# Enzyme Polymorphism and Genetic Differentiation Among Geographic Isolates of the Rice Blast Fungus

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## ABSTRACT

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Polymorphisms in 12 enzymes were examined among 335 rice isolates of *Magnaporthe grisea* from 12 rice-growing regions of the world. Six electrophoretic types (ETs) were found. Ninety-five percent of the 335 isolates belonged to two common ETs. All isolates were monomorphic at 16 of the 18 putative enzyme loci. When isolates were pooled as a single population, the proportion of polymorphic loci (P) was 0.11 with an average gene diversity ($\overline{H}$) of 0.03. In contrast, a sample of 34 nonrice isolates of *M. grisea* from several gramineous hosts showed more allelic variation (P = 0.5, $\overline{H}$ = 0.21). Among the rice isolate populations, geographic differentiation was observed at the lactate dehydrogenase-1 (Ldh1) locus, where the distribution frequencies of a functional allele ($Ldh1^{100}$) and a null allele ($Ldh1^{n}$) appeared to follow a north-south geographic trend. However, because there was also a strong association between $Ldh1^{100}$ with *japonica* rice and $Ldh1^{n}$ with *indica* rice, it was not certain whether the distribution of the Ldh1 alleles was influenced by climatic factors or by rice geographic races.

*Additional key words*: electrophoretic variation, population genetics, *Pyricularia*.

The blast fungus, genus *Pyricularia*, consists of a diverse group of pathogens parasitic on the Gramineae. The economically most important species is *Pyricularia oryzae* Cavara, which causes the rice blast disease worldwide. Other isolates morphologically indistinguishable from *P. oryzae* are found on a variety of gramineous hosts such as finger millet (*Eleucine coracana* (L.) Gaertn.), goosegrass (*E. indica* (L.) Gaertn.), weeping lovegrass (*Eragrostis curvula* (Schrad.) Ness), and crabgrass (*Digitaria sanguinalis* (L.) Scop.) (1). These *Pyricularia* isolates have been collectively referred to as *P. grisea* by some authors (1,14); however, mating experiments over the past decade have shown that *Pyricularia* isolates from various gramineous hosts were capable of producing the sexual stage under laboratory conditions (14,18,41). The perfect state is the heterothallic ascomycete *Magnaporthe grisea* (Herbert) Barr comb. nov. with bipolar control of mating (4,42). Although the degree of fertility varies among isolates from different hosts, the sexual compatibility observed among various *Pyricularia* isolates indicates that the species distinction between *P. oryzae* and *P. grisea* is unnecessary. In this paper, we will refer to *Pyricularia* isolates from rice and nonrice hosts as rice and nonrice isolates of *M. grisea*, respectively.

For more than 60 yr, pathogenic variability in the rice blast fungus has been documented extensively (31). In 1922, Sasaki first noted the existence of pathogenic races that differentially attacked rice cultivars (31). Since then, numerous races have been identified in major rice-growing regions (15,30). In the Philippines alone, more than 250 races have been reported (31). In the late 1960s, Ou and Ayad (32) and Giatgong and Frederiksen (12) reported that monoconidial isolates of a single race continued to give rise to new races. These reports, however, contrast with those of others who have not observed the same degree of pathogenic variability in single-spore isolates (15,21). Therefore, whether the reported variation is unique to a particular component of the pathogen populations remains uncertain.

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In seeking to understand the basis of the observed pathogenic variability on rice, a number of cytological and genetic studies have been made. On the basis of his observation of a high frequency of multinucleate cells in certain isolates, Suzuki (39) has suggested heterokaryosis as the primary cause for variation. Using auxotrophic markers, Yamasaki and Niizeki (43) and Genovesi and MaGill (11) have demonstrated a potential role for the parasexual cycle in generating novel recombinants. Reports of variable chromosome numbers in *M. grisea* (12,39,43) have led Ou (31) to suggest aneuploidy as a potentially important mechanism for generating variation.

In spite of the potential for these mechanisms to contribute to variation in *M. grisea*, the unusually high degree of pathogenic variability reported from the Philippines suggests a need to examine the pathogen populations more widely for other indicators of phenotypic variation. Electrophoretic procedures have made possible the examination of genetic variation among a number of enzyme loci and have become important techniques in understanding the genetic structure of many species (3,23). In recent studies with Australian cereal rusts, Burdon and co-workers have demonstrated the value of isozyme analysis in understanding the origin and nature of variation in plant pathogen populations (5–8).

