Pathogenic Variability in *Pyricularia grisea* at a Rice Blast "Hot Spot" Breeding Site in Eastern Colombia

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ABSTRACT

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Forty-five international races of *Pyricularia grisea*, representing all nine race groups, were identified in a "hot spot" breeding site (Santa Rosa) in Colombia, with the largest number included in the IA group. The international race system did not fully describe the virulence spectrum of the isolates, since several races could be further differentiated into different pathotypes when local commercial cultivars were used as differentials. Compatibility was present in the pathogen population for at least 13 known resistance genes and resistance sources tested. Frequency of virulent phenotypes on the 42 cultivars tested ranged from 0.0 to 0.86, with no cultivar susceptible to all isolates. The lowest compatibility frequencies were associated with combinations of resistance genes. It was unusual to recover isolates compatible with cultivars K-8, Peta, Ceysvoni, IR-42, Fujisaka 5, Fukunishiki, Zenith, and NP-125. No isolates were recovered that were compatible with the newly released cultivars Oryzica Llanos 4 and 5 developed at this site, and very few infected CICA 9. Analysis of the compatibility frequency of isolates recovered from commercial rice cultivars revealed a marked specialization for cultivar origin. Some cultivars were infected mainly by isolates recovered from the same cultivar. Virulence factors were accumulated in the most virulent isolates, but no isolate was virulent to all rice cultivars. Regardless, matching virulence for all resistance genes is already present in the pathogen population, indicating that new combinations of resistance factors and/or new resistance genes are needed. Rare compatibility with particular cultivars suggests that combinations of certain virulence genes may be associated with poor fitness. Differences in the distribution of virulence genes of *P. grisea* among and within cultivars support the feasibility of gene deployment strategies.

The rice blast disease, caused by *Pyricularia grisea* (Cooke) Sacc., the anamorph of *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa, is a major factor limiting yields of rice (*Oryza sativa* L.) worldwide. The pathogen produces necrotic lesions on leaves of seedlings and on leaves, nodes, necks, and panicles of mature plants, with the latter causing the most severe yield losses. Incorporation of resistance into commercial cultivars has been the preferred means to protect the rice crop from the pathogen. However, breakdown of this resistance is common in many rice-growing areas, often shortly after cultivar release.

Many pathogenic races have been identified in *P. grisea*, and this variability has been cited as the cause of resistance breakdown (1,20). There is a lingering controversy over the origins of this diversity. Great pathogenic variation has been reported from single-spore isolates originating from single lesions and monoconidial subcultures (23,24), while other studies have shown isolates were pathogenically stable (2,17). Very recently, studies on DNA fingerprints of isolates from the United States indicated that apparently clonal lineages tend to conserve pathotype, as determined by an international set of differentials, although some divergence was detected (19). Genetic analysis of host cultivar specificity in *M. grisea* suggests a simple Mendelian inheritance of virulence genes controlling such specificity. It was also found that the extent of lesion development in rice is polygenic (28). Failure of breeding lines to encounter low frequency compatible pathotypes in a population, termed "cryptic error," may explain frequent resistance breakdowns (4). Breeding strategies to minimize the probability of escape include line selection in sites favorable for the pathogen, where populations and their inoculum pressure remain high throughout the season and pathogenic diversity is high (9,11). Within these "hot spot" sites, pathogen diversity is maintained by planting spreader rows with diverse resistance sources selected to increase "rare" pathotype frequency and thus maximize the probability of an encounter between a breeding line and a compatible race. Evaluating lines at a hot spot throughout the growing season permits assessment of resistance at both the vegetative and reproductive stages of the plant. An underlying assumption in this approach is that all pathotypes for the target production system are present at the site, albeit some in very low frequency, and that pathotypes do not arise de novo, or do so very infrequently.

In 1985, the CIAT Rice Program began breeding for rice blast resistance at a hot spot site, the Santa Rosa experimental and breeding farm in eastern Colombia (9). Preliminary indications are that resistance in some lines selected at this site is stable over time and space (8,9,12). However, before such an approach can be recommended as a practical means to develop durable blast resistance, it must be critically evaluated. The initial step is to characterize and monitor the pathogenic variability of the pathogen population. In this paper we summarize studies on the pathogenic structure of *P. grisea* populations in terms of race composition, compatibility with known resistance genes, frequencies of virulent phenotypes, and variability as reflected by pathogenicity on diverse rice cultivars. Although the teleomorph of *P. grisea* has not been found in nature, genetic crosses between rice strains of the fungus have been produced in laboratory conditions (28). Given that pathogenic variability could be enhanced and that new pathotypes could be created through sexual recombination, studies were conducted to determine if the teleomorph occurred at the site.

