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**Mini-Review**

# Population Structure and Race Variation of the Rice Blast Fungus

**Seogchan Kang[1,*] and Yong-Hwan Lee[2]**

*[1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, U.S.A.*
*[2]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea*

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Worldwide, rice blast, caused by *Magnaporthe grisea* (Hebert) Barr. (anamorph, *Pyricularia grisea* Sacc.), is one of the most economically devastating crop diseases. Management of rice blast through the breeding of blast-resistant varieties has had only limited success due to the frequent breakdown of resistance under field conditions (Bonman et al., 1992; Correa-Victoria and Zeigler, 1991; Kiyosawa, 1982). The frequent variation of race in pathogen populations has been proposed as the principal mechanism involved in the loss of resistance (Ou, 1980). Although it is generally accepted that race change in *M grisea* occurs in nature, the degree of its variability has been a controversial subject. A number of studies have reported the appearance of new races at extremely high rates (Giatgong and Frederiksen, 1968; Ou and Ayad, 1968; Ou et al., 1970; Ou et al., 1971). Various potential mechanisms, including heterokaryosis (Suzuki, 1965), parasexual recombination (Genovesi and Magill, 1976), and aneuploidy (Kameswar Row et al., 1985; Ou, 1980), have been proposed to explain frequent race changes. In contrast, other studies have shown that although race change could occur, its frequency was much lower than that predicted by earlier studies (Bonman et al., 1987; Latterell and Rossi, 1986; Marchetti et al., 1976). Although questions about the frequency of race changes in *M. grisea* remain unanswered, the application of molecular genetic tools to study the fungus, ranging from its genes controlling host specificity to its population structures and dynamics, have begun to provide new insights into the potential mechanisms underlying race variation. In this review we aim to provide an overview on (a) the molecular basis of host specificity of *M. grisea*, (b) the population structure and dynamics of rice pathogens, and (c) the nature and mechanisms of genetic changes underpinning virulence variation in *M. grisea*.

## Molecular Basis of Host Specificity

A group of genes called avirulence (*AVR*) genes have been identified in many plant pathogens (Laugé and de Wit, 1998; Leach and White, 1996). A pathogen expressing one or more of these *AVR* genes is unable to infect those hosts that express any of the matching disease resistance (*R*) genes, due to the induction of host defense responses. Such a mechanism, termed a gene-for-gene interaction (Flor, 1971), governs the compatibility of plant-pathogen interactions in many cases, and thus the question of how this mechanism operates has been a subject of extensive investigations (Bent, 1996; Crute and Pink, 1996). Accumulated evidence to date indicates that the cultivar specificity of *M. grisea* follows this gene-for-gene model. Numerous *AVR* genes have been genetically identified in *M. grisea* (Chao and Ellingboe, 1997; Ellingboe, 1992; Ellingboe et al., 1990; Lau et al., 1993; Lau and Ellingboe, 1993; Leung et al., 1988; Silué et al., 1992a; Silué et al., 1992b; Valent and Chumley, 1987; Valent et al., 1991), and correspondingly, numerous *R* genes against *M. grisea* have also been identified in rice (Marchetti et al., 1987; Silué et al., 1992a; Valent et al., 1998; Wang et al., 1994; Yamada et al., 1976). In addition to *AVR* genes, two different classes of genes (*S* and *M* for suppressor and modifier, respectively) have been shown to control compatibility toward certain cultivars (Ellingboe, 1992; Lau et al., 1993; Lau and Ellingboe, 1993). The function of the *S* gene is to suppress the expression or function of specific *AVR* gene(s), whereas the *M* gene is required for the expression or function of one or more *AVR* genes. The question as to whether all the *AVR* genes in *M. grisea* have corresponding *S* and *M* genes remains unanswered.

The interfertility of *M. grisea* strains that infect different grass species has allowed for the genetic analyses of host specificity at the species level. Single genes that determine the compatibility to weeping lovegrass have been identified in independent crosses (Valent and Chumley, 1987; Valent and Chumley, 1991; Valent et al., 1986; Yaegashi, 1978). The *PWL1* gene, which prevents the fungus from infecting weeping lovegrass, was identified in a genetic cross between two grass pathogens (Valent et al., 1986). Another cross between two laboratory strains pathogenic on rice and weeping lovegrass, respectively, identified a second gene, *PWL2*, with a similar phenotype to *PWL1* (Valent and

*Corresponding author.
Phone) 1-814-863-3846, Fax) 1-814-863-7217
E-mail) sxk55@psu.edu

Chumley, 1991). Browning or auto-fluorescence of weeping lovegrass cells around developing colony margins correlated with the presence of the *PWL1* gene in an invading fungus (Heath et al., 1990). The fungus was unable to grow beyond these brown cells, suggesting that weeping lovegrass recognizes the product or by-product of *PWL1* and subsequently initiates a successful defense response.