By examining the electrophoretic profile of enzymes in a large collection of *M. grisea* rice isolates from major rice-growing regions of the world, an analysis of the inherent genetic variability of the rice blast fungus should be possible. Such a largely empirical analysis of the rice subpopulation of *M. grisea* should provide additional information on the genetic structure of the species that may be of value in understanding the nature of pathogenic variability. To investigate the potential role of sexual recombination in genetic variability of *M. grisea*, a small sample of sexually competent nonrice isolates from various gramineous hosts was included in the analysis.

## MATERIALS AND METHODS

**Fungal isolates.** Between 1979 and 1983, we acquired about 350 rice isolates of *M. grisea* from 12 geographic regions of the world and 34 isolates from a variety of gramineous hosts. The geographic

origins and characteristics of *M. grisea* isolates are summarized in Table 1. The initial collection and isolation (1979–1981) were made by S. H. Ou and Y. X. Chen. Most samples were received by mail in the form of dry, diseased leaves. Isolates from Japan and the United States were received as single-spore cultures. Classification as rice and nonrice isolates was based either on the hosts from which the fungus was isolated or on the information provided by the donors; no pathogenicity tests were conducted to confirm their host specificities. The 34 nonrice isolates were obtained from various Gramineae: 8 from *D. sanguinalis* (crabgrass), 13 from *Eleucine coracana* (finger millet), 2 from *E. indica* (goosegrass), 2 from *Eragrostis curvula* (weeping lovegrass), and 9 from unidentified species. Twenty of the 34 nonrice isolates were mating strains obtained from H. Kato (Agricultural Research Center, Tsukuba, Ibaraki, Japan) and H. Yaegashi (National Institute of Agricultural Sciences, Tsukuba, Ibaraki, Japan).

**Isolation.** Individual leaves with blast lesions were rinsed with running water (20 min), surface-sterilized in 10% (v/v) Clorox (4 min), rinsed twice in sterilized water, cut into 2-mm$^2$ pieces, and plated on acidified potato-dextrose agar (aPDA). After 4–5 days, hyphal tips were excised and transferred to fresh aPDA to eliminate bacterial contamination. Hyphae from clean colonies were excised again and maintained on rice potato-sucrose agar slants (10 g of rice straw, 40 g of potato, 8 g of sucrose, and 16 g of agar per liter of water) at room temperature. An isolate was defined as either single-spore or single-hyphal-tip culture obtained from a leaf sample. On average, one to five independent isolations were made per leaf sample.

TABLE 1. Geographic origin, sample size, host, and years of collection of *Magnaporthe grisea* isolates

| Region | No. of leaf samples | No. of isolates | Host | Type of isolation[a] | Years of collection |
|---|---|---|---|---|---|
| Southern United States[b] | ··· | 30 | Rice | S | 1975–1981 |
| Wisconsin | 4 | 8 | Crabgrass | S | 1983 |
| Cali, Colombia | 19 | 22 | Rice | H | 1979 |
| Brazil | 4 | 9 | Rice | H | 1980 |
| India | 8 | 56 | Rice | H | 1983 |
| | 1 | 6 | Grass[c] | H | 1983 |
| Ibadan, Nigeria | 9 | 38 | Rice | H | 1983 |
| Japan | ··· | 10 | Rice | S | Unknown |
| | ··· | 20 | Grass[d] | S | 1977–1981 |
| Korea | 21 | 34 | Rice | H | 1980 |
| Liaoning, China | 22 | 25 | Rice | H | 1980 |
| Hunan, China | 15 | 28 | Rice | H | 1980 |
| Guangdong, China | 31 | 35 | Rice | H | 1980 |
| Taiwan | 7 | 7 | Rice | H | 1980 |
| Philippines | 31 | 56 | Rice | H | 1979–1980 |

[a]S = single-conidial culture and H = hyphal-tip culture.
[b]Includes Louisiana, Texas, Florida, and Arizona.
[c]Unknown species.
[d]Thirteen finger millet (*Eleucine coracana*), two goosegrass (*Eleucine indica*), two weeping lovegrass (*Eragrostis curvula*), and three unknown species.

