Phenotypic Characterization of the Rice Blast Resistance Gene *Pi-2(t)*


**ABSTRACT**


The spectrum of resistance conditioned by the blast resistance gene *Pi-2(t)* in rice was characterized. Near-isogenic line (NIL) C101A51 carrying *Pi-2(t)* in the genetic background of the indica rice cultivar CO39 was resistant to 455 Philippine isolates of *Pyricularia grisea* when inoculated in greenhouse tests. The *P. grisea* isolates, collected mainly from 1983 to 1992, represented at least 18 lineages based on DNA fingerprinting data. The NIL C101A51 was planted at two sites used by the International Rice Research Institute for the evaluation of blast resistance, and for which the *P. grisea* populations had been analyzed by DNA fingerprinting. After initial resistance at the two sites, C101A51 eventually showed susceptible lesions. All isolates (n = 60) collected from C101A51 at the two sites were identified as belonging to a single lineage (more than 95% band similarity using the repetitive DNA probe MGR586), distinct (less than 80% band similarity) from the approximately 2,000 Philippine isolates previously subjected to DNA fingerprinting. The group of isolates, designated "lineage 44," was inoculated to a set of NILs in the CO39 genetic background in greenhouse tests. Lineage 44 was found to be compatible to lines carrying *Pi-2(t)* (in C101A51 and S173, the donor of *Pi-2(t)*, *Pi-4(t)* (in C102PKT), *Pi-4(t) + Pi-? (in C105TPP-4-L23), and CO39. C101A51 was found to be resistant in nursery tests at 24 of the 40 sites in 16 countries in which it was tested during 1991 to 1993 through the International Network for the Genetic Evaluation of Rice. These results suggested that *Pi-2(t)* could condition resistance to many lineages of *P. grisea* and could be useful if pyramided or otherwise deployed with genes that condition resistance to lineage 44.

Additional keywords: disease resistance, major genes

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Rice blast, caused by *Pyricularia grisea* (Cooke) Sacc. (*Pyricularia oryzae* Cavara) (teleomorph: *Magnaporthe grisea*), is a serious constraint to rice (*Oryza sativa* L.) production worldwide (18). It commonly causes leaf blast during the vegetative phase of rice plant development and infertility when plants are infected during the reproductive phase (panicle and node blast). This latter effect can result in dramatic yield and quality reductions, with obvious negative economic consequences. Because most rice farmers are resource-poor, resistant cultivars play an important role in blast management. However, resistance is often short-lived, with cultivars released as resistant showing susceptibility after only a few seasons of widespread cultivation. Although resistance conditioned by single major genes has often proved unstable (14), major-gene resistance to blast has been useful (4) and should continue to be important in rice production if resistance genes are carefully selected and managed.

The availability of a set of near-isogenic lines (NILs) provides an opportunity to characterize resistance genes in a common genetic background (17). The concept of "resistance spectrum" (the qualitative and quantitative reactions of a host plant conditioned by a resistance gene to different pathogen subpopulations) is useful (2). Characterization of resistance genes in this way should be useful in choosing appropriate genes for varietal improvement programs (24) and for designing deployment strategies for existing rice cultivars.

A set of NILs in the CO39 genetic background has been characterized in several recent studies (12,24). In these studies, the resistance spectrum of one gene, *Pi-2(t)*, was found to be quite broad. This gene, in the NIL C101A51, was derived from the breeding line 5173, developed at the Centro Internacional de Agricultura Tropical (CIAT). The resistance gene in line 5173 was backcrossed six times to cultivar CO39 (17). *Pi-2(t)* was found to be linked to DNA marker RG64 on rice chromosome 6 (23) and was considered to be allelic with *Pi-2z* (10,11).

During initial studies, no isolates compatible with this gene were found in the Philippines. Recently, however, compatible isolates have been found (6). The expression of resistance in leaf and neck tissues was examined in this study. We also report on analyses of nursery data from 40 multilocation tests in which C101A51 was included in the International Network for the Genetic Evaluation of Rice.