The cloning of several *AVR* genes from *M. grisea* provided an opportunity to study the molecular basis of their avirulence function. Characteristics of these genes are summarized below. We also summarize the progress of ongoing efforts to clone additional *AVR* genes in *M. grisea*.

**The *AVR-Pita* gene.** The *AVR-Pita* gene (formerly known as *AVR2-YAMO*), which prevents *M. grisea* from infecting rice cultivar Yashiro-mochi, is located within 1.5 kb of the tip of a chromosome in rice pathogen O-137 and encodes a protein of 223 amino acids (Valent, 1997). The protein contains a putative signal peptide for secretion and a sequence motif characteristic of neutral zinc metalloproteases. The mutation of specific residues in this motif renders the *AVR-Pita* gene nonfunctional as an *AVR* gene, suggesting that its enzymatic activity might be necessary for triggering the host defense response in cultivar Yashiro-mochi. However, recent data suggest a physical interaction between the *AVR-Pita* gene product and the product encoded by its corresponding resistance gene, *Pi-ta* (Valent et al., 1998). Some *M. grisea* isolates from *Digitaria* or *Pennisetum*, which are unable to infect rice, possess a functional gene homologous to *AVR-Pita*. Expression of *AVR-Pita* is induced *in planta*, suggesting a role as a virulence factor in those hosts that lack the *Pi-ta* gene (Valent et al., 1998).

**The *AVR-CO39* gene.** The *AVR-CO39* gene, which prevents infection of rice cultivar CO39, was cloned by chromosome walking (Farman and Leong, 1998). The gene was initially identified in a series of backcrosses between O-135, a rice pathogen isolate from China, and 4091-5-8, a hermaphroditic laboratory strain that is pathogenic on weeping lovegrass and goosegrass (Valent et al., 1991). Since O-135 is virulent on cultivar CO39, the gene appears to have been inherited from 4091-5-8, a progeny of two grass pathogen isolates that are not pathogenic on rice (Valent et al., 1986). Three additional *AVR* genes, *AVR1-M201*, *AVR1-YAMO* and *AVR2-MARA* were also inherited from 4091-5-8, suggesting that certain grass pathogens nonpathogenic on rice contain genes that act as functional *AVR* genes corresponding to certain *R* genes in rice (Valent et al., 1991). The presence of functional *AVR-Pita* in strains from *Digitaria* or *Pennisetum* spp. supports this view (see above).

The chromosome walk to *AVR-CO39* covered an approximately 610 kb region of chromosome 1 and encountered three gaps that were caused by the presence of repetitive or unclonable DNA around the *AVR1-CO39* locus. Cleavage of the genome at defined positions using a technique termed RecA-assisted Achilles Cleavage (RecA-AC) was employed to jump over the gaps (Koob et al., 1993). There existed a 14-fold variation in the relationship between genetic and physical distance (16 kb/cM to 218 kb/cM) over this 610 kb region. Two overlapping cosmid clones identified during the chromosome walk conferred avirulence to Guy11, a strain virulent on cultivar CO39. Subcloning of these cosmid clones and subsequent functional complementation tests with the resulting subclones localized the *AVR-CO39* gene on a 1.05 kb region. Several small open reading frames (ORFs) were present on this 1.05 kb region (Leong et al., 1998). Mutation of the translational start codon of individual ORFs showed that two of them, ORF1 and ORF3, are necessary for the avirulence phenotype. The ORF3 encodes a putative protein of 89 amino acids and contains a putative signal peptide.

