Pathotypes of *Pyricularia grisea* in Rice Fields of Central and Southern China

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ABSTRACT

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Blast, caused by the fungal pathogen *Pyricularia grisea*, is the most devastating disease of rice worldwide. Knowledge of the pathotype composition of the pathogen in rice fields is essential for rational deployment of resistance genes in rice breeding programs. In this study, we assayed the pathotypes of the pathogen populations using samples recently collected from 13 major rice-growing provinces of central and southern China. In all, 792 single spore isolates were tested for pathogenicity reactions using 13 host differentials consisting of six indica and seven japonica near-isogenic lines (NILs). The compositions of the pathogen populations were complex; 48 pathotypes were identified with the indica NILs, 82 pathotypes were detected with the japonica NILs, and a total of 344 pathotypes were identified with both indica and japonica NILs. There were large differences in distribution of the pathotypes among the different rice-growing areas. Even neighbor provinces seemed to differ sharply in types and frequencies of the most prevalent pathotypes. There was also a large difference in the frequencies of the isolates producing compatible reactions on the NILs, indicating the difference in frequencies of avirulence genes in the pathogen populations. The data provided very useful information for formulating strategies for improving blast resistance in rice breeding programs.

Additional keywords: cultivar development, geographical distribution, resistance gene, rice blast

Blast, caused by the fungal pathogen *Pyricularia grisea* (Cooke) Sacc., is economically the most important disease of rice in China and many rice-growing countries of the world. Heavy losses occur frequently in many rice-producing areas of China (19). For years, development of resistant cultivars has been considered the most effective strategy for protecting the crop against this disease. Although a number of cultivars with various degrees of blast resistance have been released in China in the past several decades (11), this approach has not been efficiently explored in the Chinese rice breeding programs. A major reason for the low efficiency of blast resistance breeding is the lack of knowledge of the pathotype composition of the pathogen populations for the targeted areas for which the cultivars are bred. Consequently, the breeding programs usually are not well focused on the genes for resistance that should be incorporated in the breeding programs.

Although a number of studies have been conducted in the past to assess the genetic variations of the pathogen, including pathotyping (2,14,16), isozyme electrophoresis (9), and DNA fingerprinting (10), data have remained scarce concerning the compositions and virulence spectra of *P. grisea* populations in most of the rice-producing areas of the world. Several attempts have been made to characterize the pathotype compositions of *P. grisea* populations collected from specific regions of China (17,20). The largest survey was perhaps the one conducted in the late 1970s, in which a total of 827 isolates representing 21 rice-growing provinces of China were tested against seven host differentials, including three indica and four japonica cultivars (1). The study identified 43 physiological races (pathotypes) that differed from each other in reaction to at least one of the host differentials. It also revealed that one of the races occurred at a predominantly high frequency (45.7%), and was observed in 18 of the 21 provinces. Although the data obtained were useful for understanding the complexity of the pathogen, the virulence spectra of the fungal populations was not fully understood because the resistance genes in the host differentials were not well characterized. It is highly likely that the pathotype compositions of the pathogen populations have changed greatly during the last 20 years as the widely grown cultivars have changed. Data collected 20 years ago may not be relevant to the pathogen populations currently in rice fields.

In this article, we report the pathotype composition of *P. grisea* based on a large number of samples from a recent collection, with representatives from 12 rice-growing provinces of central and southern China, tested on two sets of near-isogenic lines (NILs) as the host differentials. The objective of this study was to provide current information on the pathotype spectrum of *P. grisea* populations in the rice fields of China to allow the formulation of viable strategies for blast resistance in breeding programs, especially with respect to the deployment of specific resistance genes.

MATERIALS AND METHODS

Sampling. Diseased leaves and panicles were collected from more than 200 rice cultivars planted in the fields of rice-growing areas of central and southern China in 1996 and 1997 (Table 1 and Fig. 1). Samples were collected from 2 to 10 locations in each province to include sites representing different geographical and ecological conditions. Sampling sites also included "hot spots" where blast occurs frequently. Attention was paid in the collection to avoid specimens from fields where fungicides were sprayed during the growing season. The samples were separately bagged, air dried, and brought to the laboratory to culture *P. grisea* and obtain single spore isolates (Table 1).