MATERIALS AND METHODS

Isolates. All isolates tested were collected on different dates between 1988 and 1991 at the CIAT rice breeding and experimental station (Santa Rosa) located approximately 20 km east of Villavicencio, Colombia (310 m elevation, latitude 40° north, longitude 73° west, on alluvial soils, pH 4.5–5.0, 3.0–4.0% organic matter) in an important rice-growing area and where rice blast is the principal production constraint. The growing season extends from April to December, with mean maximum and minimum temperatures of 30 and 21 C, respectively, and 2,700 mm of well-distributed rainfall.

A total of 174 isolates of *P. grisea* were collected from rice cultivars obtained from germ plasm banks at the Centro Internacional de Agricultura Tropical (CIAT) and the International Rice Research Institute (IRRI). The cultivars from CIAT, followed by the number of isolates, were: Linea 2, 17; Oryzica 1, 16; CICA 8, 15; Metica 1, 15; CICA 9, nine; Oryzica 2, nine; CICA 6, eight; Oryzica Llanos 5, seven; CICA 4, six; CICA 7, four; and Oryzica 3, three. The cultivars from IRRI, followed by the number of isolates, were: Ceysvoni, 44; IR-42, 12; Fanny, seven; and Zenith, two. Although most of these cultivars are highly sus-

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ceptible under Santa Rosa field conditions, Ceysvoni, IR-42, and Zenith are highly resistant, having shown low incidence and disease severity for several seasons. Oryzica Llanos 5, a commercial rice cultivar released in 1989, is the most resistant cultivar, exhibiting susceptible lesions only occasionally.

Infected samples were collected mainly from cultivars representing all rice cultivars released in Colombia between 1971 and 1989. Most of these cultivars are used in infection beds or spreader rows as sources of inoculum in breeders' plots at Santa Rosa. Cultivars grown commercially in the area during 1988–1991 were Oryzica 1 (the product of a blast resistance gene-pyramiding program at CIAT [5,22]), CICA 8, Linea 2, and Oryzica Llanos 5. Because Ceysvoni is used as a main source of resistance to *P. grisea* in CIAT's rice-breeding program, a large number of isolates were analyzed to detect shifts in virulence within the pathogen population. Fanny is a susceptible cultivar under most evaluations reported in the literature (14).

All collections were made from leaves and/or panicles infected in the field with naturally occurring inoculum. All cultures were derived from either mass or single conidial isolates obtained from single lesions. Cultures were maintained on V8 juice agar (27) and multiplied for inoculations on rice-polish agar (27) at 28 C under continuous light. Medium-term storage (−20 C) of isolates was as desiccated mycelium on V8 medium-impregnated sterile filter paper disks. Infected plant tissue was also stored by drying at 30–50 C for 30 min and then freezing at −20 C.

Tester lines. A set of 42 differential lines was assembled and included the international differentials (1), resistance sources with known resistance genes (16), and resistance sources with undescribed resistance genes, all received from IRRI's germ plasm bank, plus selected commercial cultivars obtained from CIAT's germ plasm bank. The choice of rice cultivars was based on differential host responses observed previously (9). The cultivar Fanny was included in each inoculation as a check. Seedlings were grown to the three- to four-leaf stage (18–21 days after planting) in the greenhouse at 20–30 C and 12-hr day length in plastic pots (15 cm diameter) and fertilized with ammonium sulfate in three equal fractions (time of planting, 7 days later, and 1 day before inoculation) to the equivalent of 180 kg/ha of nitrogen.

Inoculation and cultivar evaluation. Each isolate was inoculated on all 42 cultivars on 18- to 21-day-old plants, 10 per pot per rice cultivar, in two replications repeated in time until uniform reactions were obtained. Plants were sprayed (10–15 psi) with a constant volume (100 ml) of conidial suspension ($1–5 \times 10^5$ conidia ml^{-1}) in 0.5% gelatin to runoff. Inoculated plants were incubated in plastic chambers under 100% relative humidity at night and opened during the day (20–32 C). Disease reaction was evaluated after 7 days.

Lesion types (26) and percentage of leaf area affected were evaluated for each seedling. A cultivar was considered susceptible when more than 20% of the inoculated seedlings exhibited either typical compatible lesions (3 mm or longer with heavy sporulation) or lesion type 3 (26), about 1–3 mm in diameter and covering 5% or more of the leaf area. Aggressiveness (leaf area affected) was not included in this study because of difficulty in distinguishing environmental effects from strictly host-pathogen interactions.

Induction of the teleomorph of *P. grisea*. Induction of the teleomorph of *P. grisea* was attempted following the method described by Valent et al (28) by placing 112 isolates in all possible combinations on oatmeal agar (27). Small plugs of mycelium of 16 different isolates to be mated were placed about 1 cm apart in the same petri dish, allowed to grow at 28 C for 10 days, then incubated at 20 C under continuous fluorescent light and observed over 2 mo for development of perithecia. Matings were also attempted by injecting mixtures of spore suspensions of the isolate combinations that produced sterile perithecia on oatmeal agar into stems of 20- to 25-day-old seedlings of Fanny and incubating the stems at 100% relative humidity and 20–32 C for 7 days. One-half of the infected plants were observed after 3 wk for external and internal production of perithecia. Stems of the other plants were cut longitudinally, incubated at 20 C in petri dishes under continuous fluorescent light, and observed during 3 wk for development of perithecia.