**The *PWL* gene family.** The *PWL2* gene was cloned by chromosome walking and was used as a probe to investigate the distribution of genes homologous to *PWL2* in more than 100 *M. grisea* strains isolated from diverse host species (Kang et al., 1995; Sweigard et al., 1995). The number of genes homologous to *PWL2* and the degree of their sequence homology were highly variable even among isolates from the same host species, suggesting that the *PWL2* gene is a member of a highly dynamic multigene family. Several *PWL* genes and their alleles, including *PWL1*, have been cloned from strains isolated from diverse hosts (Kang et al., 1995). The *PWL1* gene encodes a protein that exhibits 75% identity to the *PWL2* gene product. The *PWL3* gene, cloned from a finger millet pathogen, is allelic to the *PWL4* gene isolated from a weeping lovegrass pathogen. Both alleles are nonfunctional in triggering the defense response in weeping lovegrass. However, the *PWL4* ORF is functional as demonstrated by placing it under the control of the promoter of either *PWL1* or *PWL2* (Kang et al., 1995). The products encoded by members of the *PWL* gene family have the following common characteristics: (a) Their amino termini have features conserved among eukaryotic signal peptides for secretion. (b) A large number of glycine residues are well conserved and evenly distributed throughout the protein. (c) They are highly hydrophilic with many charged residues.

**Progress of cloning other *AVR* genes.** Several *AVR* genes have been identified following a cross of two rice pathogen field isolates, Guy11 and ML25 (Silué et al., 1992a; Silué et al., 1992b). RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers were used to identify linked DNAs to three independent *AVR* genes from this cross, *AVR1-Irat7*, *AVR1-Mednoï* and *AVR1-Ku86*. Markers linked to *AVR1-Irat7*

were used to perform a chromosome walk which resulted in the identification of a cosmid clone that when introduced into a strain virulent on rice cultivar Irat7, conferred avirulence (W. Dioh and M.-H. Lebrun, personal communication). A candidate gene for *AVR1-Irat7* in the cosmid appears different from other fungal avirulence genes identified to date as it is not a secreted small protein or peptide (M.-H. Lebrun, personal communication).

The *AVR1-MARA* gene of strain 4224-7-8 has also been localized between linked RFLP markers to a region of at least 90 kb on chromosome 2 of the consolidated RFLP map (Nitta et al., 1997). Most of this region is not represented in various chromosomal DNA libraries and parts are unclonable in *Escherichia coli*. To localize the *AVR1-MARA* within the region, a transformation-mediated locus replacement approach was taken (U. Gunawardena and M.J. Orbach, personal communication). A hygromycin phosphotransferase (hph) selectable marker was cloned between two fragments of the locus and used to transform avirulent strain 4224-7-8. Drug resistant transformants were screened for replacement of portions of the locus by the hph marker. This localized *AVR1-MARA* to a region of the locus of about 60 kb, which is currently being isolated using a combined long range and inverse PCR approach. Interestingly, the DNA content of this region is very AT rich (65-70% AT) in contrast to the *M. grisea* genome average of 45% AT. The virulent locus, *avr1-MARA*, has been isolated and contains two deletions that cover most of the locus, suggesting that virulence is due to the absence of some of these sequences (Mandel et al., 1997).

## Population Structure of Rice Pathogens

The race of a given *M. grisea* isolate is defined by its pattern of compatibility to a set of eight tester cultivars known as differential varieties (Ling and Ou, 1969). Race analysis, based on the compatibility to a set of test cultivars alone, is not sufficient however to allow for a comprehensive understanding of the genetic relationship among rice pathogens. Considering that more than one gene can control the compatibility/incompatibility to a specific cultivar, a similar pattern of compatibility to a set of test cultivars does not necessarily indicate a close genetic relationship. Analysis of the population structure of rice pathogens using genetic markers in addition to their virulence spectrum on a reference set of test cultivars has provided many new insights into the evolutionary dynamics of *M. grisea* in the field.

**Population structure at the genetic level.** A variety of markers have been used to determine the population structure at the genetic level (Shull and Hamer, 1994). The RFLPs generated with MGR586, known as MGR fingerprints, have been the most informative in elucidating the genetic diversity and variation of rice pathogens both within and between geographic regions. This is primarily because MGR586 is randomly distributed throughout the genome (Romao and Hamer, 1992) and RFLPs associated with MGR586 were sufficiently variable to distinguish strains but not so hypervariable that DNA fingerprint patterns were not generally maintained within an asexually reproducing population (Levy et al., 1991).

A large number of strains from various geographic locations, including the USA (Levy et al., 1991; Xia et al., 1993), Columbia (Correa-Victoria and Zeigler, 1993; Correa-Victoria et al., 1994; Levy et al., 1993), Europe (Roumen et al., 1997), India (Kumar et al., 1999), Korea (Han et al., 1993), and the Philippines (Chen et al., 1995; Zeigler et al., 1995), have been subjected to MGR586 fingerprinting analysis. Individual strains were grouped based on their genetic relatedness, which was estimated from pairwise comparisons between their fingerprints. These studies suggested that in most countries rice pathogens consist primarily of a few clonally (asexually) derived lineages that are genetically distinct.