**Tissue preparation and electrophoresis.** Mycelia for electrophoresis were produced in 10-ml Erlenmeyer flasks containing 8 ml of potato-sucrose broth (100 g of potato and 10 g of sucrose per liter of water). Flasks were inoculated with small mycelial pieces from individual isolates and gyrated at 125 rpm on a shaker (model G2, New Brunswick Scientific) at 25 ± 1 C for 4–5 days. Each sample was suction-filtered through Whatman No. 1 filter paper; the mycelia adhering to the filter were scraped into a pellet (about 40 mg) with a spatula and transferred to a 0.5-ml automatic analyzer beaker (Scientific Products) submerged in ice. A drop of distilled water was added to each pellet, which was then ground for 5 sec with a Teflon grinding tip attached to a homogenizer (Con-Torque, Eberbach, Fisher Scientific). The crude extract was absorbed onto a paper wick 2 mm × 10 mm (Whatman Chromatography grade paper, 3 mm), loaded onto a horizontal starch gel, and subjected to electrophoresis as described by O'Malley et al (29). All starch gels (12%, w/v) were made with Electrostarch (Connaught Laboratory, Swiftwater, PA). After completion of electrophoresis, 1.5-mm slices of gel were stained with specific enzyme stains as described by O'Malley et al (29) and Shaw and Prasad (36). Up to 60 samples including two standard isolates of known banding patterns were analyzed on each gel slab. All isolates were analyzed twice either in the same gel or in a separate run.

Four electrophoretic buffer systems (Table 2) and 26 enzyme stains were tested initially for resolution and stainability; from these, 12 enzymes producing resolvable banding patterns were selected for analysis (Table 3).

**Naming of enzyme loci.** For multiple-band enzymes, individual electromorphs (electrophoretically distinct forms of a protein) were identified by the abbreviations of the enzymes with hyphenated Arabic numbers; the most anodally migrating band was designated the lowest number. Within a putative locus, alternate alleles were described by a superscript (e.g., $Ldh1^{83}$) indicating electrophoretic mobility relative to the most common allele arbitrarily assigned a mobility of 100. Null alleles with no staining activity on the gel were designated by a superscript n.

## RESULTS

Three hundred thirty-five isolates of *M. grisea* from rice and 34 isolates from other Gramineae were surveyed for electrophoretic variation in 12 enzyme systems (Table 3). In the 12 enzyme systems analyzed, 18 unique bands were identified. Each band was tentatively assumed to be encoded by a single locus. Confirmation of Mendelian inheritance at all putative loci was not possible because of infertility among many isolates. Eight of the 18 putative loci, aspartate aminotransferase (Aat), fumarase-1 (Fum1), Fum 2, malate dehydrogenase-1 (Mdh1), malate dehydrogenase-2 (Mdh2), xanthine dehydrogenase (Xdh), glutamic-pyruvic transaminase (Gpt), and $\alpha$-esterase ($\alpha$Est), showed no allelic variation in all isolates tested. A potential $\alpha$-Est null variant was noted in several isolates from Taiwan and Brazil; however, this potential variant was not included in our analysis because of the uncertainty in ascribing a null variant without genetic analysis. Sixteen electrophoretic variants were found at the remaining 10

TABLE 2. Buffer systems and electrophoretic conditions for starch gel electrophoresis in *Magnaporthe grisea*

| System | Electrode buffer | Gel buffer | Power | Reference |
|---|---|---|---|---|
| A | 0.04 M citrate, adjust pH to 6.1 with *N*-(3-amino-propyl)-morpholine | 1:20 Dilution of electrode buffer | 220V, 50 mA, 3 hr | 10 |
| B | 0.18 M Tris, 0.1 M boric acid, 0.04 M EDTA, pH 8.0 | 1:4 Dilution of electrode buffer | 250V, 25 mA, 4 hr | 24 |
| C | 0.06 M lithium hydroxide, 0.3M boric acid, pH 8.1 | 0.03 M Tris, 0.005 M citrate, 1% electrode, buffer, pH 8.5 | 250V, 25 mA, 3 hr | 33 |
| D | 0.22 M Tris, 0.094 M citrate, pH 6.3 | 0.008 M Tris, 0.003 M citrate, pH 6.7 | 100V, 50 mA, 3 hr | 37 |

enzyme loci (Fig. 1). In another study, six loci, lactate dehydrogenase-1 (Ldh1), Ldh3, Mdh3, glycerate-2-dehydrogenase (G2dh), phosphoglucose isomerase (Pgi), and phosphoglucomutase (Pgm), were shown to conform to Medelian inheritance through genetic crosses (22). Most electrophoretic variation was found among the 34 nonrice isolates from Japan, Wisconsin, and India, whereas little variability was found among the rice isolates. Because of this lack of variation at most loci of the rice isolates, all rice and nonrice isolates from different geographic regions were pooled into two groups for comparative analysis.

