The Relationship Between Lineage and Virulence in *Pyricularia grisea* in the Philippines

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ABSTRACT

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The relationship between phylogeny and pathotype was examined for a selected group of isolates of the blast fungus *Pyricularia grisea* from two sites in the Philippines. Based on DNA fingerprinting and cluster analysis with the repetitive DNA probe MGR586, isolates fell into several distinct groups. Probing blots of the MGR-defined haplotypes with avirulence genes *AVR2-YAMO* and *PWL2* produced single or multiple copy profiles that grouped identically to those from the MGR analyses. A set of 234 isolates, representing six of these groups or putative lineages, was selected for analysis of virulence spectrum. These isolates were tested on 15 rice cultivars and six near-isogenic lines. A total of 71 distinct pathotypes (virulence patterns) was detected on the 21 hosts tested. Multiple pathotypes were seen for each of the six lineages analyzed. Although there was no obvious relationship between lineage and pathotype, the distribution of virulence was nonrandom with respect to lineage for a given cultivar. Although reactions were variable for some host-lineage combinations (both compatible and incompatible isolates were found for 43 of the 123 host-lineage combinations analyzed), consistent incompatibility was observed for the majority of host-lineage interactions. In 80 of the 123 host-lineage combinations tested (65%), no compatible reaction was found, although an average of 39 isolates was tested per lineage per host. For each lineage, between nine and 16 cultivars were resistant to all isolates. Individual cultivars showed different spectra of resistance with respect to the pathogen lineages tested. The *indica* and *japonica* cultivars tested appeared to have complementary spectra of resistance. The implications of these observations for resistance breeding are discussed.

Additional keywords: composite pathotype, disease resistance, lineage exclusion, *Magnaporthe grisea*, rice blast.

Blast disease, caused by the filamentous fungus *Pyricularia grisea* (Cooke) Sacc. (teleomorph, *Magnaporthe grisea* Barr.), is one of the most damaging diseases of rice, *Oryza sativa* L. (33). Deployment of resistant cultivars has been the preferred means for managing the disease, and considerable effort has been directed toward understanding genetic resistance (23). Blast resistance, however, has been unreliable in many cases, with previously resistant cultivars showing high levels of susceptibility in the field within a few years of release.

The numerous explanations advanced for the instability of resistance may be grouped under two broad themes: inadequate exposure of breeding materials to diverse pathogen populations and hypervariability of the pathogen. Information on the phylogenetic structure of pathogen populations, which can be efficiently generated through the application of molecular genetic markers, is relevant to these issues. Based on DNA-typing data for populations of *P. grisea* at two key screening sites for blast resistance in the Philippines, we have investigated the relationship between phylogeny and virulence phenotypes. This relationship has important implications both for an understanding of pathogen variability and diversity as well as for the improvement of screening and breeding methods for blast resistance.

The repetitive DNA element MGR586 has been widely used for fingerprinting and phylogenetic analysis of *P. grisea* (5,7, 16-18,26-28,30,39,50,54). In these studies, the diverse *P. grisea* populations analyzed were reported to consist of sets of distinct lineages, or groups of isolates with similar DNA banding patterns, which were inferred to be related by descent from a common ancestor. A lineage structure is consistent with the reproductive mode of the pathogen; although the perfect state of the fungus has been occasionally achieved in the laboratory (45), the fungus is believed to reproduce only asexually in the field. Groups of isolates defined by MGR586 are consistent with those identified by other DNA markers (4,5).

The extent of pathogenic variability of the blast pathogen has been hotly debated (24,31,53). However, the recent finding that mutation or deletion of an avirulence gene in *P. grisea* causes a shift in phenotype from avirulence to virulence on a rice cultivar is consistent with a gene-for-gene relationship between the pathogen and its host (10,11,14,41,42,44,46). Although a simple relationship between lineage and pathotype (the composite virulence phenotype on a set of cultivars/lines carrying different resistance genes) was reported for *P. grisea* in the United States (28), most studies have shown complex relationships between phylogeny and virulence phenotypes, even in the United States (4,7,8,27,50).

It is clear from these reports that there is diversity for virulence within lineages on many rice cultivars. Indeed, some *P. grisea* isolates show frequent changes in virulence, usually loss of virulence, on certain hosts in the laboratory (43, R. S. Zeigler, L. X. Cuoc, R. P. Scott, M. A. Bernardo, D. H. Chen, B. Valent, and R. J. Nelson, *unpublished data*). For certain lineage-host combinations, however, virulence was not detected (7,8,27). These observations led to the suggestion that, although some resistance genes may be easily overcome by a given lineage of the pathogen,

TABLE 1. Pathotypes of *Pyricularia grisea* encountered in six lineages on rice cultivars from the Philippines