Monoconidial isolation and culture of the fungus. Panicles and leaves with blast lesions were surface sterilized with 70% ethyl alcohol for 10 s and soaked in distilled water for 2 h to saturate the specimens. The steeped tissues were laid in glass plates containing distilled water and incubated at 25 to 26°C for 24 to 36 h to induce sporulation of the fungus. When the lesions turned gray, the diseased tissue was

Table 1. Number of cultivars from which diseased samples were collected in each province and number of *Pyricularia grisea* isolates tested in this experiment

Province	No. of cultivars	No. of isolates analyzed
Sichuan	15	70
Yunnan	7	35
Guizhou	5	32
Hubei	32	106
Anhui	11	59
Jiangxi	5	83
Hunan	21	95
Jiangsu	5	19
Zhejiang	10	34
Fujian	13	46
Guangdong	9	49
Guangxi	16	87
Total	160	715

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held over a plate containing 0.6% agar and shaken hard. Single conidia that dropped to the surface of the agar plate were removed immediately with a sharp needle under a dissection microscope. Each conidium was transferred to a slant tube and incubated at 26°C in a medium containing, per liter, 2 g of yeast extract, 20 g of sucrose, 500 mg of vitamin B1, 0.005 mg of biotin, 1 g of sodium glutamate, 500 mg of KH_2PO_4, 500 mg of K_2HPO_4, 500 mg of $MgSO_4$, 100 mg of $CaCl_2$, and 7 g of agar, with the pH adjusted to 7.0. After culturing for 5 days, hyphae were transferred to an autoclaved barley solid medium (barley grains:water = 1:1.2, wt/wt) for further propagation. After incubation, the barley grains were washed with distilled water to remove the hyphae on the surface when they were covered with white and gray hyphae. The washed grains were laid on sterilized gauze moistened with distilled water at 28°C and maintained under fluorescent light for 48 h to allow sporulation. Conidial suspensions were obtained by washing the barley grains with water and then filtering the wash solutions through four layers of gauze. Tween 20 was added to the conidial suspension at 0.05% (vol/vol). The concentration of the suspension was adjusted to approximately 10^5 conidia/ml using a hemacytometer, and the suspension was then ready to use for inoculation.

Host differentials. Two sets of NILs, kindly provided by the International Rice Research Institute (IRRI), were used as host differentials for pathotyping of the fungal isolates (Table 2). The first set consisted of five lines developed by transferring various resistance genes into the indica cultivar CO39 (13), and the second set consisted of six lines carrying various resistance genes in the background of the japonica rice cultivar Lijiangxintuanheigu (LXHG; 12). These lines carried most of the genes for blast resistance identified previously (Table 2). *Pi1* is located on chromosome 11 (24). *Pi2* may be the same as or allelic to *Piz,* which is located on chromosome 6 (7,25). *Pi3* is located on chromosome 6 (6). *Pi4*^{a}, allelic to *Pi4*^{b}, is located on chromosome 12 (25) and may also be identical to *Pita* (7), which should be allelic to *Pita*^{2} (18). *Pib* is located on chromosome 2 (15) and has recently been cloned by Wang et al. (23). *Pik*, *Pik*^{m}, and *Pik*^{p} are different alleles of the same locus located on chromosome 11 (8,21).

Seeds of the NILs used in the experiment were from bagged panicles of plants from the original seeds received from IRRI. In preparing the seedlings for inoculation, seeds of differential NILs were soaked for two days in tap water and sown in plastic trays (60 by 40 by 8 cm) filled with fungicide-treated soil (saturated with 0.1% Carbendazim for a week before sowing). Seedlings were thinned at the one-leaf stage to keep 20 plants per line.

Inoculation and disease scoring. The seedlings were inoculated at the four-leaf stage by spraying a fresh preparation of the conidial suspension at approximately 0.2 ml/plant. Inoculated seedlings were incubated for 7 days in a greenhouse maintained at 25°C and >93% relative humidity. The 0-to-5 scale rating system of Bonman et al. (2) was used in scoring the level of disease infection. Ratings 0 to 3 were referred to as an incompatible reaction, and ratings 4 to 5 as a compatible reaction. The infection of a particular line by a fungal isolate was considered to be compatible if 20% or more of the seedlings inoculated showed a compatible reaction (4).

Coding of the pathotypes. The pathotypes identified by the host differentials were coded according to the octal notation proposed by Gilmour (5). In this coding system, each of the host differentials was assigned a numeric code (1, 2, 4, 10, 20, 40, and so on). The designation of a pathotype is the summation of numeric values of the codes corresponding to the differentials on which the pathotype produces compatible reactions.

