Genetic Diversity of the Rice Blast Fungus in a Disease Nursery in Colombia

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ABSTRACT

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We determined DNA fingerprints and pathotypes on international differentials for 151 isolates of the rice blast fungus *Pyricularia grisea* that were collected from 15 rice cultivars at a blast disease breeding nursery in Santa Rosa, Colombia. The pathogen population expressed an extraordinarily high number (39) of pathotypes. DNA fingerprinting, using the *P. grisea* repetitive DNA sequence MGR586, identified 115 haplotypes that were partitioned into six discretely distinct genetic lineages. The mean genetic similarity within lineages was high, ranging from 92 to 98%. The mean similarity between lineages ranged from 37 to 85%. Each lineage was associated with a specific subset of cultivars and expressed a generally nonoverlapping subset of pathotypes. Typically, the pathotypes within a lineage had a closely related infection spectrum, differing by single compatibility differences on a particular subset of the international differentials. The cultivar and pathotype associations indicated that the lineages express historically divergent virulence spectra. MGR-DNA fingerprint analysis also deciphered the lineage associations of 31 isolates whose pathotypes could not be scored, i.e., the isolates were avirulent on all international differentials. MGR-DNA fingerprint analysis provides the genealogical framework for evaluating pathogen variation that is essential for understanding disease epidemiology and pathotype evolution. This kind of analysis may be particularly useful for selecting germ plasm sources for more durable blast-resistance breeding.

Additional keywords: disease resistance, *Magnaporthe*.

Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *Pyricularia grisea* Sacc. (teleomorph: *Magnaporthe grisea* Barr; [23]). *P. grisea* is noted for expressing a large number of virulence forms or pathotypes, especially in rainfed, upland areas where the environment is most favorable for epidemic development (20). For most rice-growing areas in Asia, Africa, and South America, rice blast disease management relies on the frequent introduction of new resistant rice cultivars. However, blast resistance in these regions is rarely effective for more than 2–3 years (1,3,4). It is not known whether the ability of the pathogen to overcome resistant cultivars reflects shifts in the frequency of formerly rare pathotypes or the frequent occurrence of genetic changes to new virulence forms or a combination of both phenomena. Consequently, detailed genetic information on population structure is essential for understanding the virulence dynamics of the pathogen and devising more effective strategies to reduce the impact of rice blast disease.

In the field, *P. grisea* reproduces asexually (18,21). Although the sexual stage (teleomorph) can be demonstrated in the laboratory (5,26), the vast majority of field isolates are infertile. Thus, a population of the rice blast fungus may be viewed as an aggregate of clones, and clonal lineages, each with a particular spectrum of virulence characteristics. The sole tool for characterizing this virulence diversity has been a pathogenicity assay. The assay sorts isolates into pathotypes ("races") by the symptoms they cause on each of a set of differentially resistant rice cultivars. Assay results can be strongly influenced by host age and condition, inoculum quality, and environmental variations during the assay (11). Furthermore, some isolates may cause a range of intermediate infection responses by one or more of the differentials, making assay scoring both difficult and subjective. Although an international set of eight differential cultivars has been established to standardize assays (14), it has become clear that this set cannot decipher all the pathotype diversity that occurs in many rice-growing regions (1,3,4). Finally, because the assay is essentially phenotypic, the genetic relatedness of pathotypes and the virulence diversities of clonal lineages cannot be ascertained directly.

Recently, a DNA probe was developed that hybridizes reliably and specifically with DNA of the rice blast fungus (8). The probe consists of a cloned fragment of repeated DNA obtained from a rice blast fungus genome and is called MGR586 (previously referred to as pCB586 [9]). This probe detects genomic profiles of about 50–60 *Eco*RI restriction fragment length polymorphisms (RFLPs) among isolates of rice blast pathogens. In contrast, the probe typically detects only one to a few restriction fragments in *P. grisea* isolates that infect other grasses. Genetic mapping has demonstrated that MGR586-RFLPs are dispersed on all chromosomes of rice pathogens and segregate as genetic loci (9,10,22). In addition, genetic linkage has been demonstrated between one such RFLP and a cultivar-specific avirulence gene (25). Thus, an MGR586-RFLP profile characterizes a large multilocus genotype, informationally equivalent to the composite RFLP haplotype of the flanking regions of 50–60 dispersed, single-copy genomic sequences, that can serve as a genealogical index among rice blast fungus isolates. We have referred to these haplotypes as MGR-DNA fingerprints and use them to define the population structure and the associated organization of pathotype variation in the rice blast pathogen (13).

MGR-DNA fingerprinting was first used to examine the genetic diversity of a historical sampling of the rice blast pathogen population in the United States (13). The sample consisted of 79 isolates collected from rice fields throughout the southern United States rice belt from 1959 to 1988. Collectively, the isolates expressed the eight most frequently observed U.S. pathotypes. MGR-DNA fingerprints partitioned the isolates into eight discretely distinct

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genetic lineages. Each lineage was associated with only one or two pathotypes, most of which were lineage specific. These results implied that rice blast disease in the United States has been caused by an aggregate of distinct clonal lineages of the pathogen, with each lineage having modal virulence characteristics that appear to have been maintained over the 30-yr sampling period. More generally, these results documented the utility of genealogical analysis for resolving the pathogen's population structure and the phylogenetic routes of pathotype evolution. However, reports of dramatically higher levels of pathogenic diversity in other rice-growing regions (3,19) suggested that the clonal population structure and the close relationship between MGR-DNA fingerprint lineages and pathotype observed in the United States might not be applicable worldwide.