Table 1. International race groups of *Pyricularia grisea* collected at Santa Rosa, Colombia, during 1988–1991

International race distribution			Most common races	
Race group[a]	Number	Isolates[b] (no.)	Race	Isolates[b] (no.)
IA	25	91	IA-103	15
IB	1	3	IA-128	12
IC	6	9	IA-99	9
ID	5	7	IA-110	8
IE	4	8	IA-104	7
IF	1	2	IA-101	6
IG	1	3	IA-97	5
IH	1	2	IA-71	4
II	1	6	II-1	6
Total	45	131		72

[a] Compatible reactions: IA to Raminad Str 3, IB to Zenith, IC to NP-125, ID to Usen, IE to Dular, IF to Kanto 51, IG to Sha-tiao-tsao, IH to Caloro, II to none.
[b] Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.

Table 2. Differentiation of six isolates[a] each of international races IA-103 and IA-128 of *Pyricularia grisea* recovered from Santa Rosa, Colombia, during 1988–1991

	IA-103 isolates[b]						IA-128 isolates[c]					
Cultivar	1	2	3	4	5	6	1	2	3	4	5	6
Fukunishiki	–[d]	–	–	–	+	+	+	+	–	–	–	–
Fujisaka 5	–	–	–	–	–	–	+	+	–	–	–	–
IR-42	–	–	–	+	–	–	+	+	+	–	–	–
Peta	–	–	–	–	–	–	–	–	–	+	–	–
PI No. 4	+	+	+	+	+	+	–	–	–	+	+	+
Metica 1	+	+	+	+	+	+	+	+	+	–	–	–
Oryzica 3	+	+	+	+	+	+	–	–	–	–	+	–
CICA 4	+	+	+	+	+	+	–	–	–	+	+	+
CICA 9	–	–	–	–	–	–	–	–	–	+	+	+
IR-22	+	–	–	+	+	+	–	–	–	+	+	+
CICA 8	–	–	–	–	–	–	+	–	–	–	–	–
Tetep	–	–	–	–	+	–	–	–	–	–	–	–
IR-8	–	+	–	+	+	+	+	+	–	+	+	+
Ceysvoni	–	–	–	–	+	+	–	–	–	–	–	–

[a] Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.
[b] 1 = 01-46, 2 = 01-85, 3 = 01-41, 4 = CEY-8-2, 5 = CEY-8-4, and 6 = CEY-19-1.
[c] 1 = CEY-7-1, 2 = CEY-5-1, 3 = CEY-14-1, 4 = C9-6, 5 = C9-11, 6 = C9-14.
[d] – = Incompatible reaction, + = compatible reaction.

RESULTS

International race structure. A total of 45 international races, out of a theoretical maximum of 256 (20) and representing all nine race groups, were recovered from the 15 cultivars sampled in this study (Table 1). Of the 131 isolates,

91 (69%) belonged to group IA and 66 (50%) belonged to only eight of the 128 possible IA races (20) (Table 1). The first seven most common races did not infect the rice differentials Zenith and NP-125, while six infected both Raminad Str 3 and Usen.

The international race system did not fully describe the virulence spectrum of the isolates. For example, six isolates each of races IA-103 and IA-128 could be further differentiated into 12 pathotypes on the basis of their virulence on commercial cultivars (Table 2).

Frequency of virulent phenotypes of *P. grisea* populations in Santa Rosa. Compatibility for all known resistance genes and donor cultivars tested was present in the pathogen population (Table 3). Frequency of virulent phenotypes (total number of compatible reactions divided by number of isolates used for inoculation of that particular cultivar) on the 42 cultivars ranged from 0.0 to 0.86 (Tables 3 and 4), with no cultivar susceptible to all isolates. Overall, it was unusual to recover isolates compatible with K-8, Peta, Ceysvoni, IR-42, Fujisaka 5, Fukunishiki, Zenith, and NP-125. No isolates were recovered that were compatible with Oryzica Llanos 4 and 5, both of which were developed at Santa Rosa.

Frequency of virulent phenotypes varied widely on commercial Colombian cultivars (Table 4). Compatibility frequencies of isolates recovered from different sources and inoculated on commercial Colombian cultivars in the greenhouse and the performance of the cultivars in the field did not correspond. For example, cultivars CICA 6, CICA 7, CICA 8, and CICA 9 are highly susceptible in the Santa Rosa field, yet frequency of virulent phenotypes on them was relatively low.