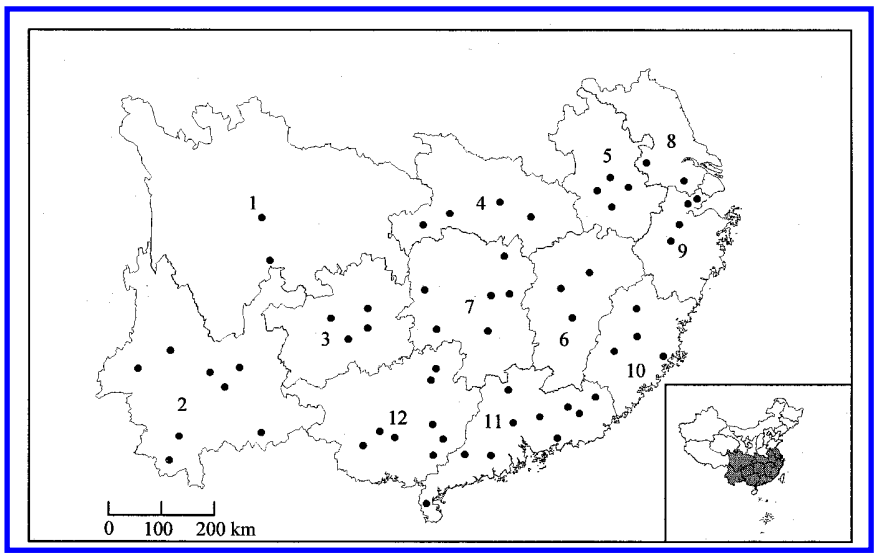

Fig. 1. Distribution of sites in central and southern China where samples of *Pyricularia grisea* used in the study were collected from the fields. Province codes: 1 = Sichuan, 2 = Yunnan, 3 = Guizhou, 4 = Hubei, 5 = Anhui, 6 = Jiangxi, 7 = Hunan, 8 = Jiangsu, 9 = Zhejiang, 10 = Fujian, 11 = Guangdong, and 12 = Guangxi.

Table 2. Differential near-isogenic lines (NILs) used for testing pathogenicity of the *Pyricularia grisea* isolates

			Code number		
NIL[a]	Subspecies	Resistance gene	Indica	Japonica	Combined[b]
C101A51	Indica	*Pi2*	1	…	1
C101LAC	Indica	*Pi1*	2	…	2
C101PKT	Indica	*Pi4*^{a}	4	…	40
C105TTP-4-L23	Indica	*Pi4*^{b}	10	…	100
C104PKT	Indica	*Pi3*	20	…	2,000
CO39	Indica	…	0.1	…	0.1
F182-1	Japonica	*Pita*^{2}	…	1	4
F145-2	Japonica	*Pib*	…	2	10
F80-1	Japonica	*Pik*	…	4	20
F98-7	Japonica	*Pik*^{m}	…	10	200
F124-1	Japonica	*Pita*	…	20	400
F129-1	Japonica	*Pik*^{p}	…	40	1,000
LXHG	Japonica	…	…	0.2	0.2

[a] Lines within each subspecies, except the two recurrent parents CO39 and LXHG, are listed in the order of increased frequencies of susceptibility to the fungal isolates.

[b] Codes are assigned to the NILs in the order of increased frequencies of susceptibility to the fungal isolates, except the two recurrent parents CO39 and LXHG.

RESULTS

Of the 792 isolates pathotyped by inoculation on the 13 rice differentials, 77 isolates did not show a compatible reaction with any of the host differentials. Consequently, data from only the 715 of the isolates that produced a compatible reaction with at least one of the host differentials were used in the analyses.

The 13 NILs differentiated the 715 isolates into 344 pathotypes (*data not shown*) that differed from each other by reaction to at least one of the NILs. To follow the convention of the two sets of NILs, the data were presented in two subsets:

pathotypes determined by indica NILs and pathotypes determined by japonica NILs.

Pathotypes identified by indica differentials. Of the 64 possible pathotypes with the six host differentials (2^6), 48 were observed in the test of the 715 isolates (Table 3). Overall, 24.5% of the isolates did not produce a compatible reaction with any of the indica NILs, 19.3% produced compatible reactions with one of the NILs, 28.7% produced compatible reactions with two NILs, 15.4% produced compatible reactions with three NILs, 9.2% produced compatible reactions with four NILs, 2.4% produced compatible reactions with five NILs, and 0.6% of the isolates produced compatible reactions with all six NILs. Among the pathotypes that showed compatible reactions on at least one of the NILs, two pathotypes (I0.1 and I20.1), showing compatible reactions with one and two NILs, respectively, occurred at predominantly high frequencies (12.3 and 14.3%). Another two pathotypes (I10.1 and I34.1), showing compatible reactions with two and four of the NILs, respectively, were also observed at fairly high frequencies (5 to 10%). The remaining pathotypes were infrequent or rare (<5%).