**MATERIALS AND METHODS**

**Plant materials.** The test materials for this study included a series of NILs carrying major genes conditioning resistance to *P. grisea*. The NILs were C101A51 [*Pi-2(t)*], C101LAC [*Pi-1(t)*], C104PKT [*Pi-3(t)*], C101PKT [*Pi-4(t)*], C102PKT [*Pi-4(t)*], and C105TPP-4-L23 [*Pi-4(t) + Pi-?*] (11,12,17). The donor for *Pi-2(t)*, S173, and the recurrent parent for the NILs, CO39, were also included as checks. Both C101PKT [*Pi-4(t)*] and C102PKT [*Pi-4(t)*] were used, because these lines showed differential reactions in some inoculation experiments (17). Although allelism tests indicated that both lines carried *Pi-4(t)* and the differential reactions were not always repeatable (11), it was possible that an additional unidentified gene(s) could be present in the line(s).

**Nursery trials.** C101A51 and its recurrent parent, CO39, were included in 40 international rice blast nursery (IRBN) trial sites in 16 countries in Asia, Africa, Europe, and Latin America from 1991 to 1993. IRBN trials were done under upland conditions. Each test entry was planted in one row (30 cm), and entries were spaced at 10 cm in a plot 1.2 m wide and 15 to 20 m long, depending on local needs. Susceptible checks were planted after every 25 or 50 test entries to check uniformity of infection. A mixture of several broadly susceptible local cultivars was planted in two to three rows lengthwise and at each end of the plot along the border to ensure presence of inoculum consisting of diverse races of the blast pathogen. A minimum of 100 to 120 kg N in the form of ammonium sulfate was applied per hectare, with half at seeding and half at 15 days after seeding. Super phosphate at the rate of 50 kg P2O5 per ha was applied before seeding. Plots were watered twice or more per day if there was no rain. At some sites, the plots were covered with a plastic sheet during the night to prolong the dew period. Although natural inocula initiated blast development in most test sites, diseased rice plants were transplanted into the plot in several places to ensure sufficient inoculum. Leaf blast and neck blast were evaluated following the standard evaluation system (SES) (9). In 1992, resistance of C101A51, CO39, and 36 other entries

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was evaluated at two blast screening sites of the International Rice Research Institute, Cavinti, and the Blast Nursery (IRRI-BN). Starting from the 1994 dry season, C101AS5 was re-evaluated in the IRRI-BN in seven sequential plantings at 15-day intervals (13). Disease severity was visually estimated as percentage of diseased leaf area (DLA) and percentage of infected panicles.

**DNA extraction and fingerprinting of P. grisea isolates.** Monocolial isolates were cultured for 7 days with constant shaking at room temperature (25 to 30°C) in Fries medium supplemented with 0.5 g of casein hydrolysate per liter. Genomic DNA of the isolates was extracted from lyophilized mycelia using a modified potassium acetate procedure (21). For DNA fingerprinting, genomic DNA was digested with EcoRI, and DNA fragments were separated by horizontal agarose (0.8%) electrophoresis at 60 volts in 0.5x TBE for 24 h with known DNA markers as size standards. DNA was denatured and transferred to nylon membrane (Hybond-N+; Amersham Corp., Arlington Heights, IL) by the alkaline transfer method, as recommended by the manufacturer. Blots were probed with labeled MGR586 (8) according to the procedure described in the Boehringer Mannheim nonradioactive DNA labeling and detection kit (Genius Kit, Boehringer Mannheim (Far East) Pte. Ltd., Singapore).

**Greenhouse inoculations.** Ten seeds of each line/cultivar were sown in a 9 × 9 cm plastic pot containing ground IRRI upland soil. There were three replicates for each line/cultivar. Plants were fertilized after sowing with ammonium sulfate at a rate of 6 g/kg of soil. Seedlings were grown in a greenhouse for 21 days before inoculation. Inoculum preparation and seedling inoculation followed the procedure described by Bonman et al. (5). Briefly, stock cultures were revived on prune agar slants. About 6 ml of sterilized distilled water was poured into the slant and the mycelial growth was macerated with a flame needle. The suspension of mycelial fragments was spread over the surface of prune agar plates. The seeded plates were incubated at 25 to 28°C for a week until the entire agar surface was covered with mycelial growth. The mycelial growth was scraped with a sterilized glass slide or spatula, and the plate was exposed to fluorescent light for 3 to 4 days to stimulate sporulation. The conidia were harvested by pouring 15 ml of sterilized distilled water with 0.02% Tween 20 onto each sporulating plate culture and scraping the surface to dislodge the spores. The spore suspensions were filtered through four layers of nylon mesh before standardizing the concentration to 5 × 10⁴ conidia per ml using a hemacytometer. Seventy-five milliliters of spore suspension was sprayed on 21-day-old seedlings (five- to six-leaf stage) in 18 pots on a rotating platform.