Genetic variability in *M. grisea* is summarized in Table 4. A locus is defined as polymorphic when the frequency of the most common allele is less than 99%. Gene diversity for a locus is defined as $H = 1 - \Sigma Xi^2$, where Xi is the frequency of the $i^{th}$ allele (28). This provides a useful measure of genetic variability by taking into account the number and frequency of alleles at a locus (35). Nonrice isolates were found to be genetically more variable than the isolates from rice on the basis of the percentage of polymorphic loci, the average number of alleles, and the mean population gene diversity ($\overline{H}$) (sum of gene diversities at all loci per total number of loci). Among isolates from rice, only two loci (11%), G2dh and Ldh1, were polymorphic with gene diversities of 0.08 and 0.49, respectively. Among isolates from nonrice hosts nine loci (50%), Mdh3, Pgi, Pgm, Ldh1, Ldh3, aconitase (Aco), hexokinase (Hk1), Hk2, and G2dh, were polymorphic with gene diversities ranging from 0.39 to 0.53. The average number of alleles per locus was 1.28 in the rice isolate population and 1.67 in the nonrice isolates. The mean gene diversity over all loci in the nonrice isolates was seven times higher than in the rice isolates ($0.21 \pm 0.22$ vs. $0.03 \pm 0.12$). The large standard deviations associated with the mean gene diversities illustrated the heterogeneity in the level of variability among enzyme loci. For instance, almost all the estimated genetic variability in the rice isolate population was attributed to two loci, G2dh and Ldh1.

TABLE 3. Enzymes from *Magnaporthe grisea* analyzed by starch gel electrophoresis

| Enzyme | Abbreviation | E.C. number | Buffer system[a] |
|---|---|---|---|
| Aconitase | Aco | 4.2.1.3 | A,B |
| Aspartate aminotransferase | Aat | 2.6.1.1 | A,D |
| α-Esterase | αEst | 3.1.1.1 | A,B,C |
| Fumarase | Fum | 4.2.1.2 | A |
| Glycerate-2-dehydrogenase | G2dh | 1.1.1.29 | A,B |
| Hexokinase | Hk | 2.7.1.1 | A,B,C,D |
| Glutamic-pyruvic transaminase | Gpt | 2.6.1.2 | B,C |
| Lactate dehydrogenase | Ldh | 1.1.1.27 | A,B,C |
| Malate dehydrogenase | Mdh | 1.1.1.37 | A,D |
| Phosphoglucose isomerase | Pgi | 5.3.1.9 | A,B,C,D |
| Phosphoglucomutase | Pgm | 2.7.5.1 | A,B,C |
| Xanthine dehydrogenase | Xdh | 1.2.3.2 | B,C |

[a] Buffer systems giving adequate separation of enzymes: A = amine-citrate, pH 6.1; B = Tris-borate EDTA, pH 8.0; C = Tris-citrate/lithium-borate, pH 8.5/pH 8.1; and D = Tris-citrate, pH 6.3.

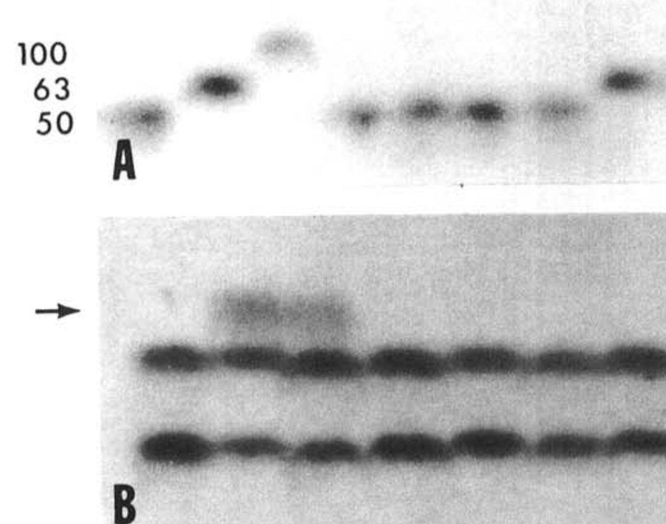


**Fig. 1.** Electrophoretic variation in enzyme loci of *Magnaporthe grisea* detected by starch gel electrophoresis. **A,** Variation at the glycerate-2-dehydrogenase (G2dh) locus showing three electrophoretic forms found among isolates from rice ($G2dh^{100}$), crabgrass ($G2dh^{50}$), and weeping lovegrass ($G2dh^{63}$). **B,** Variation at the lactate dehydrogenase-1 (Ldh1) locus (arrow) showing the functional and the null alleles found among the Korean rice isolates.

The frequency of electrophoretic types (ETs) (distinct combinations of electromorphs over all enzyme loci examined) in the total sample provides another measure of genetic variability in *M. grisea* (Table 5). Although 12 distinct ETs were identified, most of the isolates (90%) belonged to two common types (ET1 and ET2), which differed by having either the functional or the null allele at the Ldh1 locus. Other ETs were found in low frequencies ranging from 3.25 to 0.27%. Most of the ETs differed from the common types (ET1 and ET2) by one to three allelic variants, with the exception of the Wisconsin crabgrass isolates (ET4), which differed from the common types at seven loci. A single isolate that had an electrophoretic pattern identical to that of the Wisconsin isolates was found among the nonrice isolates from Japan. This isolate was probably obtained from crabgrass, though we were unable to confirm its origin.