Pathotypes identified by japonica differentials. Of the 128 pathotypes that were possible with the seven NILs (2^7), 82 were observed in the assay of the 715 isolates on the japonica host differentials (Table 4). Similar to the reactions to the indica differentials, a large proportion (15.1%) of the isolates did not show a compatible reaction with any of the japonica host differentials, but this proportion was much smaller than that observed in the tests using indica differentials. Overall, 21.1% of the isolates produced compatible reactions with one of the NILs, 25.7% produced compatible reactions with two NILs, 13.7% produced compatible reactions with three NILs, 10.5% produced compatible reactions with four NILs, 8.1% produced compatible reactions with five NILs, 3.64% produced compatible reactions with six NILs, and 1.4% of the isolates produced compatible reactions with all seven

Table 3. Reaction of the *Pyricularia grisea* isolates on the indica differential near isogenic lines (NILs)

		NIL and the gene that it carries (code number)[a]						
		C101A51	C101LAC	C101PKT	C105TTP-4-L23	C104PKT	CO39	
Pathotype	Code	*Pi2* (1)	*Pi1* (2)	*Pi4*[a] (4)	*Pi4*[b] (10)	*Pi3* (20)	(0.1)	Frequency (%)
1	I0	0	0	0	0	0	0	24.48
2	I1	1	0	0	0	0	0	0.42
3	I2	0	1	0	0	0	0	0.70
4	I4	0	0	1	0	0	0	1.54
5	I10	0	0	0	1	0	0	1.82
6	I20	0	0	0	0	1	0	2.52
7	I0.1	0	0	0	0	0	1	12.31
8	I3	1	1	0	0	0	0	0.14
9	I11	1	0	0	1	0	0	0.14
10	I14	0	0	1	1	0	0	0.56
11	I10.1	0	0	0	1	0	1	5.17
12	I21	1	0	0	0	1	0	0.14
13	I22	0	1	0	0	1	0	0.14
14	I24	0	0	1	0	1	0	0.42
15	I30	0	0	0	1	1	0	0.98
16	I1.1	1	0	0	0	0	1	1.54
17	I2.1	0	1	0	0	0	1	1.40
18	I4.1	0	0	1	0	0	1	3.78
19	I20.1	0	0	0	0	1	1	14.27
20	I7	1	1	1	0	0	0	0.14
21	I13	1	1	0	1	0	0	0.14
22	I11.1	1	0	0	1	0	1	0.14
23	I15	1	0	1	1	0	0	0.14
24	I32	0	1	0	1	1	0	0.14
25	I34	0	0	1	1	1	0	0.56
26	I5.1	1	0	1	0	0	1	0.70
27	I6.1	0	1	1	0	0	1	0.14
28	I12.1	0	1	0	1	0	1	0.42
29	I14.1	0	0	1	1	0	1	2.10
30	I21.1	1	0	0	0	1	1	0.98
31	I22.1	0	1	0	0	1	1	2.52
32	I24.1	0	0	1	0	1	1	2.66
33	I30.1	0	0	0	1	1	1	4.62
34	I35	1	0	1	1	1	0	0.14
35	I13.1	1	1	0	1	0	1	0.14
36	I15.1	1	0	1	1	0	1	0.14
37	I16.1	0	1	1	1	0	1	0.28
38	I23.1	1	1	0	0	1	1	0.42
39	I25.1	1	0	1	0	1	1	0.28
40	I26.1	0	1	1	0	1	1	0.84
41	I31.1	1	0	0	1	1	1	0.14
42	I32.1	0	1	0	1	1	1	0.56
43	I34.1	0	0	1	1	1	1	6.29
44	I27.1	1	1	1	0	1	1	0.28
45	I33.1	1	1	0	1	1	1	0.14
46	I35.1	1	0	1	1	1	1	0.70
47	I36.1	0	1	1	1	1	1	1.26
48	I37.1	1	1	1	1	1	1	0.56
Frequency (%)[b]	…	7.55	10.35	23.50	27.27	41.54	64.76	…

[a] 0 = incompatible reaction and 1 = compatible reaction.
[b] Proportion of the isolates that produced compatible reaction on the NIL.