Lineages	No. of isolates per pathotype	No. of haplotypes per pathotype	Differential cultivar set[a]																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	4	1	+	–[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	3	3	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	1	1	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
1	2	2	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
1	3	2	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	12	5	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
1	1	1	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
1	3	2	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
1	3	2	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
1	1	1	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
1	3	3	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
4	1	1	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
4	2	1	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–
4	1	1	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–
4	2	2	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–
4	3	2	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–
4	1	1	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–
4	1	1	–	–	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–
4	1	1	–	–	–	–	+	–	+	+	–	–	+	+	–	+	+	–	–
4	3	3	–	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–
4	4	2	–	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–	–
4	16	2	–	–	–	+	+	–	+	+	–	–	–	–	+	–	–	–	–
4	1	1	–	–	–	–	–	–	+	–	–	–	+	+	–	–	+	–	–
4	2	2	–	–	–	–	–	–	+	–	–	–	+	+	–	+	+	–	–
4	9	1	–	–	–	+	+	–	+	+	–	–	–	–	+	–	–	–	–
4	2	2	–	–	–	+	+	–	+	+	–	–	–	+	+	–	–	–	–
4	3	3	–	–	–	–	–	–	+	–	+	–	+	+	–	+	+	–	–
4	1	1	–	–	–	–	–	–	+	+	–	–	+	+	+	–	+	–	–
4	1	1	–	–	–	–	+	–	+	+	–	–	+	+	–	+	+	–	–
4	2	1	–	–	–	+	+	–	+	+	+	–	+	+	–	+	+	–	–
4	1	1	–	–	–	+	+	–	+	+	–	–	+	+	+	–	–	–	–
4	1	1	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–
7	1	1	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
7	1	1	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
7	5	1	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–
7	1	1	–	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–
7	4	1	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–
7	1	1	–	–	–	–	–	–	+	–	–	–	+	–	–	+	+	–	–
7	1	1	–	–	–	–	–	–	+	–	+	–	–	+	–	–	+	–	–
7	6	1	–	–	–	–	–	–	+	–	–	–	+	+	–	+	+	–	–
7	1	1	–	–	–	–	–	–	+	–	+	–	+	–	+	+	+	–	–
7	12	1	–	–	–	–	–	–	+	–	+	–	+	+	–	+	+	–	–
7	9	5	–	–	–	–	–	–	+	–	+	+	+	+	–	+	+	–	–
7	3	1	–	–	–	–	–	–	+	–	+	–	+	+	+	+	+	–	–
7	1	1	–	–	–	–	–	–	+	–	+	+	+	+	+	+	+	–	–
14	2	1	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
14	1	1	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
14	1	1	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–
14	1	1	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
14	19	6	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–
14	1	1	–	–	–	–	–	–	+	+	+	–	+	+	–	+	+	–	–
14	2	2	–	–	–	–	–	–	+	–	+	–	+	+	–	+	+	–	–
14	1	1	–	–	–	–	–	–	+	+	–	+	+	+	+	–	+	–	–
14	1	1	–	–	–	–	–	–	+	–	+	+	+	+	–	+	+	–	–
14	1	1	–	–	–	–	–	–	+	+	+	+	+	+	–	+	+	–	–
14	3	1	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	–	–
14	1	1	–	–	–	–	–	–	–	+	–	+	+	+	–	–	+	–	–
14	1	1	–	–	–	–	–	–	+	–	–	+	+	+	–	+	+	–	–
14	1	1	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
15	4	1	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
15	20	1	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–
15	5	1	–	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–
15	2	1	–	–	–	–	–	+	+	–	–	–	–	+	+	–	+	–	–
15	15	1	–	–	–	–	–	+	+	–	–	–	–	+	+	–	–	–	–
44	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
44	1	1	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–
44	2	1	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–
44	1	1	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+
44	3	1	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+
44	3	3	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	+	+

[a]Cultivar codes: 1 = IAC47; 2 = IAC165; 3 = OS6; 4 = BR21; 5 = C22; 6 = Carreon; 7 = CO39; 8 = IR8; 9 = IR36; 10 = C101LAC; 11 = C105TP-4-L23; 12 = C104PKT; 13 = C102PKT; 14 = IR50; 15 = C101PKT; 16 = 5173; and 17 = C101A51.

[b]– = Incompatible interaction; + = compatible interaction defined by presence of sporulating lesion of ≥5% of leaf area.

resulting in the general variability of these host-lineage interactions, a lineage may have certain constraints (physiological or genetic), such that it is unable to rapidly overcome particular resistance (53).

Because the collections of isolates and differential cultivars used in previous studies were not designed to allow virulence spectra of individual lineages to be rigorously assessed, the relationship, if any, between phylogeny and pathotype is poorly understood for *P. grisea*. This study was undertaken to assess the hypothesis that lineages of the blast fungus are variable for virulence on some host genotypes but not on others. In this paper, we describe the virulence spectra of several lineages of *P. grisea* from two sites in the Philippines. We also demonstrate that lineages, as defined by MGR586, are readily distinguished by avirulence gene probes (44). From the data obtained, we develop specific hypotheses about the putative virulence constraints of different lineages and their implications for blast-resistance breeding.

MATERIALS AND METHODS

Test cultivars and isolates. A monocyclic inoculation test was used to evaluate compatibility between *P. grisea* isolates and 21 rice cultivars and near-isogenic lines (NILs) (Tables 1 and 2). Isolates and cultivars were chosen based on the results of previous studies conducted at the International Rice Research Institute (IRRI), the Philippines. Cultivars were selected based on their apparently different resistance characteristics. The NILs were selected to have different blast resistance genes (19,20,29). Seeds were obtained from the International Network on Genetic Evaluation in Rice, and the Entomology and Plant Pathology Division, IRRI. In a few cases, cultivars were not challenged by all isolates in a lineage due to seed shortage. For each isolate, 12 seeds of each line were planted in a plastic pot (10 × 10 × 9 cm) containing approximately 3 g of $(NH_4)_2SO_4$ per kilogram of soil and replicated four times in separate pots. Ten days after each seeding, seedlings were thinned to 10 uniform seedlings per pot, and 0.3 g of $(NH_4)_2SO_4$ per pot was applied a few days before inoculation.