### Table 1. Performance of C101AS5 in International Rice Blast Nurseries from 1991 to 1993

<table>
<thead>
<tr>
<th>Score</th>
<th>Sites</th>
<th>Country: test sites</th>
</tr>
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<tbody>
<tr>
<td>1-3</td>
<td>24</td>
<td>Korea: Iri, Suwon, Milyang; China: Guangzhou, Luzhou, Fuyang; Indonesia: Kendari; Malaysia: Seberang Perai; Thailand: Phrae, Chiang Mai, Nakorn Si Thammarat, Prachinburi, Mae Hong Son, Bangkhen; Vietnam: Onom; India: Nellore, Pondicherry, Rajender Nagar; Nepal: Khamti; Sri Lanka: Galle; Egypt: Sakhr; Iran: Rasht; Nigeria: Ibadan, Italy: Mortara</td>
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<tr>
<td>4-6</td>
<td>10</td>
<td>Korea: Icheon; China: Fuzhou, Neijiang, Changsha, Chengdu; Taiwan (China): Chiayi; Philippines: Las Baños; Thailand: Phitsanulok; India: Haveli, Iran: Tonekabon</td>
</tr>
<tr>
<td>7-9</td>
<td>6</td>
<td>Indonesia: Sitang; Thailand: Ubon Ratchathani, Rachaburi, Phatthalung; India: Lonavala; Colombia: Sta. Rosa</td>
</tr>
</tbody>
</table>

*1-3 = Resistant, 4-6 = moderately susceptible, 7-9 = highly susceptible.*

**Fig. 1.** DNA band profiles of isolates of *Pyricularia grisea* representing different lineages defined by MGR586. Lineage designations of the isolates are labeled at the top.
Table 2. Reactions of lineage 44 isolates of 
Pyricularia grisea to the near-isogenic lines with different major genes

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<td>S</td>
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</table>

Table 3. Percentage of diseased leaf area on the top fully expanded leaf of the near-isogenic lines and CO39 inoculated with lineage 44 isolates in a greenhouse

<table>
<thead>
<tr>
<th>Isolate</th>
<th>C101A51 Pl-2(t)</th>
<th>C104PKT Pl-3(t)</th>
<th>CO39</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9240-1</td>
<td>83 A</td>
<td>71 A</td>
<td>49 B</td>
</tr>
<tr>
<td>C9240-2</td>
<td>52 A</td>
<td>60 A</td>
<td>29 B</td>
</tr>
<tr>
<td>C9240-3</td>
<td>12 A</td>
<td>5 A</td>
<td>2 B</td>
</tr>
</tbody>
</table>

* Values in the same row followed by different letters were significantly different (P < 0.05).

Panicle blast. Four isolates showing compatible reactions with C101A51 seedlings were selected to determine their ability to infect panicles of C101A51. Plants were grown in flooded condition in a greenhouse until flowering. Main culms were marked and the distance from the flag leaf collar to the panicle node was measured, with a positive value given to the distance of the panicle node above the flag leaf collar and a negative value given to the distance of the panicle node below the leaf collar. A piece of cotton was wrapped around the panicle node to retain the inoculum and to maintain a moist microenvironment (20). Five milliliters of spor suspension (5 x 10^6 sporae per ml in sterilized distilled water with 0.02% Tween 20) of the isolate compatible with C101A51 were dripped with a syringe onto each panicle. Ten panicles were exposed per replicate, and three replicates per isolate were conducted. Sterilized distilled water (with 0.02% Tween 20) was used as a check. Inoculated plants were maintained in a dew chamber for 24 h at 26°C and then placed in a mist room at 24 to 28°C for 6 and 20 days for leaf and panicle blast evaluation, respectively.