Table 4. Reaction of the *Pyricularia grisea* isolates on the japonica differential near isogenic lines (NILs)

		NILs and genes that they carry (code number)[a]							
		F182-1	F145-2	F80-1	F98-7	F124-1	F129-1	LXHG	
Pathotype	Code	*Pita*^{2} (1)	*Pib* (2)	*Pik* (4)	*Pik*^{m} (10)	*Pita* (20)	*Pik*^{p} (40)	(0.2)	Frequency (%)
1	J0	0	0	0	0	0	0	0	15.10
2	J1	1	0	0	0	0	0	0	0.42
3	J2	0	1	0	0	0	0	0	0.56
4	J10	0	0	0	1	0	0	0	1.12
5	J20	0	0	0	0	1	0	0	1.96
6	J40	0	0	0	0	0	1	0	0.56
7	J0.2	0	0	0	0	0	0	1	16.50
8	J3	1	1	0	0	0	0	0	0.14
9	J5	1	0	1	0	0	0	0	0.28
10	J22	0	1	0	0	1	0	0	0.56
11	J30	0	0	0	1	1	0	0	1.26
12	J41	1	0	0	0	0	1	0	0.14
13	J42	0	1	0	0	0	1	0	0.42
14	J60	0	0	0	0	1	1	0	0.42
15	J1.2	1	0	0	0	0	0	1	2.52
16	J2.2	0	1	0	0	0	0	1	0.56
17	J4.2	0	0	1	0	0	0	1	5.03
18	J10.2	0	0	0	1	0	0	1	2.94
19	J20.2	0	0	0	0	1	0	1	3.78
20	J40.2	0	0	0	0	0	1	1	7.69
21	J16	0	1	1	1	0	0	0	0.14
22	J23	1	1	0	0	1	0	0	0.14
23	J31	1	0	0	1	1	0	0	0.28
24	J32	0	1	0	1	1	0	0	0.42
25	J34	0	0	1	1	1	0	0	0.14
26	J43	1	1	0	0	0	1	0	0.14
27	J54	0	0	1	1	0	1	0	0.14
28	J70	0	0	0	1	1	1	0	0.28
29	J3.2	1	1	0	0	0	0	1	0.42
30	J5.2	1	0	1	0	0	0	1	0.84
31	J6.2	0	1	1	0	0	0	1	0.28
32	J11.2	1	0	0	1	0	0	1	0.28
33	J12.2	0	1	0	1	0	0	1	0.14
34	J14.2	0	0	1	1	0	0	1	0.56
35	J21.2	1	0	0	0	1	0	1	0.28
36	J22.2	0	1	0	0	1	0	1	0.42
37	J24.2	0	0	1	0	1	0	1	0.70
38	J30.2	0	0	0	1	1	0	1	2.24
39	J41.2	1	0	0	0	0	1	1	0.70
40	J42.2	0	1	0	0	0	1	1	0.56
41	J44.2	0	0	1	0	0	1	1	1.26
42	J50.2	0	0	0	1	0	1	1	0.84
43	J60.2	0	0	0	0	1	1	1	2.52
44	J53	1	1	0	1	0	1	0	0.14
45	J63	1	1	0	0	1	1	0	0.14
46	J66	0	1	1	0	1	1	0	0.14
47	J71	1	0	0	1	1	1	0	0.14
48	J72	0	1	0	1	1	1	0	0.14
49	J13.2	1	1	0	1	0	0	1	0.28
50	J25.2	1	0	1	0	1	0	1	0.14
51	J34.2	0	0	1	1	1	0	1	0.70
52	J43.2	1	1	0	0	0	1	1	0.14
53	J45.2	1	0	1	0	0	1	1	0.42
54	J46.2	0	1	1	0	0	1	1	0.28
55	J51.2	1	0	0	1	0	1	1	0.14
56	J52.2	0	1	0	1	0	1	1	0.28
57	J54.2	0	0	1	1	0	1	1	0.56
58	J61.2	1	0	0	0	1	1	1	0.42
59	J62.2	0	1	0	0	1	1	1	0.70
60	J64.2	0	0	1	0	1	1	1	0.42
61	J70.2	0	0	0	1	1	1	1	5.17
62	J25.2	1	0	1	0	1	0	1	0.14
63	J57	1	1	1	1	0	1	0	0.14
64	J73	1	1	0	1	1	1	0	0.28
65	J75	1	0	1	1	1	1	0	0.14
66	J17.2	1	1	1	1	0	0	1	0.42
67	J27.2	1	1	1	0	1	0	1	0.14

(continued on next page)

[a] 0 = incompatible reaction and 1 = compatible reaction.
[b] Proportion of the isolates that produced compatible reaction on the NIL.