P. grisea isolates were collected from two blast screening nurseries used by IRRI in studies aimed at characterizing the pathogen populations present at the blast nursery in Los Baños, the Philippines, and the upland rice screening site at Cavinti, the Philippines (M. A. Bernardo and M. Bronson, *unpublished data*). Nonradioactive DNA typing was conducted with the probe MGR586 (7). Isolates (234) were chosen to represent six lineages (coded 1, 4, 7, 14, 15, and 44; they are described and defined below), such that approximately 50 isolates of each lineage (if available) were tested. The greatest possible intralineage diversity was represented based on the following criteria (Table 3): site of origin (if possible, isolates from both sites were included for each lineage); cultivar of origin; available pathotype data; and MGR586 haplotype (isolates sharing 100% MGR586 band similarity were considered to belong to the same haplotype). The lineage identity of selected isolates (representing all haplotypes) was confirmed as part of this study.

MGR586 probing and phylogenetic analysis. Genomic DNA was extracted from lyophilized ground mycelia (38). Genomic DNA was digested with *Eco*RI, electrophoresed on 0.8% agarose, and capillary blotted on nylon membrane (Hybond-N+, Amersham Corp., Arlington Heights, IL) with 0.4 M NaOH-1 M NaCl as transfer agent. The subclone pCB586 (17), random-prime labeled with digoxigenin-11-dUTP (12), was used as a probe and detected by luminescence-based alkaline phosphatase immunosorbent assay following the manufacturer's instructions (DIG DNA labeling and detection kit, Boehringer Mannheim, Germany). Based on visual inspection of MGR586 DNA fingerprints, isolates were grouped and electrophoresed in adjacent lanes to confirm band identities. Forty-three isolates were selected to represent all the distinct banding profiles (putative haplotypes) in the six groups (putative lineages). DNA from groups of similar isolates were electrophoresed, together with representatives of other putative lineages, to allow scoring of individual band positions. To facilitate scoring, DNA from haplotype 7-16, which showed a particularly large number of bands, was run in three to four lanes on each gel.

Analysis of lineage structure. For each band position between 1.1 and 22 kb, presence or absence of the band was scored manually for each isolate. Lineage structure was inferred from similarity of MGR banding patterns. The binary matrix indicating presence (1) or absence (0) of each DNA band for each isolate was used to construct a matrix of similarities between all pairs of isolates based on Dice's coefficient [$F = 2N_{xy}/(N_x + N_y)$], where N_{xy} is the number of bands either present or absent in both isolates x and y and $N_x + N_y$ is the total number of bands observed for that pair of isolates. Based on this similarity matrix, the average linkage method of hierarchical agglomerative clustering (unweighted pair group method arithmetic mean analysis in the SAHN program of the NTSYS-pc package [34]) was used to produce a cluster dendrogram or phenogram of the isolates. A similarity level of 0.8 was used to define lineage groups.

TABLE 2. Frequency of compatible isolates from six lineages of *Pyricularia grisea* collected in the Philippines on six near-isogenic lines of rice, a resistant donor, and the recombinant parent (CO39)

Cultivar	Resistance gene[a]	Frequency of compatible isolates[b]					
		1[c]	4	7	14	15	44
C101LAC	*Pi-1*	0/36	0/59	10/41	9/36	0/46	0/11
C101A51	*Pi-2*	0/36	0/59	0/41	0/36	0/46	7/11
C104PKT	*Pi-3*	0/36	38/59	12/41	24/36	46/46	10/11
C101PKT	*Pi-4a*	0/36	12/59	34/41	11/36	2/46	0/11
C105TTP-4L23	*Pi-4b*	0/36	14/59	33/41	11/36	0/46	0/11
C102PKT	*Pi-4a*	0/36	17/59	36/38	32/36	37/46	9/11

[a] Mackill and Bonman (29).
[b] Number of compatible isolates over the total number of isolates tested.
[c] Lineage code number.

TABLE 3. Summary of origins and diversity of Philippine isolates of *Pyricularia grisea* within six MGR-defined lineages used in virulence spectum study

Lineage	No. of isolates[a]	No. of collection sites	No. of cultivars of origin	No. of pathotypes[b]	No. of MGR haplotypes	No. of unique pathotypes[c]
1	36	1	15	11	11	4
4	59	2	47	22	10	9
7	46	2	31	13	8	7
14	36	1	14	14	10	9
15	46	2	7	5	1	0
44	11	1	1	6	3	3

[a] Total number of isolates was 234.
[b] Total number of pathotypes was 71.
[c] Pathotypes found in only one lineage.

To evaluate the robustness of the groupings formed, the binary data set was subjected to bootstrapping. Using the program WINBOOT (a custom-written program based on [13]; I. Yep, *unpublished data*), the phenogram was reconstructed 2,000 times by repeated sampling of the set of possible bands with replacements, and the frequency with which a particular group was formed (indicated on the phenogram as a percentage of the iterations; Fig. 1) was considered to reflect the strength of the group (13).

Probing with avirulence genes. Isolates representing the range of pathotypes encountered in the study were selected for analysis of *avr* gene variability. Blots of genomic DNA *Eco*RI digests were prepared as above and probed with radiolabeled subclones of the *avr* genes *PWL2* and *AVR2-YAMO* (44). A 1-kb *Hpa*I fragment of the subclone pCB791 was used to detect homology with *PWL2* (J. A. Sweigard, A. M. Carroll, and B. Valent, *unpublished data*). Homology to *AVR2-YAMO* was detected with the 1.2-kb *Dra*III-*Apa*I fragment of the subclone pCB813 (L. Farrall and B. Valent, *unpublished data*). Radiolabeled probes were prepared by random priming (12). Blots were stripped between probings with 0.2 M NaOH.