Analysis of the frequency of compatibility of isolates recovered from commercial rice cultivars revealed some specialization for their cultivars of origin (Table 5) or closely related cultivars (13). Nonetheless, isolates recovered from Ceysvoni, Oryzica 1, Oryzica 2, and Oryzica Llanos 5 were compatible with most of the rice cultivars tested. None of the isolates obtained from lesions occasionally encountered on cultivar Oryzica Llanos 5 could reinfect that cultivar, although these isolates were pathogenic on most other cultivars. A similar situation was observed for isolates recovered from Ceysvoni. Only 23% of the isolates could reinfect Ceysvoni, but the frequency of compatibility was higher for other commercial cultivars.

Cultivar specificity of isolates is extreme in the case of CICA 9. Isolates recovered from this cultivar are compatible with only a few cultivars. Likewise, this cultivar was infected only by isolates recovered from the same cultivar and weakly by isolates from Metica 1 and CICA 6.

In many cases in which there is no apparent cultivar specificity there is a common genetic background (13) among the cultivars. For example, Tetep is the source of blast resistance in CICA 8 (13,25), IR-8 is a common parent for many of the cultivars (13), and Oryzica 1, Oryzica 3, and Metica 1 are all closely related (13).

Relative virulence frequencies (sum of the compatibility frequencies of all isolates recovered from one cultivar on each of the 42 rice cultivars) from Oryzica Llanos 5, Oryzica 1, and Oryzica 2 were

Table 3. Frequency of virulent phenotypes in *Pyricularia grisea* isolates collected at Santa Rosa, Colombia, during 1988–1991

Cultivar[a]	Known resistance genes	Frequency of virulent phenotypes[b]	Isolates tested[c] (no.)
Resistance donors			
K-8	...	0.03	160
Peta	...	0.09	67
Ceysvoni	...	0.10	152
IR-42	...	0.10	168
Fujisaka 5	*Pi-i, Pi-k^s*	0.14	152
Fukunishiki	*Pi-z*	0.22	135
Tsuyuake	*Pi-k^m, Pi-m*	0.25	118
Tetep	*Pi-k^h*	0.26	149
Chokoto	*Pi-k, Pi-a*	0.43	124
Bl-1	*Pi-b*	0.43	155
K-1	*Pi-ta*	0.47	138
Shin 2	*Pi-k^s*	0.54	136
Kataktara	...	0.61	156
PI No. 4	*Pi-ta^2*	0.64	159
Kusabue	*Pi-k*	0.67	141
Aichi Asahi	*Pi-a*	0.75	145
K-59	*Pi-t*	0.78	111
Taichung T.C.W.	...	0.78	145
Fanny	...	0.86	167
International differentials			
Zenith	*Pi-z, Pi-i, Pi-a*	0.04	163
NP-125	...	0.17	150
Kanto 51	*Pi-k*	0.27	136
Sha-tiao-tsao	*Pi-k^s*	0.43	131
Caloro	*Pi-k^s*	0.46	141
Usen	*Pi-a*	0.57	141
Raminad Str 3	...	0.63	142
Dular	*Pi-k^a*	0.64	154

[a] All cultivars were obtained from the germ plasm bank of the International Rice Research Institute.
[b] Calculated as the proportion of isolates tested inducing a susceptible reaction on a rice cultivar. Fanny is susceptible under most evaluations reported in the literature (14).
[c] Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.

Table 4. Compatibility frequency of virulent phenotypes of *Pyricularia grisea* recovered from commercial Colombian rice cultivars at Santa Rosa during 1988–1991

Cultivar[a]	Year of release	Frequency of virulent phenotypes[b]	Isolates tested[c] (no.)
Oryzica Llanos 5	1989	0.00	94
Oryzica Llanos 4	1989	0.00	96
Linea 2	1989	0.06	102
CICA 9	1976	0.07	168
Bluebonnet 50	1951	0.08	156
Oryzica 2	1984	0.13	168
CICA 8	1978	0.25	168
CICA 6	1974	0.33	168
CICA 7	1976	0.36	167
IR-22	1969	0.42	168
Oryzica 3	1987	0.44	162
Oryzica 1	1982	0.45	168
IR-8	1968	0.50	173
Metica 1	1981	0.54	168
CICA 4	1971	0.66	174

[a] All cultivars were obtained from the germ plasm bank of the Centro Internacional de Agricultura Tropical.
[b] Calculated as the proportion of isolates tested inducing a susceptible reaction on a rice cultivar.
[c] Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.