Table 4. (*continued from preceding page*)

		NILs and genes that they carry (code number)[a]							
		F182-1	F145-2	F80-1	F98-7	F124-1	F129-1	LXHG	
Pathotype	Code	*Pita*^{2} (1)	*Pib* (2)	*Pik* (4)	*Pik*^{m} (10)	*Pita* (20)	*Pik*^{p} (40)	(0.2)	Frequency (%)
68	J33.2	1	1	0	1	1	0	1	0.28
69	J36.2	0	1	1	1	1	0	1	0.14
70	J47.2	1	1	1	0	0	1	1	0.14
71	J56.2	0	1	1	1	0	1	1	0.28
72	J63.2	1	1	0	0	1	1	1	0.14
73	J66.2	0	1	1	0	1	1	1	0.28
74	J71.2	1	0	0	1	1	1	1	1.40
75	J72.2	0	1	0	1	1	1	1	2.52
76	J74.2	0	0	1	1	1	1	1	1.82
77	J57.2	1	1	1	1	0	1	1	0.14
78	J67.2	1	1	1	0	1	1	1	0.14
79	J73.2	1	1	0	1	1	1	1	0.70
80	J75.2	1	0	1	1	1	1	1	0.70
81	J76.2	0	1	1	1	1	1	1	1.96
82	J77.2	1	1	1	1	1	1	1	1.40
Frequency (%)[b]	…	14.69	17.06	19.86	31.47	36.36	37.06	74.13	…

NILs. Among the pathotypes that showed compatible reactions with at least one of the host differentials, one pathotype (J0.2) occurred at a predominantly high frequency (16.5%). Three additional pathotypes (J4.2, J40.2, and J70.2) were detected at fairly high frequencies (5 to 10%). The remaining 77 pathotypes were observed at low frequencies (<5%).

Relationship of the pathotypes identified by indica and japonica differentials. A total of 108 isolates (15.1% of the total) involving 25 pathotypes produced compatible reactions with at least one of the indica NILs, but not with the japonica differentials. In comparison, 175 isolates involving 46 pathotypes produced compatible reactions with at least one of the japonica NILs, but not on any of the indica differentials. Only one isolate produced compatible reactions with 12 of the 13 NILs, and no isolate produced a compatible reaction with all of the NILs.

Distribution of pathotypes in different rice-growing areas. The numbers of pathotypes and the pathotypes that occurred at the highest frequencies identified by indica and japonica differentials in each province are listed in Tables 5 and 6. These statistics should not be weighed very heavily because there was no effort in the sampling to obtain diseased samples representing the entire rice-growing area of each province, and the numbers of isolates obtained and used in pathotyping were not uniform among different rice-growing areas.

Nonetheless, useful information can be obtained by comparing the pathotype groups in samples from different provinces. For example, when assayed using indica differentials, two of the pathotypes (I0, which was not able to infect any of the NILs, and I20.1, which caused compatible reactions on resistance gene *Pi3* and the susceptible control CO39; Table 3), were found to be widespread (Table 5). Two other pathotypes (I0.1, which was only able to attack the susceptible check, and I34.1, which caused compatible reactions with four of the differentials) were also found frequently in certain provinces. There also appeared to be uneven distribution in the level of diversity among different provinces, as demonstrated by the numbers of pathotypes identified, along with the diversity values calculated using Shannon's information statistic (3), $h = -\Sigma p_i \ln p_i$, where p_i is the frequency of the ith pathotype. For example, the blast sample from Jiangxi Province was more diverse than samples from other provinces, and samples from Yunnan, Anhui, Jiangsu, and Fujian Provinces were less diverse than other samples.

The situation is quite similar when assayed using japonica differentials (Table 6), in which two pathotypes (J0, which was not able to cause compatible reactions on any of the NILs, and J0.2, which could only attack the susceptible check LXHG) occurred at high frequencies in many of the provinces. In addition, pathotypes J4.2, J40.2, and J70.2, which were able to attack the susceptible check and one or two other NILs, also occurred at substantial frequencies in certain areas. The samples from Jiangxi Province again showed the highest diversity as evaluated by both the number of pathotypes and the diversity index, while the samples from Jiangsu were the least diverse.

