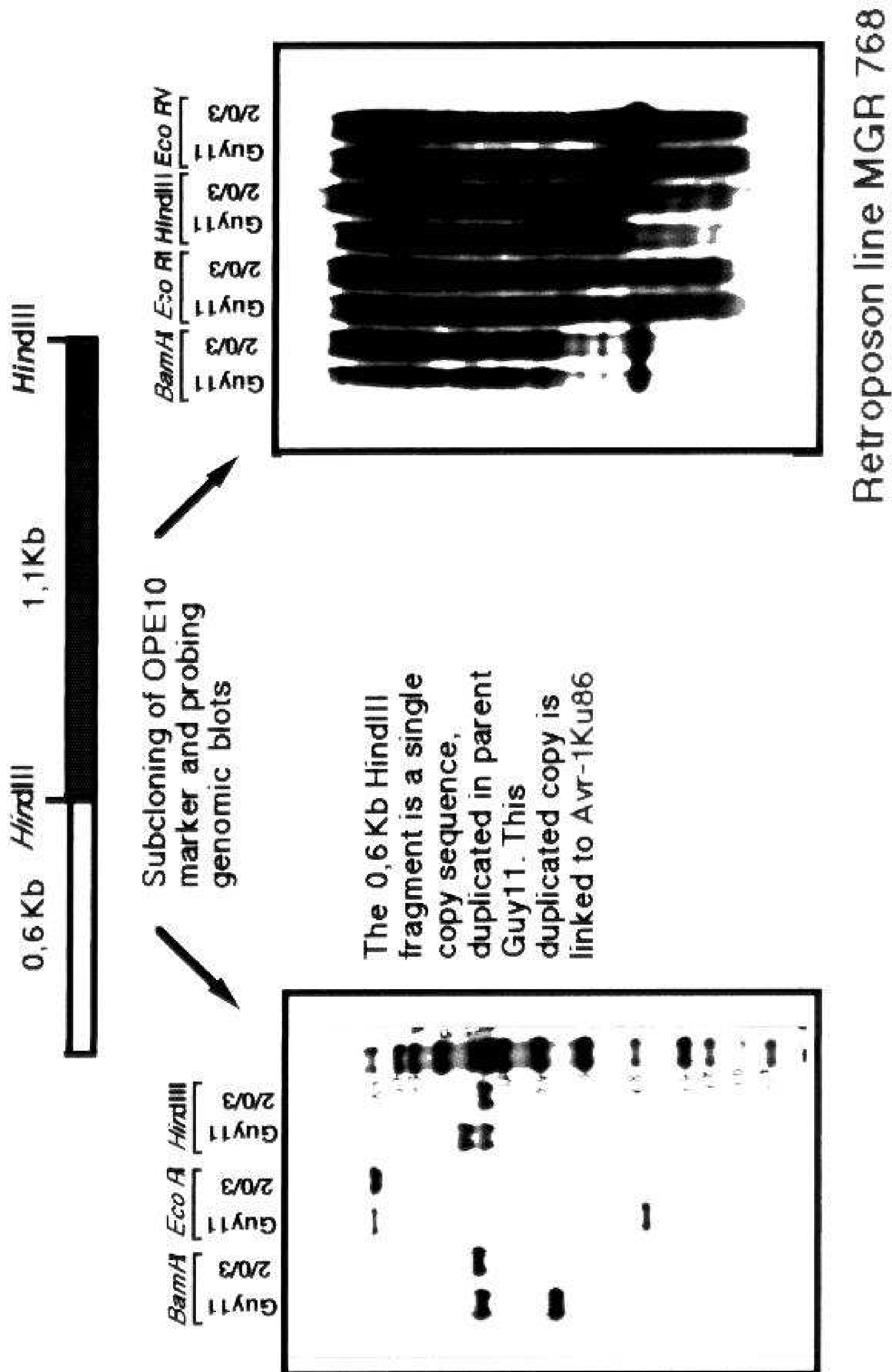
• **Zeigler R.S., Leong S.A., Teng P.S.** (1994). *Rice Blast disease*. Wallingford (UK): CAB International, 626 p.

Figure 1. Genetic maps of avirulence gene linkage groups



OPX refers to OPERON oligonucleotide number X, used for RAPD. Telomere Y refers to telomeric RFLP Y obtained with an oligonucleotide corresponding to three tandem repeats of the telomere sequence. Segregation analysis of molecular markers and avirulence genes were performed using 77 progeny from cross Guy11 by 2/0/3. Markers were mapped using MAPMAKER v2.0. Distances are in centimorgans (cM). Cosmid A11D9 is located on chromosome one of *M. grisea* strain 6043 (Sweigard et al., 1993).

Figure 2. Characterization of RAPD marker OPE10 linked to *Avr*Ku86-1



OPE10 fragment (1.7 KB) was sub-cloned with restriction enzyme HindIII. The left HindIII fragment (0.6 KB) was used as a probe on a genomic blot of both parents. It corresponded to a single copy sequence in parent 2/0/3 and a duplicated sequence in parent Guy11. The right HindIII fragment (1.1 KB) gave the same hybridisation pattern as the whole fragment, which was typical of a repeated sequence.