Inoculation and disease scoring. Stock isolates were revived from storage on prune agar slants with streptomycin at 10 mg/250 ml of medium. Mycelia from 10-day-old slants were transferred and macerated aseptically in a tube with 10 ml of distilled water. The resulting suspensions of mycelia and conidia were poured over rice-polish agar plates (2 ml per plate). After a week of incubation at 28 C, the surface of the plates was scraped with a sterile glass slide or rubber policeman. The plate surfaces were illuminated continuously under fluorescent lamps at room temperature for 3–4 days to stimulate sporulation.

Conidia were scraped from incubated plates into which 10–20 ml of distilled water was poured. Spore suspensions were filtered through nylon or gauze mesh, and spore concentration was adjusted to 5×10^4 conidia per milliliter. Tween 20 was added to 0.02% just before inoculation.

Plants were inoculated 21 days after seeding (4–5 leaf stage). Seedlings were placed on a rotating plate and sprayed uniformly with the spore suspension with a mist sprayer. A total of about 700 ml of spore suspension per fungal isolate was sprayed over a total of 21 cultivars and isolines. The inoculated plants were transferred to dew chambers and kept for 24 h at 25 C and 98–100% relative humidity. The plants were transferred to a mist room and kept at 25–28 C for 6–7 days.

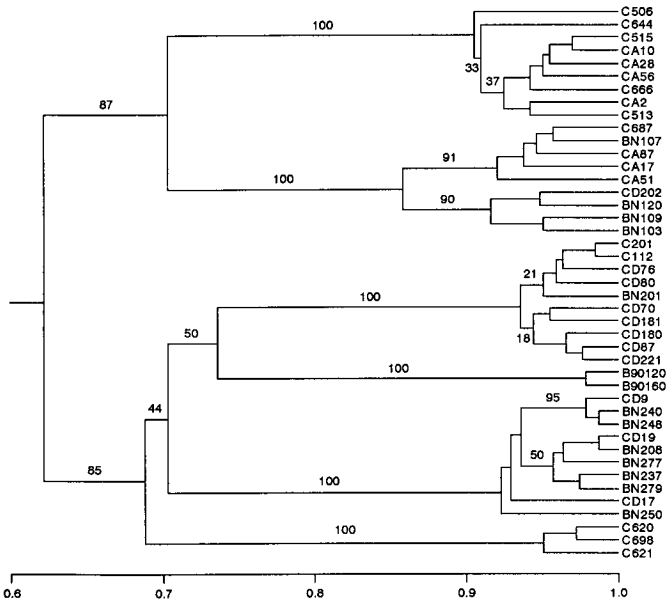

Fig. 1. Phenogram derived from the unweighted pair group method arithmetic mean analysis of the binary matrix obtained for MGR586 band comparison of 43 isolates of *Pyricularia grisea*, representing all haplotypes included in the study. The 100s indicate that in 2,000 iterations of a bootstrap procedure the groupings to the right of the indicated branch were observed 100% of the time.

Disease reactions of the inoculated plants were scored 6–7 days after inoculation by a 0–5 scale system or by modification of the evaluation system of IRRI (21), in which a score of 3.5 or higher is given to expanding sporulating lesions. Plants with no sporulating lesions were considered incompatible with the isolate, whereas those with sporulating lesions on ≥5% of the last fully emerged leaf area were considered compatible. The experiment was repeated twice. In the relatively few cases with ambiguous results, the inoculation was repeated. Results reflect repeatable isolate-cultivar interactions. Pathotypes corresponding to complete avirulence on all cultivars tested were not included because these may have lost basic pathogenicity during culturing.

Analysis of pathotype-lineage relationship. The predictability of lineage based on pathotype, and vice versa, was assessed by Goodman and Kruskal lambda statistics (40). The lambda statistic is a measure of the relative reduction in error of predicting one variable when the other is known compared to when it is not known. For this study, there are two lambda statistics: one for prediction of pathotype from lineage and one for the converse. These statistics were not necessarily equal because the two measures were asymmetrical.

The statistics were calculated by formulae given in Siegel and Castellan (40) from a contingency table of lineage-pathotype computed from Table 1 with six lineages and 71 pathotypes defined by the sequences of plus or minus responses to the differential set of cultivars.

Analysis of lineage-cultivar specificity. The interaction of lineage with the host plant factors cultivar, type, and origin in determining virulence patterns was investigated using generalized linear model (GLM) analysis. The log odds of compatible to resistant reactions were modeled as linear functions of the classifying factors, assuming a binomial distribution in each cultivar-lineage combination.

RESULTS

Lineage structure. The isolates could be clearly grouped into six lineages corresponding to the original preliminary groupings. There was typically a greater than 0.9 similarity among the haplotypes comprising the lineages and less than 0.8 similarity among lineages. Only lineage 4 showed some subclustering near the 0.8 similarity level, but the overall within-lineage similarity was greater than 0.85 (Fig. 1). There was a clear correspondence between the groupings assigned by MGR586 analysis and polymorphisms of the avirulence gene *PWL2* (Fig. 2). A similar relationship was found for *AVR-YAMO* (data not shown), although for all lineages there was only a single band detected.

Relationship between lineage and pathotype. The relationship between lineage and pathotype was examined for a selected group of *P. grisea* isolates from two sites in the Philippines. Among the 234 isolates analyzed in this study, substantial pathotypic diversity was observed. A total of 71 distinct pathotypes (virulence patterns) was detected on the 21 cultivars tested. Multiple pathotypes occurred in each of the six lineages analyzed (Tables 1–4). No compatible reactions were observed on cultivars IRAT13, HD14, CICA9, and Oryzica Llanos 5. Lambda statistics were used to assess the predictability of pathotype based on lineage data, and vice versa (40). The lambda value for prediction of pathotype based on lineage was 0.24, indicating that the probability of a false prediction of pathotype was 24% lower when the lineage was known than when it was not. Because the majority (92%) of the pathotypes were restricted to a single lineage, the predictability of lineage-based pathotype was higher, with a lambda value of 0.80. This relatively high value was presumably due to the large number of pathotypes relative to the low number of isolates per pathotype and does not necessarily reflect a useful predictive relationship between pathotype and lineage. No correspondence was detected between the banding patterns of the two *AVR* profiles of the isolates and virulence.