We also analyzed the distribution pattern of the pathotypes using the combined information provided by all 13 host differentials, including both the indica and japonica sets of NILs (Table 7). Two pathotypes, Z0.2 and Z0.3, were observed frequently in several provinces. However, these frequencies were much lower than those analyzed separately using the indica or japonica NILs. Again, the samples from Jiangxi Province were the most diverse, followed by the samples from Hubei Province.

Thus, the overall picture emerging from the comparison (Tables 5–7) is that some of the pathotypes were widespread in different rice-growing areas while others were less common. When the pathotypes that were not able to cause compatible reactions on any of the NILs were discounted, the differences in the pathotype compositions among the provinces appeared to be more pronounced and neighbor provinces seemed to differ sharply in types and frequencies of the most common pathotypes.

Susceptibility of the NILs to the fungal isolates. The frequencies of the isolates showing compatible reactions to each of the host differentials carrying various genes for resistance were also calculated (Tables 3 and 4). When analyzed on indica differentials, the NIL containing the *Pi2* gene was susceptible to the smallest proportion (7.6%) of isolates, followed by the NIL carrying *Pi1* (10.4%), while NILs carrying *Pi4*^{a}, *Pi4*^{b}, and *Pi3* were susceptible to much larger proportions (23.5 to 41.5%) of the isolates. The susceptible check, CO39, was susceptible to the largest proportion (64.8%) of the isolates.

When analyzed on the japonica differentials, approximately 15 to 20% of the isolates caused compatible reactions with host differentials carrying resistance genes *Pita*^{2}, *Pib*, or *Pik*; 30 to 40% of the isolates produced compatible reactions on differentials carrying *Pik*^{m}, *Pita,* or *Pik*^{p}. The susceptible check, LXHG, was again susceptible to the largest proportion (74.1%) of the isolates.

DISCUSSION

Knowledge about the pathotype composition of the pathogen population is crucial for the development of strategies for manipulating the disease resistance genes for crop protection. These results revealed a number of important features of the fungal pathogen populations which may enhance the understanding of the pathotype compositions of the *P. grisea* populations in central and southern China.

The main feature is the complexity of the pathotype composition of the pathogen populations in central and southern China. As many as 48 of the 64 pathotypes that were possible with the six indica host differentials were observed, and 82 of the 128 pathotypes that were possible with the seven japonica host differentials were detected. Moreover, a total of 344 pathotypes were observed out of the 715 isolates obtained using the combined information provided by both sets of the NILs. Thus, an extremely large number of pathotypes of *P. grisea* exist in the rice-growing areas of southern and central China. Mekwatanakarn et al. (14) investigated the pathotype diversity of the blast fungus in rice fields in Thailand using a similar set of host differentials and detected a total of 175 pathotypes out of 527 isolates assayed. However, the results of that survey cannot be directly compared because of the very different sampling strategy they adopted in obtaining the disease samples.

Compared with the results of the large survey of the pathogen compositions in the late 1970s (1), the number of pathotypes identified in the present study seems to be much larger, on the basis of the same number of host differentials. For example,

Table 5. Most and second-most frequent pathotypes identified by the indica differential near isogenic lines (NILs) for each province

			Most frequent pathotype		Second-most frequent pathotype		
Province	**Isolates tested**	**No. of pathotypes**	**Pathotype**	**Frequency (%)**	**Pathotype**	**Frequency (%)**	**Diversity[a]**
Sichuan	70	18	I0	38.57	I20.1	17.14	2.09
Yunnan	35	12	I0	42.86	I20.1	17.14	1.91
Guizhou	32	14	I0	25.00	I4.1	21.88	2.89
Hubei	106	21	I34.1	23.58	I0	21.70	2.42
Anhui	59	13	I0	30.51	I0.1	27.12	2.00
Jiangxi	83	27	I20.1	16.87	I0.1	12.05	2.89
Hunan	95	21	I20.1	31.58	I0.1	18.95	2.30
Jiangsu	19	9	I0	52.63	I2.1	10.53	1.66
Zhejiang	34	18	I0	17.65	I20.1	11.76	2.69
Fujian	46	10	I0	30.43	I20.1	26.09	1.85
Guangdong	49	20	I0	22.45	I34.1	10.20	2.70
Guangxi	87	23	I0	27.59	I0.1	12.64	2.56

[a] Calculated using Shannon's information statistic (3).