Disease reactions were scored 7 days after inoculation for leaf blast using a 0 to 5 scale (17). Percentage of neck blast and spikelet infection were evaluated 21 days after inoculation. The effectiveness of different isolates in causing neck blast and spikelet infection was analyzed by Duncan's multiple range test. To determine the relationship between panicle age and occurrence of neck blast and spikelet infection, the original percentage data from counts transformed using arcsine, and the transformed data for four isolates were pooled by replicate for regression analysis.

RESULTS

Reaction of C101A51 to P. grisea under nursery conditions. C101A51 developed little or no blast at most sites where the IRBN was planted (Table 1). Among 118 trials conducted at 40 IRBNs from 1991 to 1993, C101A51 was rated as highly susceptible (blast scores 7 to 9) in only nine trials. In the Philippines trial, blast on C101A51 was scored as 4 (moderately resistant). Among 27 neck blast evaluations, scores greater than 7 were observed in 10 trials. The reactions of C101A51 to leaf blast and neck blast did not always coincide (r = 0.65 from correlation analysis between leaf blast and neck blast scores).

Susceptible lesions on C101A51 were first observed at Cavinti in 1992, and then in IRRI-BN in 1994. No infection of C101A51 was observed at IRRI-BN in 1992 in either the dry or the wet season experiments, although other cultivars were heavily infected in the dry season and moderately infected in the wet season. At Cavinti in the 1992 wet season, no disease was observed until flowering, while many susceptible cultivars were killed during seedling stage. However, susceptible lesions were observed on the flag leaf of C101A51 at the flowering stage. Panicle (neck and spikelet) blast was observed thereafter, with 30% of the panicles showing disease.

At the IRRI-BN during the 1994 dry season, no disease was observed at the seedling stage in the first planting. Disease increased gradually during sequential plantings from the second to the seventh, when the average DLA reached 40%. For CO39, the disease rating was consistently high, with DLA of 60 to 80% recorded even during the first three plantings.

Lineage and pathotypic analysis of isolates attacking C101A51. C101A51 was free of disease in the IRRI-BN in 1992, as noted above, and none of the 901 IRRI-BN isolates that were subjected to DNA fingerprinting were found to belong to lineage 44 (6,7). When disease was observed on C101A51 in 1994, 47 isolates were collected from C101A51 in IRRI-BN and subjected to DNA fingerprinting using MGR586. All the isolates showed a single DNA banding profile (haplotype). This haplotype was very similar to those of isolates taken from C101A51 in Cavinti in 1992, differing only in one band from the isolates previously designated as belonging to lineage 44 (Fig. 1) (6,7).

To evaluate the virulence spectra of isolates of lineage 44, 16 monoclonal isolates were inoculated onto C101LAC [Pl-1(t)], C101PKT [Pl-P(t)+], C102PKT [Pl-P(t)+], C105TPP-4.L23 [Pl-P(t)+ + Pi-7], C104PKT [Pl-3(t)], C101A51 [Pl-2(t)], 5173 (donor of Pl-2(t)), and CO39 (recurrent parent of the NILs) seedlings. Compatible reactions were observed on C101A51 [Pl-2(t)], 5173 (donor of Pl-2(t)), C104PKT [Pl-3(t)], C102PKT [Pl-P(t)+], C105TPP-4.L23 [Pl-P(t)+ + Pi-7], and CO39, but incompatible reactions on C101LAC [Pl-1(t)] and C101PKT [Pl-P(t)+] (Table 2). These results suggest that lineage 44 is compatible with CO39 and the CO39 NILs carrying Pl-2(t), Pl-3(t), and Pl-P(t)+, but is incompatible with the NILs carrying Pi-7. The differential reactions of C101PKT [Pl-P(t)+] and C102PKT [Pl-P(t)+] to lineage 44 isolates suggest that C101PKT carries an additional unrecognized resistance gene that is apparently absent in C102PKT and C105TPP-4.L23.