The relationship between lineage designation of isolates and

their virulence on individual hosts also was examined. Both compatible and incompatible isolates were detected within a single lineage for 43 of the 123 (35%) host-lineage interactions tested (Tables 1 and 4). In all the variable cultivar-lineage combinations, compatible isolates comprised >10% of the isolates, and the number of incompatible isolates detected often exceeded half of those tested. Similar results were obtained for the NILs (Table 2). Compatibility of all isolates from a lineage with a given cultivar or NIL was rare, with only two cases observed among the 123 host-lineage combinations tested: cultivars Carreon and PKT104 with 46 isolates of lineage 15.

Although the reactions of individual isolates within lineages were often variable, consistent incompatibility was observed for the majority of host-lineage interactions. In 90 of the 123 host-lineage combinations tested (65%), no compatible reaction was found, although many isolates (from 11 to 59, mean = 39) were tested per lineage per host. For each lineage, between nine and 16 cultivars were resistant to all isolates.

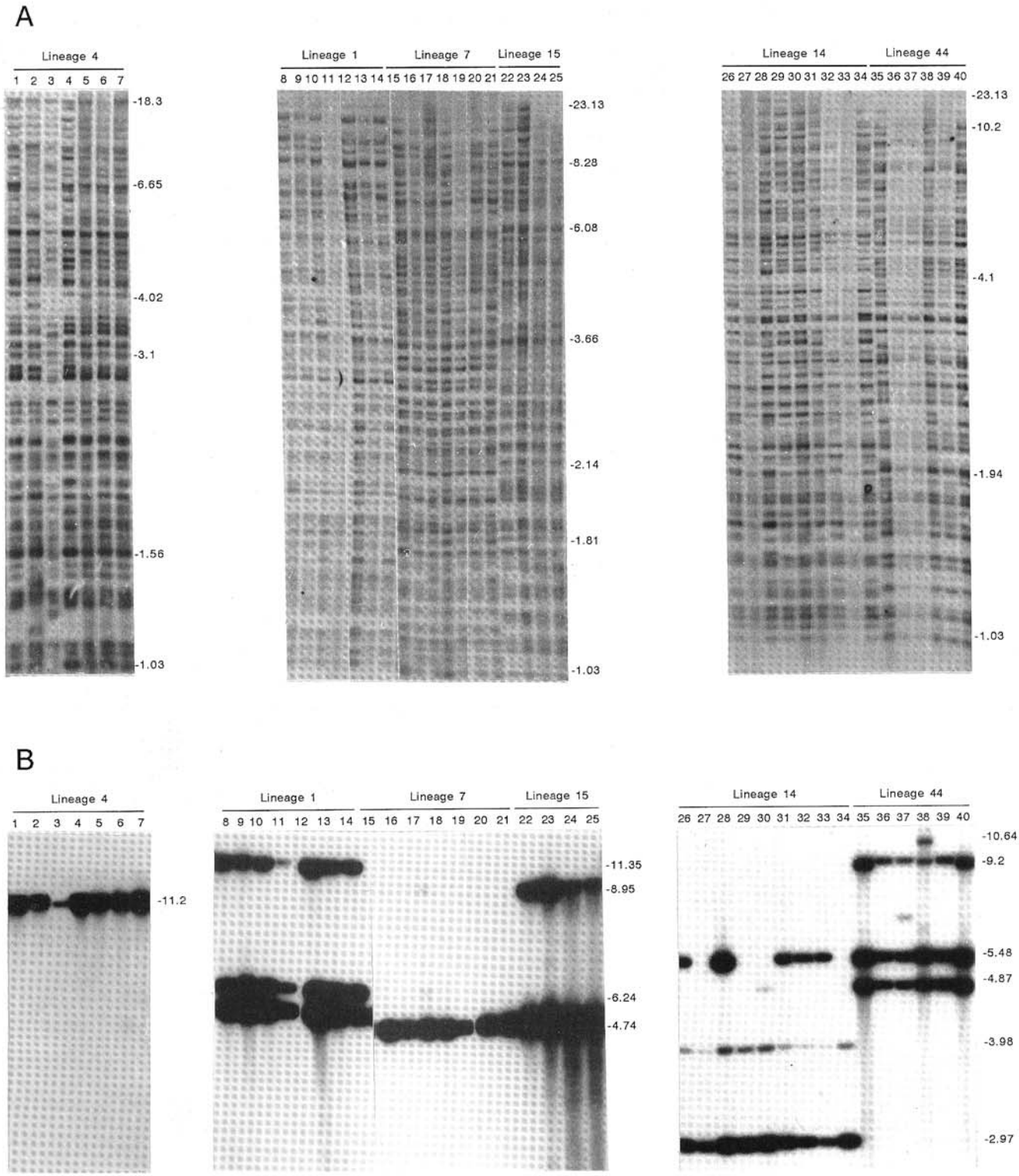

Fig. 2. DNA profiles of representative isolates of *Pyricularia grisea* used in this study, probed with **A,** MGR586 and **B,** *PWL2*. Differentiation among lineages was based on MGR DNA-band similarity, using Dice's coefficient. The cut-point for lineage was 80% of DNA band similarity.