In this study, we used MGR-DNA fingerprinting and pathotyping to examine the population structure and associated virulence properties of the rice blast fungus in an ecological setting that contrasts sharply with the setting in the United States, namely, a blast disease "hot spot" used as a resistance-breeding nursery. This paper is the third in a series of ongoing studies of pathogen diversity and resistance gene deployment at this site (3,4). The nursery is part of the Santa Rosa Experimental Station, located in a blast endemic, upland region of central Colombia. The Santa Rosa rice fields are managed to favor blast disease development and maximize pathogen diversity. In this 30-ha area, ideal climate conditions and continuous plantings of a diverse set of susceptible cultivars support chronic, epidemic levels of blast disease over the 270-day growing season. The local blast fungus population contains more than 40 pathotypes (3) or more than five times that typically found throughout the United States.

The primary issue we addressed is whether the much higher levels of virulence diversity at Santa Rosa reflect a concomitant increase in lineage diversity or a more complex organization of lineage-pathotype association. Our results demonstrated that the virulence diversity in the Santa Rosa population was organized hierarchically into six distinct clonal lineages, each with a generally nonoverlapping cultivar range and an associated set of several related pathotypes. As a first study of the genetic organization in a pathotype-rich population of *P. grisea*, our analysis also provided us with a glimpse of the possible routes of pathotype variation and allowed us to speculate on breeding strategies that may be more effective for controlling blast disease. Finally, our results demonstrate that the genealogical analysis of the population structure of the rice blast fungus, afforded by MGR-DNA fingerprinting, can provide meaningful information on the dynamics of the pathogen in a variety of rice-growing areas, including those under high disease pressure.

MATERIALS AND METHODS

Collaborative arrangements. The collection, isolation, and pathotyping of rice blast materials were conducted at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. MGR-DNA fingerprinting was conducted at Purdue University, West Lafayette, IN. Data analysis and interpretation were conducted jointly.

Cultivars and fungal strains. Collections of the fungus were taken from 15 rice cultivars that, with the exception of Ceysvoni, Fanny, Zenith, and IR 42, have been grown commercially in Colombia over the last 20 years. Isolates were obtained from lesions resulting from field infections on plants growing at CIAT's Santa Rosa Experimental Station, located approximately 20 km east of Villavicencio, Colombia (elevation 310 m, 4° N latitude, 73° W longitude). The lesions were collected during the period from May 1988 to October 1990. Isolate cultures were developed by placing thoroughly washed leaf pieces bearing a single lesion on a water-agar plate and subsequently transferring a small sector of the growing mycelium to plates containing rice polish agar. In most cases, monoconidial isolates were subsequently obtained from each single-lesion culture; the monoconidial isolates are noted hereafter as a numbered version of the original lesion culture. The identity of isolates as *P. grisea* was confirmed by light microscopy. Samples of each isolate were stored on dried filter papers (5) and mailed to Purdue University under APHIS permits for further analysis. Isolates were reactivated by placing slivers of filter paper on oatmeal-agar plates. A total of 151 isolates from field lesions were analyzed.

Isolate pathotyping. Pathotypes were determined by a pathogenicity assay with the eight cultivars of the international differential set. For reasons discussed later, a test of the ability of each isolate to cause disease on the cultivar of origin under greenhouse assay conditions was conducted concurrently. Isolates that did not infect any of the differentials also were inoculated on other cultivars to confirm their pathogenicity for rice. Assay procedures were those used by F. J. Correa-Victoria and R. S. Zeigler (3). Ten seedlings of each differential host were grown to the three- to four-leaf stage (18–21 days after planting) in 15-cm plastic pots and then spray-inoculated with aqueous conidial suspensions containing a minimum of 10^5 conidia per milliliter. The seedlings were maintained in a 100% humidity chamber placed in a screenhouse with a 12-h photoperiod per day and 20–30 C ambient temperatures. The seedlings were fertilized at planting, 1 wk later, and 1 day before inoculation with equal amounts of an ammonium sulphate solution that provided an equivalent of 180 kg of N per hectare. Host responses were scored visually for the average percentage of leaf area covered by lesions and for lesion type 7 days after inoculation. Host response was judged as compatible (+) if the majority of seedlings exhibited fully sporulating lesions (lesion type 4 or greater [12]) or if the average percentage of leaf area affected was 5% or greater with at least some sporulating lesions present. All other responses were judged as incompatible (−). The generally compatible cultivar, Fanny, was included in each assay as a check for infection efficiency. Pathotypes were determined only from assays in which Fanny seedlings developed fully sporulating lesions. Pathotypes were defined by the nomenclature of Ling and Ou (14).

DNA isolation and MGR-DNA fingerprinting. DNA was isolated as previously described (6,24). DNA concentrations were determined in a TKO 100 minifluorometer (Hoeffer, San Francisco, CA) with the DNA-specific fluorescent dye Hoechst 33258 according to manufacturer's directions.

One microgram of chromosomal DNA from each sample was digested with *Eco*RI and fractionated by electrophoresis on 0.8% agarose gels. A reference standard, consisting of an *Eco*RI digest of DNA from an *M. grisea* laboratory strain was cofractionated on all gels to facilitate direct comparisons between electrophoresis experiments (22). This reference strain exhibited an MGR-DNA profile of 30 RFLPs of known length ranging from 0.9 to 38.2 kb under the electrophoretic conditions (Fig. 1, lane 1). The fractionated DNA was blotted to Hybond-N membranes (Amersham Corp., Arlington Heights, IL) according to the manufacturer's suggestions. The DNA blots were probed with radioactively labeled MGR subclone MGR586 (9). MGR-DNA fingerprints were visualized by autoradiography after high-stringency washes as previously described (13).