In the United States for example, eight distinct clonal lineages were identified among 42 isolates representing the eight races collected over 30 years (Levy et al., 1991). The average similarity of MGR fingerprints among isolates within individual lineages exceeded 90%, while similarity between lineages ranged from 30-80%, suggesting that sexual or parasexual recombinations between rice pathogen isolates have occurred rarely, if at all, in the United States. If sexual or parasexual recombination had been occurring frequently, one would expect to see continuous variation in MGR fingerprints rather than the presence of a small number of discrete lineages. The relationship between race and genetic lineage within these USA field isolates exhibited a one-to-one relationship in six lineages (Levy et al., 1991). The remaining two lineages contained two races. However, a larger number of isolates collected from two rice fields in Arkansas revealed a slightly more complex picture than the previous study (Xia et al., 1993). Although no new lineage, different from the previously identified eight lineages, was identified, three of the four USA lineages detected in this study contain more than one race. A study with 41 isolates from five European countries showed the presence of five lineages (Roumen et al., 1997), and surveys of isolates from Colombia and the Philippines showed that more lineages (17 and 10, respectively) are present there. However, races within individual lineages typically differed by only one or a few compatible/incompatible interactions on the international differential cultivar set, suggesting that genetic lineage can still serve as a good indicator for the spectrum of virulence among rice pathogens in these countries (Chen et al., 1995; Levy et al., 1993).

**Recombination vs. clonal.** Several lines of evidence suggest that the role of sexual recombination in the population dynamics of rice pathogens in many regions has, if any, been minimal (Zeigler, 1998). These data include the presence of distinct genetic lineages within a single geographic region, the predominance of a single mating type in most areas, very low fertility and a high degree of karyotype diversity. However, it was pointed out that the existence of small scale parasexual recombinations between individuals could not be completely ruled out based on the results from MGR586 fingerprinting analyses (Zeigler et al., 1994). Subsequently, evidence for parasexual recombination between field isolates under unselected conditions has been reported (Zeigler et al., 1997). Another piece of evidence challenging the exclusive clonality of rice pathogens came from a survey of strains in the Indian Himalayas, a center of rice diversity (Kumar et al., 1999). Based on a high degree of diversity, dynamic fungal populations and the presence of hermaphroditic isolates, it was suggested that sexual recombination may have affected, to some extent, the structure of some populations in this region. The test for gametic disequilibrium using single- or low copy-RFLP markers failed to reject the null hypothesis of gametic equilibrium (i.e., random mating), hence supporting the possibility of sexual recombinations in this location (Kumar et al., 1999).

## Mechanisms Underpinning Race Variation

Although questions about the frequency of race changes in *M. grisea* remain unanswered, accumulated information on the genetic basis of its host specificity and population structure has started providing new insights into the question as to how race changes occur in some strains. Since the compatibility of interactions between *M. grisea* and rice follows the gene-for-gene model, the mutation of *AVR* genes or genes controlling the expression and function of *AVR* genes can cause race changes. Considering the mainly clonal population structure of rice pathogens in many countries, such a mechanism is likely to play a major role in race dynamics. Indeed, some of the *AVR* genes, including *AVR-Pita*, *AVR1-TSUY*, and *PWL2*, have been shown to be unstable (Valent and Chumley, 1994). Strains carrying one or more of these unstable *AVR* genes frequently produce spontaneous gain-of-virulence mutants. In addition, the pattern of race shifts in Arkansas strongly suggests that the mutation of certain *AVR* genes has caused the evolution of new races in the field.

**Mechanisms of mutations causing the gain of virulence on rice cultivar Yashiro-mochi.** Gain of virulence on cultivar Yashiro-mochi was often associated with the alteration or disappearance of telomeric restriction fragments linked to *AVR-Pita* (Valent and Chumley, 1994). Analysis of eight spontaneous virulent mutants of a laboratory strain, 4375-R-6, revealed that three different types of mutation were responsible for the gain of virulence on Yashiro-mochi (Valent and Chumley, 1994). Five of the mutants had a deletion, ranging in size from 100 bp to over 12.5 kb, that spans either a part of *AVR-Pita* or the whole gene plus its flanking sequences. Two mutants had a point mutation within the ORF of *AVR-Pita*. Insertion of a transposable element, Pot3 (Farman et al., 1996), in the putative promoter region of *AVR-Pita* was responsible for the gain of virulence in the remaining mutant.