For the analysis of genetic differentiation among geographic populations of the rice isolates, only the Ldh1 locus is informative. Among all rice isolates tested, the frequencies of the functional allele ($Ldh1^{100}$) and the null allele ($Ldh1^{n}$) were 57.5 and 42.5%, respectively. The frequency distributions of the two alleles in geographic populations are shown in Table 6. Of the 12 populations, seven were fixed for one allele and five had both alleles at intermediate frequencies. In an attempt to establish the nature of the geographic distribution of the Ldh1 alleles, two hypotheses were tested. First, we tested whether the Ldh1 allelic distribution followed a north-south latitudinal trend, and second, whether there was an association between the Ldh1 alleles and the types of rice from which the fungus was isolated.

To test the existence of latitudinal trend, frequencies of $Ldh1^{100}$ allele from 10 rice isolate populations were used for regression analysis. The Taiwan and Brazil populations were not included in the analysis because of the small population size. We found a significant positive correlation ($r = 0.67$, $P < 0.05$) between $Ldh1^{100}$ frequencies and latitudes (Fig. 2). Although the correlation was significant, the latitudinal effect did not account for the observed allelic frequencies in certain populations. In the Korean population (36.3° N), the $Ldh1^{100}$ (43%) and $Ldh1^{n}$ (57%) alleles were equally frequent, whereas the nearby populations of Japan (36.0° N) and North China (41.0° N) were fixed for the $Ldh1^{100}$ allele. Furthermore, only the null allele was found in the crabgrass isolates from Wisconsin (the northernmost population in the collection).

In testing the hypothesis of association between Ldh1 alleles and rice hosts, we considered two geographic races of rice (*Oryza sativa* L.), *japonica* and *indica*, which are grown predominantly in the temperate and tropical regions, respectively. With the assistance of the International Rice Research Institute, Los Baños, The Philippines, and the Institute of Crop Germplasm Resources at Beijing, China, 133 rice lines and cultivars were identified as either *japonica* or *indica*. The null hypothesis of independence between the Ldh1 alleles and the rice geographic races was tested using a chi-square $2 \times 2$ contingency table (38) (Table 7). The calculated $\chi^2$ value (42.5, 1 df adjusted) was highly significant ($P = 0.001$), suggesting a strong association between $Ldh1^{100}$ allele with *japonica* rice and $Ldh1^{n}$ allele with *indica* rice. However, the

TABLE 4. Electrophoretic variation at 10 enzyme loci of rice and nonrice isolates of *Magnaporthe grisea*