Fingerprint comparisons. MGR-DNA fingerprints were scored visually. The fingerprints were first sorted into groups on the basis of obvious similarities between RFLP profiles. In practice, about one-third of the isolates were subsequently reanalyzed on gels with isolates typical of each profile group to confirm isolate associations. Fingerprint variation in all isolates was quantified by visually scoring the presence and absence of all restriction fragments in the 1.5- to 18.2-kb size range. (Examples of the raw scores of fingerprint variation are available on request from M. Levy.)

Variation within a profile group was evaluated from pairwise comparisons of the proportion of shared RFLPs between fingerprints, i.e., two times the number of shared fragments divided by the total number of fragments in the fingerprints being compared. This method was equivalent to calculating Nei and Li's (16) index of genetic similarity (S_{xy}) for RFLP comparisons. Isolate relationships within a profile group were evaluated by converting the similarity values to their distance-value complements ($D_{xy} = 1 - S_{xy}$), analyzing the distance-value matrix with

a phenetic cluster analysis by the unweighted pair group method for arithmetic averages (UPGMA), and plotting the results in a phenogram. Statistical confidence intervals for the branching arrangements of phenograms were determined by a bootstrap procedure (7) using 1,000 repetitive samplings of the RFLP data and computing the 95% range of the branch point values in the resultant UPGMA phenograms (modified from [17]). The confidence intervals were used to delimit group membership. All isolates occupying phenogram branches related by overlapping confidence intervals (and that also included some 100% similarity values) were judged to be not significantly different from one another. In other words, the isolates within such a group exhibited a continuous distribution of variation with no significant subclustering. Consequently, the group was considered to represent a single genetic lineage.

The relationships between groups were assessed as previously described but were based on the consensus MGR-DNA fingerprint (all RFLPs present in 50% or more of the isolates) of each group. We used a consensus fingerprint for each lineage to avoid the prohibitively long computational time for bootstrap analyses of the entire set of 151 isolates. This method was justified by the small range of RFLP variation and the absence of significant subclustering within groups and the large distances (discontinuous variation) between groups. All groups were significantly different from each other (i.e., confidence intervals did not include 100% similarity values) and were considered to represent distinct genetic lineages.

All computations and statistical analyses were performed by SAS (version 6) programs (SAS Institute, Cary, NC).

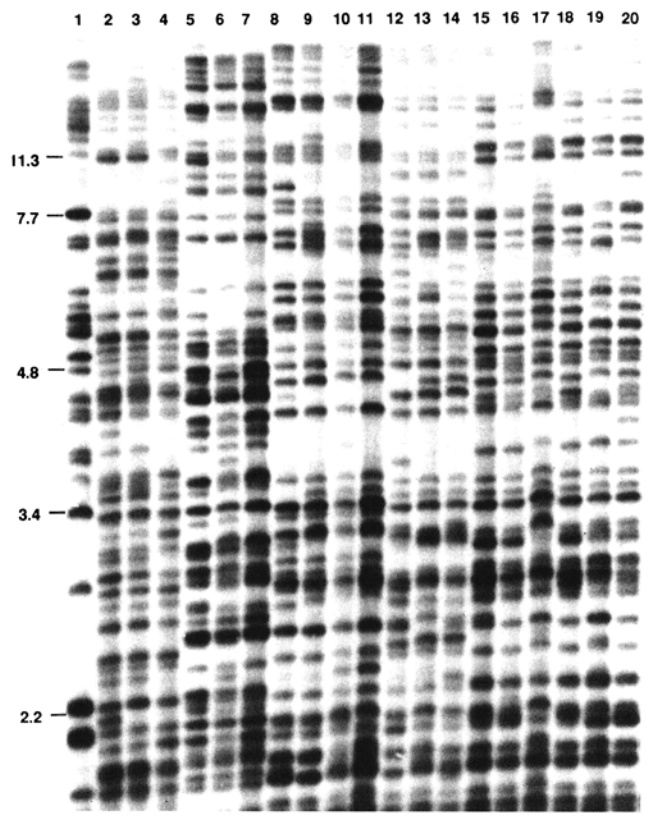

Fig. 1. MGR-DNA haplotypes of representative Santa Rosa isolates. The cultivar origin (pathotype) of isolates by lane number are: 1, laboratory reference standard; 2, Cica 9 (IA-128); 3, Cica 9 (IA-128); 4, Linea 2 (II-1); 5, Metica 1 (IC-32); 6, Metica 1 (II-1); 7, Fanny (IC-16); 8, Ceysvoni (IA-126); 9, Metica 1 (IG-2); 10, Metica 1 (II-1); 11, Zenith (IH-1); 12, Cica 8 (IH-1); 13, IR 42 (IE-5); 14, IR 42 (IF-2); 15, Ceysvoni (IA-103); 16, Cica 4 (IA-102); 17, Fanny (IA-104); 18, Oryzica 1 (IA-103); 19, Oryzica 2 (II-1); and 20, Oryzica Llanos 5 (IA-33). Lineage associations are: lanes 2–3 = SRL-1; 4 = SRL-2; 5–7 = SRL-3; 8–11 = SRL-4; 12–14 = SRL-5; and 15–20 = SRL-6.

RESULTS

Pathotype diversity. The primary goal of this study was to examine the relatedness of a collection of *P. grisea* isolates that represent a broad collection of pathotypes from a small geographical area. A total of 39 pathotypes were detected among the 151 isolates (Table 1). Most pathotypes occurred only in one to two isolates in the sampling. Typically, multiple (two to seven) pathotypes were recovered from each cultivar; the exceptions were IR 22, Linea 2, and Oryzica Llanos 4, which collectively provided only five isolates (Table 2). The most frequently observed pathotype (31 isolates or 21% of the sampling) was II-1, which was recovered from nine cultivars (Table 2). II-1 isolates have uncharacterized pathogenicity traits because they are avirulent on all international differentials. However, infection assays on certain Colombian cultivars showed that II-1 isolates had different infection spectra (Table 3).

