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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, AvrI7-1,
AvrKu86-1). 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 AvrKu86-1). 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 gri-
sea, 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 correspon-
ding 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 resis-
tance 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 posizio-
nal 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 indi-
rectly 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, Avr2-YAMO and Pw2 (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. This strategy was successfully used in
isolating peptide elicitors encoded by avirulence genes of the tomato pathogen Cladosporium fulvum (avr4 and
avr9, 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 pathoge-
nic to rice. Such crosses were used to identify molecular markers closely linked to avirulence genes. These mark-
ers 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 fer-
tility (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: *AvrMedNoï-1*, *AvrIrat7-1*, *AvrKu86-1*. 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 *AvrMedNoï-1*, in the unrelated rice cultivars MedNoï and Cica8. Another resistance gene interacting with *AvrIrat7-1*, 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 (*AvrMedNoï-1* and *AvrKu86-1*) 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 *AvrIrat7-1* 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, *AvrMedNoï-1* and *AvrKu86-1*, were located near the chromosome tips (3 to 5 cM). Two other *M. grisea* avirulence genes mapped near chromosome tips (*Avr2-YAMO* and *Avr1-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 *Avr2-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 *AvrIrat7-1*, gave a pattern typical of a repeated sequence. OPJ16 (0.7 Kb) was completely linked to *AvrIrat7-1* 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
HindIII fragment giving the same pattern as the whole marker, while the 0.1 Kb HindIII fragment revealed another repeated sequence. These results showed that OPJ16 was composed of two different repeated sequences. The avirulence gene AvrKu86-1 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 HindIII, 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 AvrKu86-1. Finally, the RAPD fragment OPD16 (0.3 Kb), mapping 3 cM from avirulence gene AvrMedNoï-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 AvrMedNoï-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 Pwl2 and Avr2-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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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).
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.