| | | Grouping | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rice isolate[c] | | Nonrice isolate[d] | | All isolates | |
| Locus[a] | Allele[b] | No. of isolates | % | No. of isolates | % | No. of isolates | % |
| Hk1 | 100 | 335 | 100.0 | 25 | 73.5 | 360 | 97.6 |
| | 90 | 0 | 0.0 | 9 | 26.5 | 9 | 2.4 |
| Gene diversity (H)[e] | | | 0.0 | | 0.39 | | 0.05 |
| Hk2 | 100 | 335 | 100.0 | 25 | 73.5 | 360 | 97.6 |
| | 57 | 0 | 0.0 | 9 | 26.5 | 9 | 2.4 |
| H | | | 0.0 | | 0.39 | | 0.05 |
| Aco | 100 | 335 | 100.0 | 25 | 73.5 | 360 | 97.6 |
| | 133 | 0 | 0.0 | 9 | 26.5 | 9 | 2.4 |
| H | | | 0.0 | | 0.39 | | 0.05 |
| Pgm | 100 | 335 | 100.0 | 26 | 76.5 | 361 | 97.8 |
| | 107 | 0 | 0.0 | 2 | 5.9 | 2 | 0.6 |
| | 80 | 0 | 0.0 | 6 | 17.6 | 6 | 1.6 |
| H | | | 0.0 | | 0.38 | | 0.04 |
| Pgi | 100 | 335 | 100.0 | 24 | 70.6 | 359 | 97.3 |
| | 113 | 0 | 0.0 | 1 | 2.9 | 1 | 0.27 |
| | 92 | 0 | 0.0 | 9 | 26.5 | 9 | 2.44 |
| H | | | 0.0 | | 0.43 | | 0.05 |
| Mdh3 | 100 | 334 | 99.7 | 24 | 70.6 | 358 | 97.0 |
| | 67 | 0 | 0.0 | 9 | 26.5 | 9 | 2.44 |
| | 266 | 1 | 0.3 | 0 | 0.0 | 1 | 0.27 |
| | Null | 0 | 0.0 | 1 | 2.9 | 1 | 0.27 |
| H | | | 0.0 | | 0.43 | | 0.06 |
| G2dh | 100 | 323 | 96.4 | 24 | 70.6 | 347 | 94.00 |
| | 75 | 12 | 3.58 | 0 | 0.0 | 12 | 3.25 |
| | 63 | 0 | 0.0 | 1 | 2.9 | 1 | 0.27 |
| | 50 | 0 | 0.0 | 9 | 26.5 | 9 | 2.44 |
| H | | | 0.07 | | 0.43 | | 0.12 |
| Ldh1 | 100 | 189 | 56.4 | 23 | 67.6 | 212 | 57.5 |
| | Null | 146 | 43.6 | 11 | 32.4 | 157 | 42.5 |
| H | | | 0.49 | | 0.44 | | 0.49 |
| Ldh2 | 100 | 334 | 99.7 | 34 | 0.0 | 368 | 99.7 |
| | 93 | 1 | 0.3 | 0 | 0.0 | 1 | 0.3 |
| H | | | 0.01 | | 0.0 | | 0.01 |
| Ldh3 | 100 | 334 | 99.7 | 21 | 61.76 | 355 | 96.21 |
| | 83 | 0 | 0.0 | 4 | 11.76 | 4 | 1.08 |
| | 91 | 1 | 0.3 | 0 | 0.0 | 1 | 0.27 |
| | 93 | 0 | 0.0 | 9 | 26.5 | 9 | 2.44 |
| H | | | 0.01 | | 0.53 | | 0.07 |

[a] G2dh = glycerate-2-dehydrogenase, Ldh = lactate dehydrogenase, Hk = hexokinase, Aco = aconitase, Pgm = phosphoglucomutase, Pgi = phosphoglucose isomerase, and Mdh = malate dehydrogenase.
[b] Numbers indicate relative electrophoretic mobility, with 100 designating the most common allele.
[c] Isolates from 12 geographic regions.
[d] Isolates from Japan, Wisconsin, and India.
[e] Gene diversity = $1 - \Sigma Xi^2$, where Xi is the frequency of the $i^{th}$ allele in a population (28).

TABLE 5. Frequency and origin of electrophoretic types (ET)[a] of *Magnaporthe grisea*

| | | | | Enzyme locus[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ET | No. of isolates | Frequency (%) | Host | Ldh1 | Ldh2 | Ldh3 | Hk1 | Hk2 | Aco | Pgm | Pgi | Mdh3 | G2dh |
| 1 | 189 | 51.22 | Rice and nonrice[c] | 100[d] | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2 | 146 | 39.57 | Rice | n | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 3 | 12 | 3.25 | Rice | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 75 |
| 4 | 9 | 2.44 | Crabgrass[e] | n | 100 | 93 | 90 | 57 | 133 | 100 | 92 | 67 | 50 |
| 5 | 6 | 1.63 | Unknown | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 |
| 6 | 3 | 0.81 | Finger millet | 100 | 100 | 83 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 7 | 1 | 0.27 | Weeping lovegrass | n | 100 | 83 | 100 | 100 | 100 | 107 | 100 | n | 100 |
| 8 | 1 | 0.27 | Finger millet | 100 | 100 | 100 | 100 | 100 | 100 | 107 | 100 | 100 | 100 |
| 9 | 1 | 0.27 | Weeping lovegrass | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 113 | 100 | 63 |
| 10 | 1 | 0.27 | Rice | 100 | 100 | 91 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 11 | 1 | 0.27 | Rice | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 266 | 100 |
| 12 | 1 | 0.27 | Rice | 100 | 93 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

[a] Electrophoretic types are distinct combinations of electrophoretic alleles over all enzymes examined.
[b] Ldh = lactate dehydrogenase, Hk = hexokinase, Aco = aconitase, Pgm = phosphoglucomutase, Pgi = phosphoglucose isomerase, Mdh = malate dehydrogenase, and G2dh = glycerate-2-dehydrogenase.
[c] Includes finger millet (*Eleucine coracana*), goosegrass (*E. indica*), and weeping lovegrass (*Eragrostis curvula*).
[d] Numbers indicate relative electrophoretic mobility with 100 designating the common allele; n = null allele.
[e] *Digitaria sanguinalis*, host of one isolate unconfirmed.

association was not absolute because populations from India and Hunan, China, were fixed for the Ldh1[100] allele even though *indica* rice predominates in the two regions.