Although a systematic sampling strategy was not employed in the collection of the isolates, the 39 pathotypes observed in this sampling were typical of the blast population at Santa Rosa (3). This diversity at a single site is strikingly greater than that observed in the United States, where across the six-state southern region of rice cultivation only about 20 international races have been detected since the 1950s and only eight of these have been considered common (15).

TABLE 1. Pathogenicity variation among Santa Rosa, Colombia, isolates of *Pyricularia grisea*

Pathotype	No. of isolates	International differentials[a]							
		A	B	C	D	E	F	G	H
IA-7	2	+	+	+	+	+	−	−	+
IA-33	2	+	+	−	+	+	+	+	+
IA-67	1	+	−	+	+	+	+	−	+
IA-71	3	+	−	+	+	+	−	−	+
IA-78	1	+	−	+	+	−	−	+	−
IA-84	1	+	−	+	−	+	+	−	−
IA-92	1	+	−	+	−	−	+	−	−
IA-96	1	+	−	+	−	−	−	−	−
IA-97	5	+	−	−	+	+	+	+	+
IA-99	10	+	−	−	+	+	+	−	+
IA-101	5	+	−	−	+	+	−	+	+
IA-102	6	+	−	−	+	+	−	+	−
IA-103	5	+	−	−	+	+	−	−	+
IA-104	5	+	−	−	+	+	−	−	−
IA-110	8	+	−	−	+	−	−	+	−
IA-124	1	+	−	−	−	−	+	−	−
IA-126	1	+	−	−	−	−	−	+	−
IA-128	10	+	−	−	−	−	−	−	−
IC-16	2	−	−	+	+	−	−	−	−
IC-20	2	−	−	+	−	+	+	−	−
IC-28	2	−	−	+	−	−	+	−	−
IC-32	3	−	−	+	−	−	−	−	−
ID-1	2	−	−	−	+	+	+	+	+
ID-6	1	−	−	−	+	+	−	+	−
ID-7	3	−	−	−	+	+	−	−	+
ID-8	6	−	−	−	+	+	−	−	−
ID-15	1	−	−	−	+	−	−	−	+
ID-16	4	−	−	−	+	−	−	−	−
IE-1	2	−	−	−	−	+	+	+	+
IE-4	4	−	−	−	−	+	+	−	−
IE-5	1	−	−	−	−	+	−	+	+
IE-6	1	−	−	−	−	+	−	+	−
IE-8	5	−	−	−	−	+	−	−	−
IF-1	1	−	−	−	−	−	+	+	+
IF-2	1	−	−	−	−	−	+	+	−
IG-1	1	−	−	−	−	−	−	+	+
IG-2	5	−	−	−	−	−	−	+	−
IH-1	5	−	−	−	−	−	−	−	+
II-1	31	−	−	−	−	−	−	−	−
Total	151								

[a] Letters A–H designate the international blast differential rice cultivars Raminade 3, Zenith, NP 125, Usen, Dular, Kanto 51, Sha-tia-tsao, and Caloro, respectively (12,14).

Fingerprint diversity. MGR-DNA fingerprints among Santa Rosa isolates contained an average of 50 (ranging from 41–57) resolvable *Eco*RI fragments. A total of 115 MGR-DNA fingerprints were detected among the 151 isolates. However, many isolates expressed such highly similar profiles that all fingerprints were sorted visually into five basic groups (Fig. 1). Examples of the five groups that were initially recognized are arranged in numerical order in Figure 1 as follows: lanes 2–4, 5–7, 8–11, 12–14, and 15–20. When fingerprint similarities within groups were calculated and the variation analyzed by bootstrap analysis, group 1 isolates were separated into two significantly different and internally cohesive clusters. No significant subgrouping was detected by bootstrapping in the other groups, bringing the total number of statistically distinct MGR-defined groups to six. Subsequent analysis of the variation between consensus fingerprints of each group showed that all groups were significantly different from each other. Each group represented a distinct genetic lineage. Hereafter, the lineages is designated as SRL-1 through -6.

Isolates within each lineage expressed very similar fingerprints (Fig. 2). Average similarities within lineages ranged from a maximum of 98% in SRL-2 (Fig.2A) to a minimum of 92% in SRL-4 (Fig. 2D). In contrast, the lineages were well differentiated from each other (Fig. 3). The maximum consensus similarity between lineages was 85% (SRL-1 versus SRL-2), the minimum was 37% (SRL-1 and SRL-3 versus SRL-4; SRL-2 versus SRL-3), and the average among all lineages was 49%. All lineages were significantly different from one another at the $P < 0.001$ confidence level, i.e., 1,000 bootstrap resamplings of the consensus fingerprints never produced a case of 100% similarity in pairwise comparisons between lineages. The bootstrap analysis of the consensus MGR phenogram also indicated that, in terms of the degree of difference between lineages, there were three significant ($P < 0.05$) higher order branch points: one separating lineages SRL-4, -5, and -6 from the others, another separating SRL-3 from the preceding lineages, and a third separating SRL-1 and -2 from all other lineages.