If pathogenicity of isolates within the sample population was completely unrelated to lineage, the distribution of virulence should have been random with respect to lineage for a given cultivar. This, however, was not the case. Lineage 44 had a relatively narrow virulence spectrum, showing compatibility with NIL C101A51 and its donor (5173), which were resistant to all isolates of all the other lineages assayed. Specific pathogenicity of lineages to cultivars was confirmed by GLM analysis of the data in Table 4 (residual deviance after fitting lineages and cultivar was 1,272 with 67 degrees of freedom [df]). The analysis of NIL-lineage combinations in Table 2 also showed significant interaction (residual deviance = 392; df = 30). This indicates that the pattern of virulence of the lineages is specific to hosts. Furthermore, the relationship with the NILs indicates that single resistance genes may condition resistance to entire lineages.

For lineage 1, out of 729 inoculations on 36 isolates (21 hosts), 101 compatible interactions were recorded. These were limited to five hosts; all of which were *japonica* and those susceptible only to lineage 1 (Table 4). Lineage 14 showed 58 compatible interactions from 691 inoculations. None of the *japonica* were compatible with isolates from lineage 14, whereas most of the *indica* cultivars were compatible with some isolates of the lineage.

Resistance spectra of rice cultivars in relation to pathogen lineages. A few cultivars (i.e., IRAT13, HD14, CICA9, and Oryzica Llanos 5) were resistant to all isolates tested. Each of the cultivars tested was resistant to all isolates of at least one lineage. Even cultivars considered to be highly susceptible, such as CO39 and IR50 (used as susceptible checks in many experiments at IRRI; e.g., 29), were resistant to some lineages. Conversely, cultivars such as OS6, IAC47, and IAC165, which are considered highly and durably resistant and which have been widely used as donors in resistance-breeding programs (35,36), were highly susceptible to isolates of certain lineages.

Two main varietal types of rice, designated *indica* and *japonica*, can be discriminated based on morphological characters, isozymes, and restriction fragment length polymorphisms (6,15, 22,48). The *japonica* and *indica* cultivars tested showed distinct resistance spectra (Tables 1 and 4). Three *japonica* cultivars with origins in Africa and Latin America (OS6, IAC47, and IAC165) were susceptible only to lineage 1. The two *japonica* cultivars with Asian origins (C22 and BR21) were susceptible only to lineages 1 and 4. It is noteworthy that lineages 1 and 4 are more similar to one another than to the remaining lineages (Fig. 1). The *indica* cultivars were resistant to all isolates of lineage 1. Whereas the *japonica* cultivars were resistant to lineages 7, 14, and 15, only *indica* cultivars were susceptible to these lineages.

The GLM analysis of host type (*indica* versus *japonica*) and lineage showed significant interaction (deviance = 28.9; df = 2). Similarly, host origin and lineage interacted (deviance = 535; df = 5). Even when the interaction of these two factors (origin and type) was taken into account in a three-factor analysis, the residual deviance was significant (deviance = 886; df = 63), indicating that three-factor interaction may be important in determining specificity.

IR36 is known to possess a high level of partial resistance (PR), while IR50 has a low level of PR (47,51). In this study, IR36 and IR50 showed qualitatively similar spectra of resistance relative to lineages of *P. grisea*. Of the 71 pathotypes detected, six showed differential interactions between IR50 and IR36. Five of these were compatible with IR50 but not with IR36. One isolate was compatible with IR36 but not with IR50. Thus, IR36 and IR50 each appear to carry at least one major gene not present in the other. These genes, however, do not condition resistance against any of the lineages of the pathogen tested in this study.

DISCUSSION

In this study, a complex relationship between lineage and pathotype was found. This is consistent with the results of previous studies (4,5,7,8,50) and supports the view that changes in virulence

TABLE 4. Frequency of compatible isolates of six lineages of *Pyricularia grisea* inoculated on 15 rice cultivars

Host			Lineage[a]					
Cultivar	Group	Origin[b]	1[c]	4	7	14	15	44
IRAT13	*japonica*	Af	0/36	0/59	0/41	0/36	0/46	0/11
OS6	*japonica*	Af	31/36	0/59	0/41	0/36	0/46	0/11
IAC47	*japonica*	LA	28/36	0/59	0/41	0/36	0/46	0/11
IAC165	*japonica*	LA	7/12	0/53	0/16	0/11	0/46	...
BR21	*japonica*	As	10/36	33/59	0/41	0/36	0/46	0/11
C22	*japonica*	As	25/36	43/59	0/41	0/36	0/46	0/11
IR8	*indica*	As	0/36	48/59	0/41	7/36	0/46	0/11
IR50	*indica*	As	0/36	10/58	38/46	9/36	0/46	0/11
IR36	*indica*	As	0/35	6/59	27/41	8/36	0/46	0/11
Carreon	*indica*	As	0/34	0/59	6/46	20/34	46/46	3/11
CO39	*indica*	As	0/36	57/59	40/46	14/36	22/46	0/11
HD14	*indica*	As	0/36	0/59	0/41	0/36	0/46	0/11
CICA9	*indica*	LA	0/12	0/10	0/14	0/16	0/13	...
Oryzica Llanos 5	*indica*	LA	0/36	0/42	0/35	0/36	0/42	0/11
5173	*indica*	LA	0/24	...	0/22	0/18	0/14	10/11

[a]Number of compatible isolates over the total number of isolates tested.
[b]Af = Africa; LA = Latin America; As = Asia.
[c]Lineage code number; ... = no data.

on many host cultivars may occur rapidly relative to the genomic changes that lead to the divergence of MGR586-defined lineages of the pathogen. In lineage 4, in which two subclusters were observed (Fig. 1), there was no corresponding clustering of virulence or pathotypes.