For isolates of lineage 44, the DLA was greater for some lines carrying major genes (C101A51 carrying Pl-2(t) and C104PKT carrying Pl-3(t)) than for CO39 (Table 3). The DLA seen on CO39 for these isolates is rather low, suggesting that CO39 carries a gene(s) that reduces disease for lineage 44. It is unclear why this
Table 4. Resistance spectrum of Pi-2(t) in C101A51 to virulent isolates of Pyricularia grisea, collected in the Philippines from 1975 to 1994

| Lineage identity | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | Unknown |
| No. of isolates  | 49| 1 | 3 | 84| 2 | 79| 1 | 2 | 1 | 1 | 1 | 1 | 54 | 48 | 3 | 5 | 3 | 1 | 16 | 1 | 116|
| Compatibility %  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100|
| No. of hosts     | 24| 1 | 3 | 58 | 1 | 35 | 1 | 1 | 2 | 1 | 1 | 1 | 33 | 9  | 3 | 4 | 1 | 1 | 1 | 1 | 65 | 7 |
| No. of locations | 1 | 1 | 4 | 2  | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2  | 1 | 1 | 1 | 1 | 2 | 2 | 1  | 1 |
| Collection years | 2 | 1 | 1 | 4  | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Fig. 2. Relationship between panicule infection and panicule age.

effect is not evident in the lines carrying the unlinked genes Pi-2(t) and Pi-3(t).

Reaction of C101A51 to P. grisea under greenhouse conditions. To determine the resistance spectrum of Pi-2(t) to subpopulations of P. grisea, 471 virulent monochromidal isolates representing at least 19 lineages were inoculated onto C101A51 [Pi-2(t)] seedlings, along with C101LAC, C104PKT, C101PKT, and CO39, in a greenhouse at IRRI. These isolates were collected from diverse rice cultivars and locations, mainly from 1983 to 1992. Because most of the isolates with available DNA fingerprint data were collected from the IRRI-BN and Cavinti, additional isolates lacking DNA fingerprint data (n = 116) were selected to represent a broader diversity of locations, hosts, and years of collection (Table 4). C101A51 carrying Pi-2(t) had a very broad resistance spectrum to P. grisea (Table 4). Among 355 isolates representing 19 lineages, and an additional 116 isolates of unspecified lineage, Pi-2(t) was resistant to all except the 16 isolates of lineage 44.

The four compatible lineage 44 isolates tested caused neck and spikelet blast on C101A51, ranging from 8 to 23% for neck blast and 59 to 71% for spikelet infection. No significant differences in neck blast and spikelet infection were observed among the isolates. To determine whether the variation in panicule infection was due to variation in panicule tissue age, the relationship between occurrence of neck blast and panicule age was evaluated. The age of panicule tissue is correlated with the distance between the panicule node and the flag leaf collar, with the basal tissue being the youngest. Panicles were grouped into age classes based on distance between the panicule node and the flag leaf collar: <5 cm, -5 to 0 cm (panicle node not emerged from flag leaf sheath), 0 to 5 cm and >5 cm (emerged). There was a strong negative correlation between panicule age and the occurrence of neck blast or spikelet infection (r = -0.91 for neck blast and r = -0.76 for spikelet infection) (Fig. 2). Neck blast occurred following inoculation of the three youngest classes, but no neck blast occurred when the panicule node was more than 5 cm above the flag leaf collar. These results suggest that C101A51 was fully susceptible to both leaf and neck blast caused by lineage 44 isolates, although the degree of susceptibility to neck blast decreased with panicule age.

DISCUSSION

The use of major gene resistance is an inexpensive and environment-friendly strategy for disease management, but resistance has often been short-lived. While the use of single major genes is associated with instability (14), major genes may contribute to stable resistance if properly used (4). Durable resistance may be achieved by the careful design of "gene pyramids" (especially resistance genotypes with multiple major and minor genes for resistance) and prudent deployment and management of such genotypes.