**Multiple genetic changes associated with the gain of virulence on cultivar Tsuyuake.** Rice pathogen O-137, an isolate from China, is unable to infect a number of rice cultivars, including Tsuyuake and Yashiro-mochi, due to the presence of the *AVR* genes *AVR1-TSUY* and *AVR-Pita*, respectively (Valent and Chumley, 1991). However, this strain frequently produces spontaneous mutants that have become virulent on one or both of these rice cultivars. Similar to *AVR-Pita*, *AVR1-TSUY* is very closely linked to a telomere and mutates frequently (S. Kang and B. Valent, unpublished). Six independent spontaneous mutants of O-137 (designated as CP714, CP726, CP819, CP820, CP821 and CP822) that had become virulent on cultivar Tsuyuake were isolated following infection of Tusyuake with monoconidial cultures of O-137. All six mutants remained virulent on Sariceltik, a cultivar susceptible to O-137, and avirulent on weeping lovegrass, a host that is resistant to O-137. Thus, spontaneous genetic change(s) in O-137 specifically altered its compatibility to cultivar Tsuyuake without changing its compatibility to weeping lovegrass and cultivar Sariceltik.

A specific chromosome end of O-137 was found to be missing in all six mutants. The nature of mutation in this telomere appears complex, involving deletion and/or rearrangement. DNA fragments corresponding to this telomeric region, as well as any other loci that might be deleted in these mutants, were cloned by genomic subtraction. This technique specifically enriches for DNA fragments corresponding to loci present in one genome, but absent in the other (Straus and Ausubel, 1990). Genomic DNAs of two of the virulent mutants, CP821 and CP822, were used individually for subtracting DNA of O-137 to isolate DNA fragments deleted in each mutant. Southern analyses using these fragments as probes showed that in addition to deletion and/or rearrangement at a specific telomere, multiple deletion mutations were present at specific areas of the genome in these mutants. Some of the mutations were present in all six mutants, and both CP821 and CP822 contained at least one deletion mutation unique to each strain. Cloning and characterization of the sequences spanning the deletion breakpoints suggests that recombination between

homologous transposable elements is responsible for some of the deletions (S. Kang, unpublished).

**Gain of virulence toward weeping lovegrass.** The *PWL2* gene in strain 4375-R-6 was inherited from Guy11 through three genetic crosses (Sweigard et al., 1995). The descendants of Guy11, including 6043, 4360-17-1 and 4375-R-6, that are avirulent on weeping lovegrass, frequently produce spontaneous virulent mutants on weeping lovegrass (Sweigard et al., 1995). Analysis of the spontaneous mutants derived from these strains showed that the *PWL2* gene and more than 30 kb of flanking DNA sequences had been deleted. However, Guy11 failed to produce such mutants. The segregation pattern of *PWL2* in a cross between Guy11 and 2539 suggested that its stability in Guy11 is due to the presence of an additional gene conferring avirulence toward weeping lovegrass. Low-stringency hybridization showed that Guy11 contains two copies of *PWL2* (*PWL2-1* and *PWL2-2*) and a single copy of *PWL3* (S. Kang and J.E. Hamer, unpublished). Both *PWL2* genes are functional in conferring avirulence to weeping lovegrass, and 6043 inherited *PWL2-1*, but not *PWL2-2*, from Guy11. These *PWL2* genes and their flanking regions (> 2 kb to both directions) are identical in sequence, but are located on different chromosomes.

The genetic stability of the two identical, but unlinked, *PWL2* genes in Guy11 has been investigated using nine progeny from a cross between Guy11 and 2539 (Kang and Hamer, unpublished results): three of the progeny carried only *PWL2-1*, three progeny contained only *PWL2-2*, and three progeny contained both of them. Two monoconidial isolates from each progeny were individually cultured to produce conidia for an infection assay. Five pots of weeping lovegrass were sprayed with $1 \times 10^6$ conidia per pot. The total number of lesions in each pot was counted as a way of estimating the mutational frequency. Both *PWL2* genes were unstable, frequently producing spontaneous virulent mutants. Strains carrying *PWL2-1* produced 40-100 lesions per pot ($4 \times 10^{-5}$ to $1 \times 10^{-4}$), and those carrying *PWL2-2* produced 2-10 lesions per pot ($2 \times 10^{-6}$ to $1 \times 10^{-5}$). Strains containing both *PWL2* genes also produced lesions (one or two lesions per pot). The mutational frequency of each gene was not significantly different among progeny carrying the same gene, suggesting that the genetic background of the rest of the genome does not significantly affect the mutational frequency. However, since *PWL2-2* is more stable than *PWL2-1* by an order of magnitude, local chromosomal contexts around these *PWL2* genes appear to significantly influence their genetic stability. In these spray inoculum assays, fewer than half the spores sprayed onto the plants actually land on plant tissue. In addition, experiments with low inoculum densities suggested that only 1% of the pathogenic spores that actually land on plant tissue produce lesions (Sweigard et al., 1995). Considering these factors. the actual mutational frequency was probably two orders of magnitude higher than that listed above.