Population structure of plant pathogens is usually analyzed by assessing the virulence spectrum of isolates on a set of cultivars carrying different resistance genes, termed differential cultivars. A group of isolates sharing a common phenotype on a defined set of differential cultivars is termed a race or pathotype (49). A relationship between virulence and lineage was detected here but not the simple relationship proposed for the southern United States population (28). A large number of pathotypes was detected in this study, and multiple pathotypes were found even within lineages, defined as statistically robust groups of isolates sharing 80% band similarity in DNA fingerprinting. One might conclude that lineage information is not a useful basis for predicting virulence phenotypes of *P. grisea*, especially when inferences are intended to inform a resistance-breeding program (50). Pathotype, however, is not necessarily the most appropriate phenotypic unit to examine in this context. For instance, Wolfe and Schwarzbach (49) pointed out that the isolate-cultivar combination may be a more useful unit of phenotypic interaction for some purposes.

We propose that an informative unit of phenotypic interaction for *P. grisea* is the lineage-host combination, or if possible, the lineage-resistance gene. There are several advantages to analyzing the virulence spectra of the blast pathogen in this way. First, it is possible to group the target pathogen population into a reasonable number of units. Although there may be a virtually infinite number of isolates and pathotypes present in a population, there appear to be fewer distinct phylogenetic groups based on available data and on the supposition that populations are wholly or predominantly asexual. Indeed, the nature of the individual is ambiguous when a clonal organism is considered, and it is reasonable to use lineage as a unit.

The presence of both virulent and avirulent isolates of a certain lineage for a given host may indicate that it is easy for isolates in the lineage to generate derivatives virulent on that host. That is, there appears to be promiscuous change within apparently clonal lineages on some hosts, as a result certain incompatible interactions are likely to be unstable. Although some host-lineage combinations analyzed in this study were variable, there was at least one host for which no virulent isolate was found for each of the lineages analyzed. This observation is consistent with the hypothesis that certain resistances are more difficult than others for a given lineage to overcome. While one resistance gene or set of genes may be readily overcome by isolates in one lineage, that same resistance may be a formidable obstacle for isolates in another lineage, and vice versa.

Variable and invariable cultivar-lineage incompatibilities offer a means to simplify complex pathotype relationships. We propose to identify the composite phenotype of a variably compatible lineage on a given host as compatible on that host. A host would be considered resistant to a lineage only if a certain number of nonidentical members of the lineage are uniformly incompatible. Based on this approach, a composite pathotype can be developed for each lineage (Table 5). The extreme pathotypic variation in a population could be represented by a limited number of isolates if those with the broadest spectra of virulence (or a few isolates that collectively possessed the maximum number of virulences for the lineage) are selected.

The laborious task of population virulence characterization, thus, can be greatly simplified. Using conventional pathotypes as a basis for selecting potentially useful resistance sources, it is impossible to discriminate between the most unstable incompatible interaction and a putatively stable incompatible interaction. In contrast, using the concept of a composite host-lineage interaction phenotype allows a more stable interaction to be distinguished from a less stable one. It also allows the analyst to test a minimum number of host-isolate combinations before making a judgment. If several compatible interactions have been detected for a certain host-lineage combination, the lineage can be scored as compatible with that host with no need for further inoculation data. If no compatible isolate has been detected for a certain host-lineage combination, diverse isolates from that lineage should be tested until some credible, arbitrary number has been analyzed (we suggest $n = 50$). In this way, resistance sources likely to provide unstable resistance to lineages in the target region can probably be identified with a minimum number of inoculations. Such an approach may prove useful for other pathosystems characterized by high pathotypic diversity within primarily asexually reproducing populations.

Understanding pathotype diversity at the lineage level allows a novel interpretation of the controversy over virulence stability in *P. grisea* (2,24,25,32). If a pathotype is variable for some host-lineage combinations but not others, the contradictory reports on pathotypic stability may reflect, at least in part, differences in the particular host-lineage combinations used by different researchers.

Just as lineages can be characterized as compatible or incompatible with selected hosts, it is useful to examine the reciprocal relationship (i.e., the resistance spectra of rice cultivars). For instance, the *japonica* cultivars OS6, IAC47, and IAC165 were susceptible only to lineage 1 of the pathogen. In contrast, widely-susceptible *indica* cultivars, such as IR50 (1), were resistant to this lineage. Thus, even cultivars widely considered to be susceptible may carry resistance against certain lineages and be useful in a breeding program for which blast resistance is an objective. In a site where multiple lineages are present, the degree of field susceptibility of a cultivar or line may not be a good indicator of the potential utility of the genes it carries.

Other analyses of the resistance spectra of *japonica* and *indica* cultivars also have suggested that the lineages infecting them can be distinct (8,18). Early divergence within the *O. sativa* species is apparently reflected in complementary patterns of susceptibility to its presumably coevolved pathogen (37). At the IRRI blast screening nurseries, *indica* and *japonica* cultivars have been grown together for many years, yet certain lineages of the pathogen have apparently remained specific to one type or the other. It is possible that genes from these cultivars could be combined to give complementary resistance to the entire spectrum of lineages present in a region. Transferring blast resistance genes between

TABLE 5. Composite pathotype of six lineages of *Pyricularia grisea* from the Philippines

	Compatible isolates[a]										
Lineage	5,4[b]	1–3	6	7	8	14,9	17,16	10	11	12,13	15
1	S	S	R	R	R	R	R	R	R	R	R
4	S	R	R	S	S	S	R	R	S	S	S
7	R	R	S	S	R	S	R	S	S	S	S
14	R	R	S	S	S	S	R	S	S	S	S
15	R	R	S	S	R	R	R	R	R	S	S
44	R	R	S	R	R	R	S	R	R	S	R

[a]S = Compatibility with the rice cultivar encountered with some isolates of the lineage; R = all isolates of a lineage incompatible with the rice cultivar.