Information on the resistance spectrum of available genes should be useful in the selection of gene combinations for breeding and deployment (1,16,19,25). The availability of a set of near-isogenic lines of rice carrying different blast resistance genes makes it possible to carefully characterize the resistance spectra of single major resistance genes (17). Based on available data, Pi-2(t) in C101A51 appeared likely to be useful for cultivar improvement. In a previous study, C101A51 [Pi-2(t)] was reported to be susceptible only to lineage 44 isolates among the 234 isolates representing six lineages tested (6,24).

This study was undertaken to further characterize and document the phenotypic reactions of Pi-2(t). The resistance gene Pi-2(t), likely an allele to Pi-2(T) (10), has an extremely broad resistance spectrum. In 118 trials conducted at 40 sites over 3 years as part of the International Network for the Genetic Evaluation of Rice, C101A51 was highly susceptible in only nine trials. At two sites in Laguna, Philippines, C101A51 was found to be infected only by lineage 44 of the pathogen. Pi-2(t) conditioned resistance to over 450 isolates representing most of the known lineages of P. grisea in the Philippines and was compatible only with isolates of lineage 44, which has only been isolated from C101A51 (6,7).

Although lineage 44 was only isolated from C101A51, isolates of this lineage could attack CO39 and CO39 NILs carrying Pi-2(t) (C101A51), Pi-3(t) (C104PKT), and Pi-4(t) (C102PKT), but could not attack C101LAC carrying Pi-1(t) and C101PKT carrying Pi-4(t). C101PKT and C102PKT were classified into two groups based on their reactions to five Philippine isolates of P. grisea (17). Inukai et al. (11) reexamined these reactions, conducted allelism tests, and concluded that C101PKT and C102PKT both carried Pi-4(t), which was allelic or closely linked and possibly identical to Pi-ta. The consistent differential reactions observed for C101PKT and C102PKT to lineage 44 isolates tested in this study indicate that C101PKT carries an additional gene conferring resistance to lineage 44. Genetic analysis of this additional resistance gene is underway.

The broad effectiveness of Pi-2(t) suggests that it could be an extremely useful gene in rice blast resistance breeding programs, in combination with other genes with complementary resistance spectra. Combining Pi-2(t) with a resistance gene(s) effective against lineage 44 should provide effective resistance, as proposed for a "lineage exclusion" breeding approach (25). Lines carrying Pi-2(t) in combination with other major genes have been constructed, and their effectiveness against blast is under investigation (S. Hittalmani and N. Huang, IRRI, personal communication).

Because field infection of C101A51 at Cavinti was first observed on flag leaf and panicule, we suspected that Pi-2(t) may confer only seedling resistance. However, controlled inoculations and sequential plantings showed that isolates could infect the rice plant at both the vegetative and the reproductive phase. The occurrence of neck blast was negatively correlated with panicule age. This result was consistent with the findings of Willis et al. (22) and Amin (3). In contrast, Roumen (20) reported that lesion length, but not infection efficiency, was reduced with panicule age.

The late season appearance of lineage 44 could simply reflect its low frequency in the pathogen population. Alternatively, lineage 44 could have been a recent mi-
grant to the site or could have arisen from a recombination event. Parasexual recombinants detected by MGR586 DNA fingerprinting in laboratory studies, however, have shown evidence of exchange of only small DNA fragments (R. S. Zeigler, R. P. Scott, H. Leung, A. A. Bordeos, and R. J. Nelson, IRRI, unpublished). The DNA profile of lineage 44 is very different from those of the other lineages present at the site, so parasexual exchange of small chromosomal segments is unlikely to explain the appearance of this lineage.

In this study, C101A51 was found to have a broad spectrum of resistance. Field infection was shown to result from susceptibility to only one of the lineages of the blast pathogen known in the Philippines. Further analysis is required to determine the resistance spectrum of the gene at other sites. Available evidence, however, suggests that the broad spectrum of resistance may be a general feature of this gene. When C101A51 [P2-2(t)] was planted in Santa Rosa, Colombia, it was infected predominantly by lineage SRL-1 (F. Correa-Victoria, Centro Internacional de Agricultura Tropical, personal communication). The MGR586 fingerprint patterns of lineages 44 and SRL-1 isolates are very different (15, 25), but both are correlated with narrow virulence spectra.

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LITERATURE CITED