Lineage-cultivar-pathotype associations. A summary of pathotype diversity and host cultivar specificity among the six MGR-defined lineages is given in Table 2. In these data, we included a measure of the virulence of isolates on their host of origin to more accurately assess isolate-cultivar compatibility. Many isolates (91) failed to reinfect the host of origin under greenhouse assay conditions (Table 2). We infer that this means the original field infections represent opportunistic encounters fostered by the extremely favorable conditions for disease development at Santa Rosa and do not reflect full compatibility. For example, at Santa Rosa, cultivar Oryzica Llanos 5 is infected by isolates from SRL-2 and -6. None of these isolates are capable of reinfection under greenhouse assay conditions. Furthermore, under cultivation, Oryzica Llanos 5 has proven to be reliably blast resistant since 1988. The occurrence of opportunistic blast infections is not uncommon at the Santa Rosa disease nursery, but such infections are infrequent in cultivated fields and are generally associated with plant wounding (3).

Taking into account the reinfectivity data, each MGR-DNA fingerprint lineage was composed of isolates that infected a specific and limited subset of cultivars and expressed a specific subset of pathotypes. The degree of cultivar specificity and pathotype diversity varied between lineages. For example, *P. grisea* isolates belonging to SRL-1 were found exclusively on cultivar Cica 9 and expressed only two pathotypes. Broader sampling of *P. grisea* isolates from Cica 9 planted throughout Colombia has confirmed the generality of this association (data not shown). The specificity of SRL-1 isolates for Cica 9 also was confirmed by greenhouse assay (3). Strong host specificity also was observed for SRL-2 isolates; all had the pathotype II-1 on cultivar Linea 2 (Tables 2 and 3). Some SRL-2 isolates infect Cica 9 under greenhouse assay conditions (Table 3; F. J. Correa-Victoria, *unpublished data*). Lineages SRL-3, -4, and -5 were each compatible with two cultivars and expressed a total of four, eight, and 11 pathotypes, respectively. SRL-6 was compatible with a broad range of cultivars and expressed a total of 23 pathotypes.

Broader sampling in the rest of Colombia has demonstrated that SRL-4, -5, and -6 are the most frequently encountered rice blast fungus lineages (J. Manry, F. J. Correa-Victoria, and M. Levy, *unpublished data*). Their high frequencies may be a reflection of cultivar-specific compatibility. Cica 8 (compatible with SRL-4 and -5) and Oryzica 1 (compatible with SRL-6) are the most widely planted rice varieties in Colombia. Thus, although the sampling of the Santa Rosa nursery analyzed in this report was not systematic (e.g., 37 isolates came from cultivar Ceysvoni and three isolates came from cultivar Linea 2) and concentrated

TABLE 2. Pathogenic organization of the rice blast fungus population at Santa Rosa, Colombia

Lineage	Source cultivar	No. of isolates	No. reinfecting[a]	Pathotypes expressed
SRL-1	Cica 9	10	10	IA-128, II-1
SRL-2	Linea 2	3	3	II-1
	Oryzica Llanos 5	8	0	II-1
SRL-3	Metica 1	6	2	IC-28, IC-32, II-1
	Fanny	1	1	IC-16
SRL-4	Ceysvoni	17	0	IA-78, IA-110, IA-126, IA-128, IG-2, II-1
	Cica 8	2	1	IH-1, II-1
	Metica 1	6	3	IG-2, II-1
	Oryzica 1	1	0	IH-1
	Zenith	3	0	IG-1, IH-1, II-1
SRL-5	Ceysvoni	6	0	IC-20, ID-8, IE-8, II-1
	Cica 4	1	0	IE-4
	Cica 6	2	2	IE-4, IE-6
	Cica 8	6	5	IE-4, IE-8, IH-1, II-1
	IR 42	5	0	IE-1, IE-5, IF-1, IF-2
	Metica 1	1	0	II-1
SRL-6	Ceysvoni	14	7	IA-7, IA-67, IA-71, IA-99, IA-101, IA-103, ID-8
	Cica 4	7	2	IA-101, IA-102, IA-124, IE-4, II-1
	Cica 6	3	3	IA-84, IA-92, IA-96
	Cica 8	1	0	IA-99
	Fanny	9	7	IA-97, IA-99, IA-101, IA-104, IC-16, ID-8
	IR 22	1	1	IA-97
	Metica 1	2	2	IA-104
	Oryzica 1	7	6	IA-99, IA-103, IA-104, ID-6, ID-8
	Oryzica 2	10	2	IA-97, IA-99, ID-15, ID-16, II-1
	Oryzica Llanos 4	1	0	IA-102
	Oryzica Llanos 5	16	0	IA-33, IA-102, ID-7, ID-8, ID-16, IE-8, II-1
	Zenith	2	0	ID-1

[a] To further evaluate cultivar specificity, the number of isolates capable of causing disease on their source cultivar also was determined by greenhouse seedling assay.

TABLE 3. Cultivar- and lineage-specific pathogenicity of isolates having an II-1 pathotype

Isolate[a]	Lineage	Cultivar				
		Cica 9	Linea 2	Metica 1	Cica 8	Ceysvoni
Cica 9 (4)	SRL-1	+	−	−	−	−
Linea 2 (15)	SRL-2	+	+	−	−	−
Metica 1 (33-20)	SRL-3	−	−	+	−	−
Metica 1 (33-2)	SRL-4	−	−	+	−	−
Cica 8 (61)	SRL-5	−	−	−	+	−
Ceysvoni (50-1)	SRL-6	−	−	−	−	+

[a] Isolate names indicate the cultivar of origin followed by the number of the lesion or monoconidial version tested.

on isolates from Colombian commercial varieties, the lineage-cultivar relationships we identified are consistent in other areas of Colombia. The results strongly suggest that MGR-DNA fingerprint lineages are reliable indicators of cultivar compatibility. More systematic sampling of plantings in the Santa Rosa nursery over the next few years will permit us to monitor the evolutionary dynamics of this population in more detail.