[b]Cultivar codes: 1 = IAC47; 2 = IAC165; 3 = OS6; 4 = BR21; 5 = C22; 6 = Carreon; 7 = CO39; 8 = IR8; 9 = IR36; 10 = C101LAC; 11 = C105TP-4-L23; 12 = C104PKT; 13 = C102PKT; 14 = IR50; 15 = C101PKT; 16 = 5173; and 17 = C101A51.

the two groups may be a promising application of the emerging rice gene-transfer systems (9), given the generally poor *indica-japonica* combining ability.

Resistance to entire lineages, apparently, could be conditioned by individual resistance genes, based on the analysis of resistance spectra of NILs (Table 2). *Pi-2*(t) conditioned resistance to five of the six lineages tested but not to lineage 44. Lines carrying *Pi-1*(t) and *Pi-4a*(t), in contrast, were susceptible to other lineages but resistant to lineage 44. It is likely that lines carrying both *Pi-2*(t) and either *Pi-1*(t) or *Pi-4a*(t) would be resistant to all the lineages tested. The virulence spectrum of isolates from lineage 44 is quite narrow, and it is the only lineage shown to infect NIL C101A51 carrying *Pi-2*(t). This type of narrow specificity is similar to that observed between the cultivar CICA9 and lineage SRL1 from Santa Rosa, Colombia (26): Lineage SRL1 was only recovered from CICA9 from the field and would infect only CICA9 and a few other broadly susceptible cultivars in greenhouse assays, while no isolates from other lineages (except the very similar SRL2) could infect CICA9.

The NILs included in this study were evaluated in the Santa Rosa breeding fields. NIL C101A51 showed susceptibility in the field and isolates of lineage SRL1 predominantly were recovered (F. J. Correa-Victoria, *personal communication*). Compatibility with the *Pi-2*(t) gene appears to limit compatibility with a broad range of other resistance genes, and vice versa. This is consistent with the proposal that certain virulence-avirulence configurations in the pathogen may be favored (53) and that such configurations may arise repeatedly in different populations, albeit in different genetic backgrounds.

If the gene in C101A51 is different from that in CICA9, it suggests there are sets of resistance genes that interact with pathogen avirulence genes such that a compatible interaction precludes compatibility with other genes. This might be the case with the observed *japonica-indica* complementarity. In these cases, the complementarity suggests strategic combination of resistance genes may be effective across most, or even all, components of a population.

C101PKT and C102PKT are supposed to carry the same gene, *Pi-4a* (29), but they show striking differences in their reactions to isolates from lineages 14, 15, and 44. This suggests that C101PKT may carry an additional resistance gene from the donor parent. CO39, the recurrent parent of the NILs, also obviously carries a major resistance gene, based on the high frequency of incompatibility with isolates from lineages 1, 14, 15, and 44. If C102PKT does not carry the CO39 resistance genes, then the higher frequency of incompatibility of C101PKT with isolates from these lineages could result from epistatic interactions between the two genes. Further study into the genetic composition of the NILs is clearly warranted.

IR36 has been shown to carry significantly greater PR than IR50 (3,47,52), and the two cultivars show corresponding differences in disease development in the field (1). Earlier studies reported that there was a good correlation between susceptibility at the isolate level between IR50 and IR36 (3). Here we show that this extends to the lineage level. The lineage resistance spectra of these cultivars is the same, suggesting that for the lineages in question (representing the predominant lineages at the IRRI screening sites), these cultivars are equivalently susceptible. Thus, when evaluating germ plasm, quantitative differences in levels of disease in the field of entries with identical spectra of lineage resistance may indicate significant and exploitable levels of PR, while differences between cultivars with very different resistance spectra may be incorrectly ascribed to differences in PR.

If it were the case that each lineage of the pathogen could be reliably excluded from infection by one or more resistance gene(s), it would be useful to combine such invariant resistances to exclude all known lineages in a target region, as proposed by Zeigler et al (53). Ideally, genes for qualitative resistance would be selected on this basis, and would be combined, together with genes conditioning quantitative resistance, by marker aided selection. Before this lineage-exclusion approach is tested as part of a resistance-breeding strategy, it is necessary to establish that the assumptions of the approach are valid. First, it must be demonstrated that for each lineage present in the field, at least one resistance gene can be identified for which no virulent isolates are found. Second, it must be demonstrated that for progeny of an avirulent isolate from a variable host-lineage combination, isolates virulent to the host in question can be recovered more frequently than from progeny of an avirulent isolate from a nonvariable host-lineage combination.

The results of this study are consistent with one assumption of the lineage-exclusion strategy for resistance breeding. Based on these data, however, we cannot conclude that incompatibility of all isolates within a lineage reflects a major barrier to variation within that lineage. Lineage-wide incompatibility may simply reflect a founder effect. That is, isolates within some or all lineages may have no significant constraints to variation in virulence, and therefore, no resistance gene is more effective than any other against the lineage. Studies are currently underway to test the second assumption of the lineage-exclusion proposal: For an avirulent isolate from a variable host-lineage combination, virulent derivatives can be recovered more frequently than from an avirulent isolate from a nonvariable host lineage combination.

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