## DISCUSSION

The many pathogenic races of *M. grisea* continue to pose a major problem for plant breeders in the development of durable forms of blast resistance in rice. In spite of a number of hypotheses attempting to explain the high degree of pathogenic variability in the fungus, there is yet a need for more genetic information on the pathogen population from rice to provide a basis for understanding the reported variability. By randomly selecting isolates from a variety of rice cultivars in major rice-growing regions of the world and analyzing their electrophoretic variability at various enzyme loci, it should be possible to provide a better understanding of the extent and distribution of variability in the global population of the species. Such largely empirical information may be of value in interpreting data on pathogenic variation.

Although no attempt was made in this study to characterize pathogenic variation among the isolates sampled, a basic assumption was made that considerable pathogenic variation existed among the isolates. This assumption is likely to be valid because these isolates were obtained from more than 100 cultivars and breeding lines of both upland and paddy rice from 12 major rice-growing regions of the world. Most of these rice lines carry blast resistance of diverse origins, and therefore, isolates of *M. grisea* taken from them should represent a diversity of pathogenicity.

In contrast to the assumed high degree of genetic diversity conditioning pathogenicity, when electrophoretic analysis of 18 putative enzyme loci was carried out on 335 isolates of *M. grisea* from rice, relatively little variability was detected (proportion of polymorphic loci [P] = 0.11, mean gene diversity [$\bar{H}$] = 0.03). Among the 18 enzyme loci tested, only two, Ldh1 and G2dh, showed allelic diversity. On a global basis, six of the 12 regional populations were monomorphic for all loci. Populations from the United States, Colombia, Korea, Nigeria, and South China were polymorphic for Ldh1. A population from India was polymorphic for G2dh; however, this low variability is not characteristic of the species *M. grisea*. In the limited number of nonrice isolates examined, measures of genetic variation (P = 0.5, $\bar{H}$ = 0.21) are comparable to those found in natural populations of other species (see 23 for review). This higher level of variability in the nonrice isolates probably reflects the greater diversity of the hosts from which these isolates were derived. Patterns of niche-dependent genic variation have been reported in other species. Second (34) found a higher level of electrophoretic variability in the wild African rice (*O. breviligulata*), which presumably occupies more diverse habitats than the cultivated African rice (*O. glaberrima*). Also, among mushroom-feeding *Drosophila*, the polymycophagous species have more genic variation than the specialized feeders (20).

In spite of the considerable allelic diversity observed in the nonrice isolates, no electrophoretic variants were found in common between the nonrice and rice isolates. Three of the four variants (Ldh2[93], Ldh3[91], and Mdh3[266]) in the rice isolates were found only once in the entire collection at a frequency of 0.3% and are considered rare. The uniqueness of electrophoretic variants limited to the nonrice isolates (Table 5) provides no positive evidence for gene flow between the rice and nonrice isolates. A systematic sampling and analysis of rice and nonrice isolates from hosts in proximity would provide more substantial evidence for potential gene flow between subpopulations.

An important aspect of this study has been the unusually high degree of homogeneity observed among the rice isolates. These observations raise the question of how such genetic uniformity is maintained over a broad geographic range. One possible explanation is that individual pathogen populations were established by a few founder isolates that originated from a common ancestral population and hence only carried a fraction of the genetic variability that existed in the species (25). According to Chang (9), most rice cultivars grown in North America, South America, and Africa were introduced from either temperate or tropical Asia. If the dissemination of *M. grisea* was effected through the distribution of rice germ plasm, many isolates around the world could have originated from a few common ancestral populations. Furthermore, the use of blast-resistant rice cultivars may have caused drastic reductions in population size within a region, leading to further reduction in genetic variability of the population.

Although the founder effect may account for genetic invariability within a region, it is inadequate to explain the uniformity of enzyme phenotypes over a broad geographic range. Even within a founder population, it is anticipated that subsequent population growth and environmental heterogeneity would restore most variability over time (25). Mutation rates for enzyme loci based on a number of species (barley [17], *Drosophila* [26], and human [27]) have been estimated in the range of $10^6$ per locus per

TABLE 6. Geographic distribution of the functional and null allele at the lactate dehydrogenase-1 locus of *Magnaporthe grisea*

| Country or region | Latitude | No. of isolates | Allelic frequency (%) | |
|---|---|---|---|---|
| | | | Functional | Null |
| Liaoning, China | 41.0° N | 23 | 100 | 0 |
| Korea | 36.3° N | 28 | 43 | 57 |
| Japan | 36.0° N | 10 | 100 | 0 |
| United States[a] | 30.5° N | 30 | 90 | 10 |
| Hunan, China | 28.0° N | 25 | 100 | 0 |
| Taiwan | 23.3° N | 7 | 100 | 0 |
| Guangdong, China | 23.1° N | 32 | 19 | 81 |
| India | 20.3° N | 56 | 100 | 0 |
| Philippines | 14.4° N | 55 | 0 | 100 |
| Nigeria | 7.2° N | 38 | 21 | 79 |
| Colombia | 3.3° N | 22 | 20 | 80 |
| Brazil | 9.0° S | 9 | 100 | 0 |

[a] Includes Louisiana, Texas, Florida, and Arizona.