A total of 39 pathotypes were present in our sampling of the Santa Rosa nursery. The pathotypes were not distributed randomly among the six lineages but rather were highly lineage specific (Table 2). When all 151 isolates are considered, 33 of the 39 pathotypes (85%) were lineage specific. When nonreinfecting isolates are excluded from the data set, 21 of the 23 remaining pathotypes (91%) were lineage specific. Considering the two nonspecific pathotypes, one of these was recovered only from Cica 8 (IH-1) and occurred in lineages SRL-4 and -5. The remaining pathotype that was not specific to a lineage was II-1, which was found in each lineage. The virulence characteristics of this pathotype are not decipherable on the international differential set. However, greenhouse assays of II-1 isolates that can reinfect their host of origin have shown that their virulence spectra on Colombian cultivars is strongly lineage- and cultivar-specific (Table 3).

In summary, the lineage-cultivar-pathotype associations detected in these results indicate that the organization of the Santa Rosa rice blast population may be viewed as hierarchical. The population consists of a small set of genetic lineages, each with a largely nonoverlapping virulence spectrum, in terms of cultivar specificity and pathotype variation. This organization would not be revealed by pathotyping alone.

DISCUSSION

In this study, we found that MGR-DNA fingerprinting can be used to study complex rice blast fungus populations in environments exhibiting high disease pressure and a diverse array of

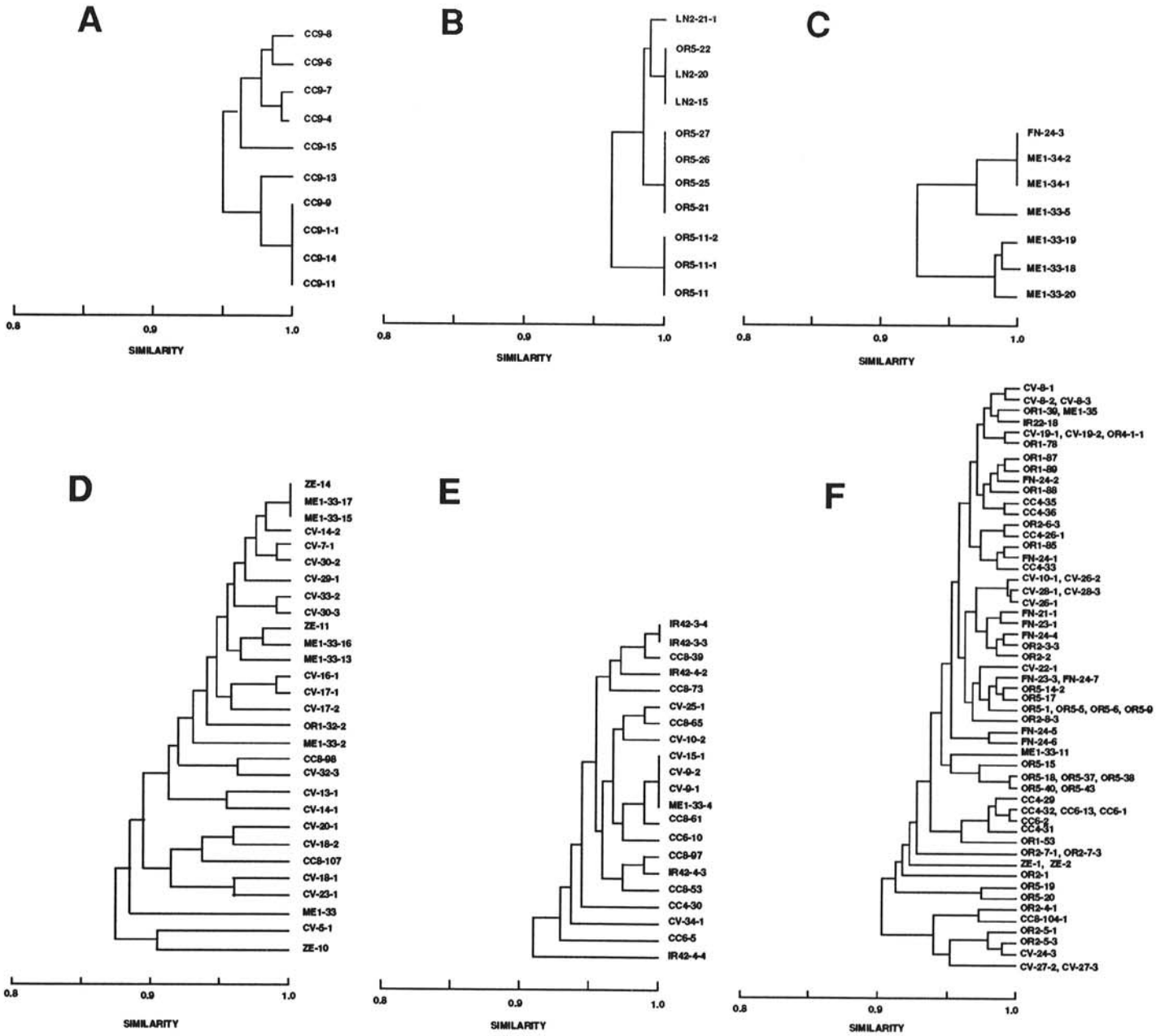

Fig. 2. Phenograms of MGR-DNA haplotype variation within lineages. Lineages SRL-1 through -6 are represented in **A–F**, respectively. Isolates are noted by cultivar name/abbreviation and plant number, with culture-subculture numbers following when pertinent. Cultivar abbreviations: CV = Ceysvoni; CC4 = Cica 4; CC6 = Cica 6; CC8 = Cica 8; CC9 = Cica 9; FN = Fanny; IR22= IR 22; IR42 = IR 42; LN2 = Linea 2; ME1 = Metica 1; OR1= Oryzica 1; OR2 = Oryzica 2; OR4 = Oryzica Llanos 4; OR5 = Oryzica Llanos 5; and ZE = Zenith.