Table 5. Pathogenicity in *Pyricularia grisea* isolates collected from different rice cultivars at Santa Rosa, Colombia, during 1988–1991

Cultivar	Frequency of virulent phenotypes in cultivars giving origin to blast isolates (no. of isolates recovered)[a]								
	CEY (44)	O1 (16)	C8 (15)	M1 (15)	C9 (9)	O2 (9)	C6 (8)	C4 (6)	OL5 (7)
Ceysvoni (CEY)	0.23	0.13	0.00 (6)[b]	0.07	0.00	0.00	0.13	0.00	0.00
Oryzica 1 (O1)	0.48	0.88	0.13	0.20	0.00	0.67	0.00	0.67	0.71
CICA 8 (C8)	0.11	0.19	0.93	0.20	0.00	0.33	0.00	0.00	0.71
Metica 1 (M1)	0.68	0.94	0.13	0.73	0.00	0.44	0.00	0.17	0.71
CICA 9 (C9)	0.00	0.00	0.00	0.13	1.00	0.00	0.13	0.00	0.00
Oryzica 2 (O2)	0.07	0.13	0.13	0.07	0.00	0.78	0.00	0.00	0.71
CICA 6 (C6)	0.18	0.19	0.33	0.13	0.00	0.89	1.00	0.17	1.00
CICA 4 (C4)	0.50	0.94	0.27	0.40	1.00	0.89	1.00	0.67	0.71
Oryzica Llanos 5 (OL5)	0.00 (20)[b]	0.00 (5)	0.00 (8)	0.00 (12)	0.00 (7)	0.00 (8)	0.00	0.00 (4)	0.00
Oryzica Llanos 4	0.00 (20)	0.00 (5)	0.00 (8)	0.00 (12)	0.00 (7)	0.00 (8)	0.00	0.00 (4)	0.00
Tetep	0.18	0.29 (14)	1.00 (11)	0.07	0.00	0.22	0.13	0.00 (4)	0.67
CICA 7	0.45	0.81	0.00	0.33	0.00	0.78	0.00	0.17	1.00
IR-22	0.39	0.69	0.07	0.27	0.00	0.67	0.50	0.33	0.86
Oryzica 3	0.41	0.81	0.40	0.13	0.33	0.44	0.25	0.33	0.57
IR-8	0.41	0.50	0.00	0.20	0.89	0.89	0.75	0.67	1.00
Fanny[c]	0.84	1.00	0.33	0.87	0.89	1.00	0.63	1.00	1.00

[a]Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.
[b]Number of isolates tested different from one indicated is given in parentheses.
[c]Susceptible check.

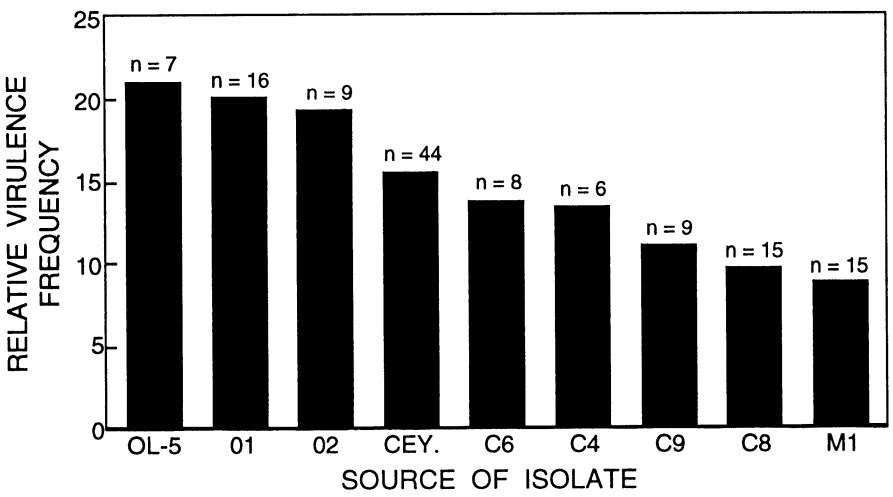

Fig. 1. Relative virulence frequency of *Pyricularia grisea* isolates recovered from nine rice cultivars (OL-5 = Oryzica Llanos 5, O1 = Oryzica 1, O2 = Oryzica 2, CEY = Ceysvoni, C6 = CICA 6, C4 = CICA 4, C9 = CICA 9, C8 = CICA 8, M1 = Metica 1) and inoculated onto 42 rice cultivars; n = number of isolates.

markedly higher than those of the other isolates (Fig. 1). A high frequency of isolates recovered from Oryzica Llanos 5 infected both Oryzica 2 and Oryzica 1. Most isolates from Oryzica 2 infected Oryzica 1, but a very low number of isolates from Oryzica 1 infected Oryzica 2 and none infected Oryzica Llanos 5. Isolates from CICA 8, CICA 9, CICA 4, CICA 6, and Metica 1 had a low relative virulence frequency (Fig. 1).