**Evolution of new races in Arkansas.** Analysis of new races that appeared in Arkansas in the 1980s provides some support for the hypothesis that the breakdown of blast resistance in the field may be caused by spontaneous mutations of *AVR* genes (Correll et al., 1998). The *M. grisea* population in the USA is primarily composed of eight genetic lineages (Levy et al., 1991), making it relatively easy to trace the origins of any new races. In Arkansas four genetic lineages (A, B, C, and D) have been identified in the contemporary *M. grisea* population. Three lines of evidence suggest that the new races IC-17 and IC-1k capable of infecting cultivars Newbonnet (released in 1983) and Katy (released in 1989), respectively, originated from race IG-1 (avirulent on both Newbonnet and Katy) in the resident population. Historical evidence (Lee, 1994) indicated that there had been a progression of races in Arkansas from IG-1 to IC-17 (virulent on Newbonnet), and then to IC-1k (virulent on both Newbonnet and Katy). The population data suggest that all three races (IG-1, IC-17, and IC-1k) belong to genetic lineage B. As described above, it has been demonstrated in the laboratory that some *AVR* genes undergo frequent spontaneous mutations in certain strains, resulting in the generation of new races. If this mechanism underpins the observed race shift in Arkansas, strains in genetic lineage B should be able to produce the two races, IC-17 and IC-1k in the laboratory. Indeed, it has been demonstrated that, using certain IG-1 isolates in genetic lineage B, the observed changes of race in the field can be experimentally reproduced. Spontaneous mutations affecting certain *AVR* genes occur more readily among isolates in this lineage.

**Mechanisms of genetic instability in genes other than *AVR* genes.** Genetic instability is not unique to *AVR* genes. The *BUF1* gene, which encodes an enzyme involved in the melanin biosynthetic pathway, spontaneously mutates at a high frequency in some rice pathogens, but the frequency of mutation differs significantly from strain to strain (Chumley and Valent, 1990). The *SMO1* gene, whose mutation causes abnormal spore morphology and reduces pathogenicity, also mutates frequently (Hamer et al., 1989). In contrast to these unstable genes, other *M. grisea* genes exhibit normal mutation frequencies (Valent, 1997), indicating that genetic instability may affect only certain parts of the genome and that the affected parts can vary from strain to strain. It has been proposed that recombination between homologous repetitive DNA elements might be responsible for frequent mutations and chromosomal rearrangements (Talbot et al., 1993; Valent and Chumley, 1991). Depending on the relative orientation and position of the repetitive elements that are recombined, homologous recombination

between these elements could cause deletion, inversion, duplication, or translocation. The *AVR-Pita* gene and many members of the *PWL* gene family are closely associated with certain types of repetitive DNA. No repetitive DNA element is present within 20 kb of a stable *BUF1* allele in WGG-FA40, but a rice pathogen carrying an unstable allele appears to have several types of repetitive DNA elements around the locus (M. Farman, personal communication).

## Conclusions

Considering the importance of resistance breeding in controlling rice blast disease, addressing the question as to how virulence changes arise, resulting in the generation of new races, is critical for sustainable rice cultivation, especially in many developing countries in which chemical control is often economically impractical. The practical significance of investigating the question is not limited to the rice blast system, since the breakdown of disease resistance seems to be a common problem among other crops (Mundt, 1990; Mundt, 1991). A better understanding of the mechanisms underlying the evolution of new races of pathogens could theoretically allow a survey of pathogen populations for their potential for the evolution of new races, which will assist us in minimizing the incidence of rapid breakdown of disease resistance by assisting the identification of durable *R* genes for breeding and/or engineering. Such information will also facilitate the deployment of disease resistance in a way that minimizes the evolution of new races.

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