cultivar plantings. Our sampling of the rice blast disease nursery at Santa Rosa, Colombia, contained six well-differentiated genetic lineages. Each lineage infected a limited number of cultivars and expressed an essentially specific array of pathotypes. These results support the view that cultivar specificity and pathotype evolution at the site has developed within constraints imposed by the genetic background of each lineage. Given the lineage-cultivar specificity detected (Table 2) it remains possible that sampling infections on a even more diverse set of cultivars might reveal the presence of more pathogen lineages at the site. Experiments to test this hypothesis are underway. However, the organization of the pathogen's virulence diversity currently revealed at this site allows us to discuss several key points relating to the use of blast nurseries, pathotype evolution, and disease resistance breeding.

Rice blast nurseries are used routinely in many rice-growing areas for testing the resistance of breeding lines and new cultivars. An accurate definition of pathogen diversity at these sites is crucial to understanding the meaning of observed cultivar responses. Clearly, genetic lineages of the rice blast fungus that do not reside in the nursery population pose a potential threat to cultivars that are resistant in nursery trials and may be viewed as one source of cryptic error in resistance breeding (2,3).

The inclusion of genetic lineage identification for definition of the structure of rice blast pathogen populations in these nurseries may provide a more reliable estimate of the durability of cultivar resistance than only the consideration of pathotype or inferred virulence gene diversity. Our results suggest that the presence or absence of a particular pathotype in a region may not accurately predict the disease vulnerability of a given cultivar. For example, according to our results the rice cultivar Cica 9 will be vulnerable to severe infection in any Colombian region where isolates of SRL-1 are present, regardless of whether pathotype IA-128 is detected. IA-128 is one of the SRL-1–associated pathotypes found on Cica 9 (Table 2). Isolates with the same pathotype but from lineage SRL-4 are not capable of infecting Cica 9 (3). Additionally, the SRL-6 lineage contains numerous pathotypes, several of which are expressed by isolates infecting cultivars Ceysvoni and Oryzica 1 (Table 2). Again, we predict that these cultivars are likely to be vulnerable to blast infection in any Colombian region where isolates of lineage SRL-6 occur, regardless of the particular pathotypes that may be detected.

The environment and management of the Santa Rosa disease nursery promotes a chronically high level of disease that also results in a high level of pathotype variation. Viewing this polymorphism in combination with genetic lineage information provides a glimpse of the dynamics of pathotype evolution at the site. First, many pathotyped isolates proved to be nonpathogenic when inoculated on their source cultivar under greenhouse assay conditions. These apparently opportunistic infections were clearly caused by members of the resident rice pathogen genetic lineages present at Santa Rosa. They do not appear to be an artifact because all lineages were not generalized on all hosts, i.e., noninfecting isolates demonstrated some specificity (Table 2). Thus, we speculate that opportunistic infections may reflect partial compatibility on their field host cultivars. As previously suggested (3), these infections may indicate the potential for future cultivar breakdown by a specific lineage. This hypothesis can be confirmed by monitoring the eventual breakdown of the cultivar Oryzica Llanos 5 under field conditions. Our results (Table 2) predict that, among the lineages currently present in the nursery, virulent pathogens would most likely arise from within lineages SRL-2 and/or SRL-6. Alternatively, this cultivar may become infected by lineages that were not present at Santa Rosa. Future experiments can be aimed at determining the significance of opportunistic infections detected at Santa Rosa.

A second feature of the Santa Rosa population revealed by combining pathogenicity assays and genetic lineage analysis is that each lineage expressed a specific subset of the large number of pathotypes present. Moreover, the pathotypes in each subset appear to be closely related in virulence spectrum. For example, the SRL-6 isolates recovered from Cica 6 collectively express three pathotypes, IA-84, IA-92, and IA-96. These pathotypes form a small network of virulence forms distinguished from one another by single differences in compatibility on the international differentials (Table 1). Similar pathotype networks are apparent in all lineages. A more extensive analysis of pathotype relatedness within lineages is in progress and will be reported elsewhere. However, the strongly hierarchical lineage-cultivar-pathotype associations revealed at Santa Rosa suggests that, although lineages may have an aggregate virulence phenotype containing numerous variants, their constituent pathotypes evolve under limits defined by the genetic background of the lineage. The genes that ultimately determine cultivar specificity within a specific lineage await characterization.

Previous analysis of the Santa Rosa rice blast population by pathogenicity assay found strong cultivar specificity for certain isolates. For example, Correa-Victoria and Zeigler (3) noted that Cica 9 was heavily infected in the disease nursery but that the frequency of isolates that could infect this cultivar in greenhouse assay was extremely low. In general, only isolates recovered from Cica 9 had the capacity to reinfect Cica 9 under greenhouse assay conditions. A genetic interpretation of this phenomenon is now provided by the MGR-DNA fingerprinting. All the isolates we recovered from Cica 9 belong to lineage SRL-1. Other lineages also showed strong cultivar specificity at Santa Rosa (Tables 2 and 3).