Accumulation of virulence factors in *P. grisea* isolates. The six isolates shown in Table 6 have accumulated virulence factors to most known resistance genes, but none was virulent to all of them. The most resistant cultivar was Fujisaka 5, known to have resistance genes *Pi-i* and *Pi-k*^{s}. Only isolate IR-42-5-2 (race IC-17) recovered from IR-42 was virulent on Fujisaka 5. A broad spectrum of resistance was also observed in cultivars Fukunishiki, which has resistance gene *Pi-z*, and Zenith, which has genes *Pi-z*, *Pi-i*, and *Pi-a*.

Cultivars IR-42, Peta, and K-8 (unknown resistance genes) were also resistant to most of these highly virulent isolates. Isolate IR-42-5-2 (race IC-17) was virulent to cultivars IR-42 and K-8. Isolate CEY 26-2 (race IA-7) was virulent on cultivar K-8, and isolate CEY 27-1 (race IA-99) was virulent on cultivar Peta.

The same isolates shown in Table 6 were pathogenic to most commercial cultivars, although no isolate was virulent to the commercial cultivar CICA 9. Cultivars Bluebonnet 50 and Ceysvoni were also resistant to five and four of these isolates, respectively. Isolate IR-42-5-2 (race IC-17) was virulent to Bluebonnet 50 and isolates CEY 26-2 (race IA-7) and O1-87 (race IA-67) were virulent to Ceysvoni. Isolate IR-42-5-2 was not virulent to some of the cultivars most susceptible in the field, such as Metica 1, Oryzica 1, CICA 7, CICA 9, and IR-22.

Induction of teleomorph. The perfect stage of *P. grisea* was not produced in any observed mating. In 26 of the matings on oatmeal agar, small masses of hyphae started forming 1 wk after incubation at 20 C and developed into dark brown, globose bodies with no beaks. These structures were partially or wholly embedded in the media, and no asci or ascospores developed. These isolates did not produce perithecia when injected into the sheaths of rice plants.

DISCUSSION

Development of durable blast resistance for environments highly conducive for the disease should be possible if breeding programs are based on a complete understanding of pathogen diversity in the target area. Breeding lines developed from such programs should then be evaluated and selected at hot spots under pathogen populations representing all the diversity. Enhancing the probabilities of all breeding lines in their evaluation process to encounter a matching compatible pathotype will reduce frequent resistance breakdowns as defined by the cryptic error hypothesis (4). Here we report on the initial steps to characterize the pathogen diversity at a hot spot used to develop durable blast resistance in Colombia.

The population of *P. grisea* in the Santa Rosa breeding station is pathogenically very diverse. Virulence factors to all the international differentials and cultivars with at least 13 different genes for resistance have been identified at the site.

Race recovery varied by cultivar, and several races could be recovered from one cultivar. Compatibility with all international differentials was observed, although compatibility with Zenith and

NP-125 was rare. Avirulence to all of the differentials is present in the pathogen population as well.

Isolates within an international race may be further separated in different phenotypes according to their virulence on other rice cultivars known to have different sources of resistance. Several isolates classified as the same race using a set of differentials may also possess different virulence genes not detected with such a differential set. For example, six pathogenicity patterns are described for the two more common races designated IA-103 and IA-128 (Table 2). This common phenomenon (3) suggests that the international race designations do not fully describe the entire pathogenic variability of the pathogen but merely a subset of this variability. For a complete understanding of the pathogen diversity it is very important to increase the number of differentials, including local commercial cultivars and other sources of resistance.

Compatibility with the resistance in all cultivars tested was identified in this sample of the pathogen population, except for the newly released commercial cultivars, and frequencies varied widely. The overall frequency of virulent phenotypes to K-8, Peta, Ceysvoni, IR-42, Fujisaka 5, Fukunishiki, Zenith, and NP-125 was low and may reflect potential virulence changes in the pathogen population.

Compatibility was low with combinations of some known resistance genes, such as those present in cultivars Fujisaka 5 and Zenith. This suggests that the genes *Pi-i* and *Pi-z*, or unknown genes associated with them, are highly effective against the present pathogen population. The gene *Pi-i* is present in both cultivars, whereas the gene *Pi-z* is present in Zenith as well as Fukunishiki, which also was infected at a low frequency. Isolates compatible with the other resistance genes present in Fujisaka 5 (*Pi-k*^{s}) and Zenith (*Pi-a*) were common, as indicated by the frequency of virulent phenotypes on cultivars Aichi Asahi, Usen, Shin 2, and Dular. However, matching virulence for *Pi-i* and *Pi-z* is already present in the population and very likely would not be particularly durable. Thus, the potential for cryptic error or escapes may be reduced, as these rare pathotypes are already detectable and probably would increase rapidly in frequency if the corresponding resistance genes become common in the breeding population.