TABLE 7. Association of *Magnaporthe grisea* isolates containing the functional and the null allele of lactate dehydrogenase-1 (Ldhl) with *japonica* and *indica* rice[a]

| Ldhl allele | Rice geographic race | | Total |
|---|---|---|---|
| | *japonica* | *indica* | |
| Functional | 46 | 18 | 64 |
| Null | 10 | 59 | 69 |
| Total | 56 | 77 | 133 |

[a] Chi-square test for association: adjusted $\chi^2 = 42.5$, 1 df, $P = 0.001$.

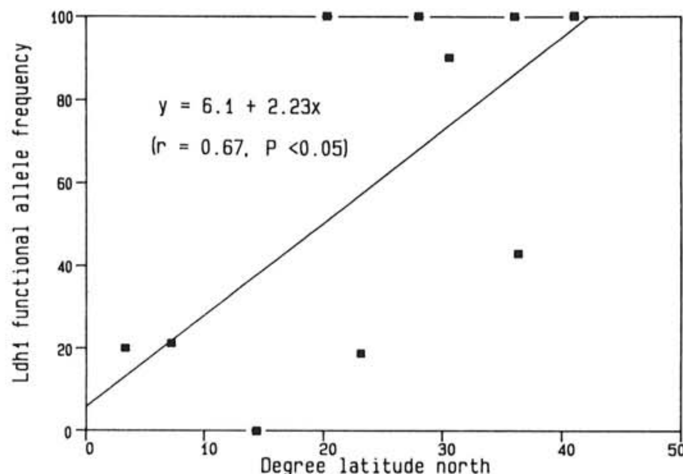


**Fig. 2.** Regression of the frequencies of lactate dehydrogenase-1 (Ldh1) functional allele on latitudes of 10 geographic populations of *Magnaporthe grisea* isolates from rice.

generation. If this mutation rate is applied to the enzyme loci in *P. oryzae*, and given the high reproductive potential of the fungus (4,000 conidia per lesion per night) (32), considerable variability would be expected. The fact that little enzyme variability is observed in the rice isolates of *M. grisea* suggests that either most electrophoretic variants are deleterious or that a set of coadapted enzyme loci in *M. grisea* has been selected under the influence of the rice host. Indeed, the strong associations between $Ldh1^{100}$ with *japonica* rice and $Ldh1^{n}$ with *indica* rice (Table 7) supports the notion of possible linkage between Ldh1 and factors conditioning host specificity. However, because the distribution of *japonica* and *indica* rice follows a north-south trend, the distribution of the Ldh1 alleles might have resulted from direct climatic or geographic effects on the fungus (Fig. 2).

In a comparative study of enzyme and pathogenic variation in the Australian wheat rust flora, Burdon et al (5) found no enzyme variation among 58 isolates of the stem rust fungus (*Puccinia graminis* f. sp. *tritici*) or among 66 isolates of the leaf rust fungus (*P. recondita* f. sp. *tritici*) though the two pathogen populations were shown to be pathotypically diverse. In studies with other animal and plant species, there has been evidence indicating ecological divergence of morphological characters in the absence of appreciable enzyme differentiation (2,13,16,19,40). For instance, Giles (13) found little variation in enzyme phenotypes in natural populations of wild barley (*Hordeum murinum* L.), which showed variation in morphological and reproductive characters. Also, Jain et al (16) observed a low level of electrophoretic variation in *Amaranthus* species, which displayed ubiquitous morphological variation.

These observations suggest that enzyme loci, at least in some instances, might represent a unique set of characters that are subject to different modes of selection and hence show a different pattern of variability. If this variance between the level of heterogeneity in enzyme loci and the presumed pathogenic diversity in *M. grisea* reflects fundamental differences in the evolution of the two sets of characters, then the analysis of enzyme variability as a probe into the basis of pathogenic variation in *M. grisea* will be of limited value. Before such a conclusion can be drawn, a more comprehensive analysis of the pathogenic variability of isolates of known enzyme phenotypes must be completed.

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