Correa-Victoria and Zeigler (3) have further suggested that useful combinations of resistance genes might be generated from crossing cultivars that show complementary, but strongly specific, susceptibilities to narrow components of a resident pathogen population. As indicated in this study, identifying such cultivars would be expedited by characterizing the lineage composition of the pathogen population by MGR-DNA fingerprinting. Such a breeding approach would combine resistances against all six of the lineages detected in the Santa Rosa region. For example, cultivars Cica 9 and Oryzica 1 appear to be susceptible only to lineages SRL-1 and -6, respectively, although both are resistant to the other four Santa Rosa lineages. In theory, the combination of the resistances of these two cultivars might provide corporate resistance against all resident Santa Rosa lineages. Thus, the possibility now exists to test whether there are specific resistance genes for *P. grisea* rice-pathogen lineages and whether such a combination of resistance genes can be durably effective. Clearly, genetic lineage analysis of the pathogen will play an important

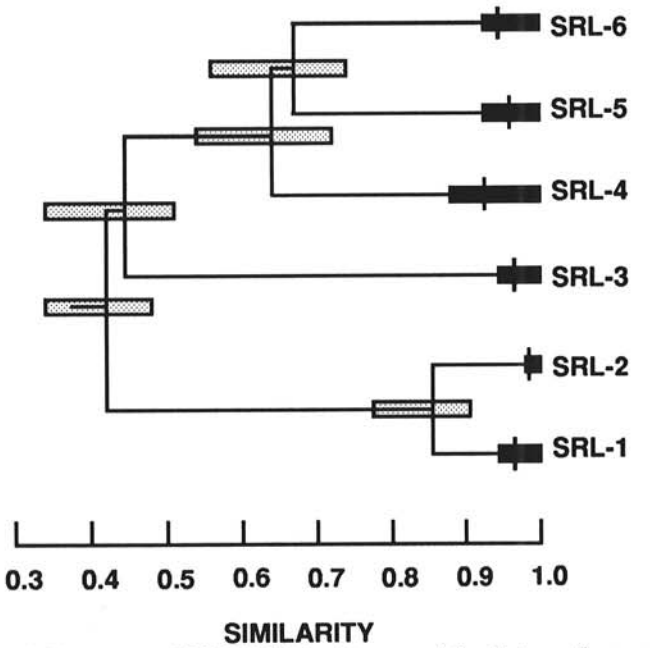

Fig. 3. Phenogram of MGR-DNA haplotype variation between lineages based on consensus haplotypes. Stippled error bars denote the 95% confidence interval of branch point values from bootstrap analysis of consensus haplotypes. Filled bars denote the range of haplotype variation within lineages. Vertical lines denote mean similarity within a lineage.

role in testing and developing such a breeding strategy.

The pathogenic diversity of the rice blast pathogen at Santa Rosa is much greater than that previously detected in the United States (13), although the number of genetic lineages detected is similar, six at Santa Rosa and eight in the United States. Our Santa Rosa sampling contained 39 pathotypes, and each Santa Rosa lineage expressed multiple (although largely lineage specific) pathotypes (Table 2). In the six-state southern rice belt region of the United States, only eight pathotypes are considered common (15), and each U.S. lineage is associated with only one to two of these pathotypes (13). Several factors might contribute to these different levels of pathotype polymorphism. First, the U.S. study analyzed an archival collection of isolates representing a geographically broad sampling collected over a long period of time and under episodic, rather than chronic epidemic, disease conditions. Consequently, the samples may represent only the modal, and perhaps the most highly fit, pathotypes in the United States. Second, the isolates in the U.S. archive collection may have been preserved because of especially definitive reactions on the international differentials. Third, until recently, there has been little rice blast resistance breeding in the United States, and the diversity of resistance genes and blast-resistant cultivars used as germ plasm sources is historically small compared to Colombia (15). Thus, it is not surprising that a detailed study of a recent U.S. blast epidemic in a specific area might reveal pathotype polymorphism not sampled previously (27). The Santa Rosa *P. grisea* population clearly shows that high levels of pathotype variability can occur over limited geographical areas providing there is a range of host genotypes and permissive climatic conditions. We conclude that the number of pathotypes within MGR-DNA fingerprint lineages is conditioned by a variety of factors and will need to be assessed in a number of rice-growing areas under a variety of ecological conditions.

The distinctiveness of the genetic lineages detected in Colombia in terms of MGR-DNA fingerprints parallels that deciphered in the archived collections of U.S. isolates (13). In both cases, a majority of the lineages was easily scored and sorted by visual inspection. Statistical treatments were needed to quantify variation within lineages and to confirm the discontinuous variation relationships between lineages. These results demonstrate that, even in regions of high pathogen genetic diversity, lineages are easily recognized. This result could only be obtained if pathogen populations are strictly clonal. The high degree of pathotype diversity at Santa Rosa does not obscure the fundamental genetic structure of the pathogen as revealed by MGR-DNA fingerprinting. At first, this may seem paradoxical, i.e., that fingerprints remain highly related while pathotypes vary. However, MGR586-associated sequences are well dispersed throughout the *P. grisea* genome (9,22), and thus, subtle changes in virulence within a lineage are not likely to produce major discontinuities in fingerprint profiles.

Preliminary studies on rice blast pathogen population structures in other countries also have indicated that each is composed of a small number (six to 18) well-differentiated genetic lineages (M. Levy, F. J. Correa-Victoria, R. S. Zeigler, and J. E. Hamer, *unpublished results*). Future efforts should be directed to compiling an international reference base consisting of blast fungus lineages, their geographic distribution, and their associated virulence characteristics. This international atlas of rice blast pathogen diversity will assist in the selection of resistant germ plasm sources for specific rice-growing regions.

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