Very few of the known resistance genes appear to be immediately useful in this area, and new resistance genes, or combinations thereof, seem to be needed. However, there was no cultivar susceptible to all isolates recovered in the trials, indicating that the resistance genes remain effective against segments of the pathogen population. Fanny, a cultivar that is considered nearly universally susceptible and is killed by blast 15–20 days after planting in Santa Rosa, has some unknown resistance genes, as inferred from the compatibility frequency (0.86) of isolates tested (Table 3).

The frequency of virulent phenotypes to a cultivar and the susceptibility of that cultivar in the field do not correspond. For example, the frequency of compatibility to CICA 9 and Oryzica 2 was low in the pathogen population (Table 4), while these cultivars were highly susceptible in the field. The virulence range of isolates obtained appears to be strongly influenced by the cultivar of origin. Therefore, to adequately sample a blast population in a breeding site, care must be taken to sample a broad range of cultivars, resistance sources, or the air spores (7). There were also differences in frequency of virulent phenotypes on the commercial cultivars released in Colombia, with recovered isolates showing a fairly high degree of complementarity in their pathogenicity patterns, suggesting that these cultivars have distinct resistance (Table 5). No isolate was found that could infect Oryzica Llanos 4 and 5, and very few isolates recovered from different cultivars in-

Table 6. Accumulation of virulence in *Pyricularia grisea* isolates collected at Santa Rosa, Colombia, during 1988–1991

Cultivar	Known resistance genes	Source of isolate (race)[a]					
		O1 (IA-67)	C8 (IA-97)	CEY (IA-7)	CEY (IA-99)	IR-42 (IC-17)	OL5[b] (IB-35)
Aichi Asahi	*Pi-a*	+[c]	+	+	+	+	+
Bl-1	*Pi-b*	+	+	+	+	−	+
Shin 2	*Pi-k*^{s}	+	+	+	+	+	+
Dular	*Pi-k*^{a}	+	+	+	+	+	+
Fukunishiki	*Pi-z*	+	−	+	−	−	−
Fujisaka 5	*Pi,i, Pi-k*^{s}	−	−	−	−	+	−
Chokoto	*Pi-k, Pi-a*	+	+	+	+	+	−
Kanto 51	*Pi-k*	+	+	−	+	+	+
K-1	*Pi-ta*	+	+	+	+	+	+
K-59	*Pi-t*	+	+	+	+	+	+
PI No. 4	*Pi-ta*^{2}	+	+	+	+	−	+
Tsuyuake	*Pi-k*^{m}, *Pi-m*	+	+	−	+	+	+
Zenith	*Pi-z, Pi-i, Pi-a*	−	−	+	−	−	+
Tetep	*Pi-k*^{h}	+	+	−	+	+	+
IR-42	...	−	−	−	−	+	−
K-8	...	−	−	+	−	+	−
Peta	...	−	−	−	+	−	−
Metica 1	...	+	+	+	+	−	+
Oryzica 1 (O1)	...	+	+	+	+	−	+
Oryzica 2	...	+	+	−	+	+	+
Oryzica 3	...	+	+	+	+	+	+
CICA 4	...	+	+	+	+	+	+
CICA 6	...	+	+	−	+	+	+
CICA 7	...	+	+	+	+	−	+
CICA-9	...	−	−	−	−	−	−
IR-22	...	+	+	+	+	−	+
CICA 8 (C8)	...	+	+	−	+	+	+
Bluebonnet 50	...	−	−	−	−	+	−
IR-8	...	+	−	+	+	+	+
Ceysvoni (CEY)	...	+	−	−	−	−	−

[a]Each isolate was inoculated on 18- to 21-day-old plants, 10 per pot per cultivar, in two replications repeated in time until uniform reactions were obtained.
[b]Oryzica Llanos 5.
[c]− = Incompatible reaction, + = compatible reaction.

fected CICA 9, under greenhouse conditions. Those that could infect CICA 9 were almost always isolated from CICA 9. The former two cultivars are symptom-free in the field, while the latter is always heavily diseased.

Because some of the commercial cultivars are highly susceptible in the field, and because isolates virulent to them are recovered only infrequently from other cultivars, useful combination of resistance genes could be generated from crosses between such complementary groups. Long-term observation of virulence/avirulence frequencies and accumulation of virulence/avirulence would be needed to monitor the success of this strategy. Pathotypes with multiple virulences simply may be rare events with little practical relevance. However, recovery of isolates with the greatest combinations of virulence from the most recently released cultivar, Oryzica Llanos 5 (Table 6, Fig. 1), which combines the most diverse sources of resistance, suggests that relevant virulences are being accumulated. The same situation is observed on isolates recovered from the source of resistance, Ceysvoni.

Isolates often were unable to reinfect their cultivar of origin, this being most common for highly resistant cultivars such as Ceysvoni, a commonly used source of resistance in the breeding program for 7 yr, and Oryzica Llanos 5, which combines resistance from a gene-pyramiding program at CIAT with such sources as C 46-15 from Africa, Aiwini and Apura from Suriname, Colombia 1 from Colombia, and Tadukan from Taiwan (13,18). From these cultivars, isolates were typically recovered from mature panicle tissue that may have been in the early stages of senescence when infection occurred. Although these isolates were not pathogenic on the cultivar of origin, they were pathogenic on other cultivars. Saprophytic growth on senescent tissue of highly resistant cultivars is a common phenomenon observed under field conditions. However, the fact that most of these isolates accumulated a large number of virulence factors conferring compatibility with most of the 42 cultivars tested suggests that the interaction observed may be more than a simple saprophytic growth. Studies are under way to determine if these isolates are associated with the first steps of resistance breakdown of both cultivars Oryzica Llanos 5 and Ceysvoni. This is suggested by the fact that isolates recovered from Ceysvoni and capable of reinfecting it were only recovered in 1990, whereas those incapable of reinfection were recovered earlier as well, implying the sequence of a resistance breakdown. Because the relationship among these isolates is unknown, it is impossible to tell at this time if compatibility with Ceysvoni resistance comes from a formerly very rare member of the population that was favored by increasing frequency of a compatible virulent type in the field or whether mutation within previously existing individuals resulted in compatibility.

Mundt (21) has indicated that reported data do not support the probability hypothesis. According to this theory, resistance combinations confer durability because of the very improbable accumulation of random, resistance-matching mutations in a given pathogen population. Mundt cites evidence countering the assumption that mutations to virulence at different loci are necessarily independent and that combinations of virulence will occur in frequencies determined by the product of probabilities of individual mutations. A large number of virulence factors and combinations are present in the *P. grisea* population at Santa Rosa. However, isolates combining genes compatible with known resistance genes seem to be rarer than those having only one (Table 3) and might be interpreted as supporting the probability hypothesis. The number of virulence/avirulence genes apparently combined in some isolates (Table 6) would seem to be very improbable (or practically impossible) if the occurrence of random combinations were determined by the product of their individual frequencies.

We have found in Santa Rosa combinations of virulence genes for combinations of resistance genes that do not exist in the program, at least not produced intentionally by breeders. The combination of virulence genes in isolates recovered from the most recently developed cultivars suggests that the hot spot indeed permits detection of formerly rare phenotypes or that the pathogen is accumulating genes either by mutation or by recombination.

The range of virulence in the pathogen population suggests that virulence/avirulence genes may be retained, or perhaps generated, in the absence of a compatible host, as in the case of Bluebonnet 50, a cultivar that has not been grown in the region for at least 20 yr, and CICA 9, which was grown commercially for only 1 yr (1976). Compatibility with the international differential Raminad Str 3 yields an IA race group designation, the most common group in Santa Rosa and in commercial fields in the region (*unpublished*). Isolates lacking this gene are capable of infecting CICA 8 and Oryzica 1, the most common cultivars grown in the region over the last 9 yr.

Thus, the commonly encountered virulence factor for Raminad Str 3 may be unnecessary, yet persist in the population at a fairly high frequency. The gene rotation concept assumes that eliminating Raminad Str 3 will eventually eliminate corresponding virulence genes in the pathogen population, although it seems not to be the case for this host-pathogen interaction. On the other hand, the high specialization observed in the interaction of the cultivar CICA 9 and the isolates that infect it, plus the fact that compatible virulence genes are not detected in isolates from other sources, even in isolates accumulating a high number of virulence genes, suggest that eliminating CICA 9 or genetically related cultivars will probably eliminate matching phenotypes.

With the persistence of apparently unnecessary virulence, it is unclear why there is such cultivar specificity. It is possible that certain virulence combinations confer poor fitness in the pathogen (6,7). However, it also may be possible that some pathotype combinations are simply more unlikely to occur or develop from a preexisting pathotype or virulence combination.

It is very important from an ecological, epidemiological, and breeding perspective to know how genetic diversity is maintained in the pathogen and how new, well-adapted complex races arise. The combinations of virulence/avirulence observed, and the apparent increase in the number of genes combined (Fig. 1), suggest that some mechanism may exist for recombination. However, no evidence for sexual reproduction at Santa Rosa has been observed. Attempts to locate the teleomorph in nature and to induce it under controlled conditions failed. However, a parasexual cycle (10) may play a role. Analysis of the population by use of DNA fingerprints (19) may suggest whether the population is clonal in nature or if recombination occurs at a scale that would obscure lineages. The critical evaluation of the blast pathogen population at Santa Rosa conducted in this study proves that this site exhibits a great pathogen diversity. Virulent phenotypes observed in low frequency may be increased in the pathogen population by inoculating them in the field on identified susceptible cultivars, or they may be used to identify sources of resistance under greenhouse conditions. The results should help to identify effective and diverse germ plasm, which could be resistant or susceptible in the field, and consequently help to design appropriate breeding strategies for development of durable blast resistance (15).

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