Table 6. Most and second-most frequent pathotypes identified by the japonica differential near isogenic lines (NILs) for each province

			Most frequent pathotype		Second-most frequent pathotype		
Province	**Isolates tested**	**No. of pathotypes**	**Pathotype**	**Frequency (%)**	**Pathotype**	**Frequency (%)**	**Diversity[a]**
Sichuan	70	24	J0.2	27.14	J0	11.43	2.69
Yunnan	35	16	J4.2	31.43	J0.2	20.00	2.28
Guizhou	32	14	J0.2	43.75	J0	12.50	2.03
Hubei	106	34	J0	16.04	J40.2	11.32	3.10
Anhui	59	25	J70.2	18.64	J0.2	16.95	2.72
Jiangxi	83	41	J70.2	9.64	J0.2	7.23	3.46
Hunan	95	27	J0	33.68	J0.2	15.79	2.46
Jiangsu	19	9	J1.2	31.58	J0	21.05	1.91
Zhejiang	34	18	J0.2	23.53	J0	11.76	2.61
Fujian	46	22	J0[b]	13.04	…	…	2.87
Guangdong	49	28	J0	16.33	J40.2	12.24	3.02
Guangxi	87	32	J0.2	19.54	J0[c]	11.49	2.94

[a] Calculated using Shannon's information statistic (3).
[b] Another pathotype (J40.2) in the same province is equally frequent with the one listed in this cell.
[c] Another pathotype (J40.2) in the same province is equally frequent with the one listed in this cell.

Table 7. Most and second-most frequent pathotypes identified by all the 13 host differentials for each province

			Most frequent pathotype		Second-most frequent pathotype		
Province	**Isolates tested**	**No. of pathotypes**	**Pathotype**	**Frequency (%)**	**Pathotype**	**Frequency (%)**	**Diversity[a]**
Sichuan	70	46	Z0.2	10.10	Z0.3[b]	5.71	3.63
Yunnan	35	29	Z20.2	14.29	Z0.2	8.57	3.18
Guizhou	32	25	Z40.3	15.63	Z0.2	9.38	3.07
Hubei	106	75	Z3140.3	7.55	Z3744.3	3.77	4.14
Anhui	59	45	Z0.3	4.21	Z0.2[c]	3.16	2.59
Jiangxi	83	76	Z410[d]	2.41	…	…	4.30
Hunan	95	55	Z2000.1	13.68	Z0.1	8.42	3.64
Jiangsu	19	14	Z4.2	26.32	Z0.2	10.53	2.45
Zhejiang	34	34	…	…	…	…	3.53
Fujian	46	37	Z0.3[e]	4.35	…	…	3.56
Guangdong	49	44	Z1000.2	6.12	Z0.1[f]	4.08	3.74
Guangxi	87	65	Z2100.3[g]	6.90	Z1000.2	4.60	3.92

[a] Calculated using Shannon's information statistic (3).
[b] Another pathotype (Z1000.2) is equally frequent with the one listed in the cell.
[c] Other pathotypes (Z1000.2, Z1600.3) are equally frequent with the one listed in the cell.
[d] Another pathotype (Z2200., Z334.2, Z3600.3, Z3700.3, Z3770.3) are equally frequent with the one listed in the cell.
[e] Other pathotypes (Z20.2, 400, Z400.3, Z1000.2, Z1400.2, Z2000.1, Z3000.3, Z3020.3) are equally frequent with the one listed in the cell.
[f] Other pathotypes (Z 0.2, Z40.3) are equally frequent with the one listed in the cell.
[g] Another pathotype (Z0.2) is equally frequent with the one listed in the cell.

the seven japonica NILs identified 80 pathotypes and the six indica NILs resolved 48 pathotypes; both totals are much larger than the 43 pathotypes that were detected by the seven cultivars used as host differentials in the previous study. The increase in the number of pathotypes may be related to an increase in diversity of the fungal pathogen population or, alternatively, may reflect different resolution of the host differentials used in the two different studies, because it seems likely that NILs may have better resolution than ordinary cultivars as host differentials.

Another noticeable feature concerns the distributional patterns of the various pathotypes. In the previous survey, one pathotype, which was able to cause compatible reaction only on the susceptible check LXHG, occurred at a high frequency (45.7%) and was found to be widespread in the majority of the provinces. The results from the present study are not directly comparable with those of the previous study; being able to attack only LXHG does not guarantee the same virulence spectrum in the two studies because of the very different differentials used in the two studies. However, as in the previous study, isolates that were able to cause compatible reactions on the recurrent parents that were used as susceptible checks occurred frequently. The reason for the widespread distribution of such low-virulence pathotypes is not clear and needs further study, although this phenomenon has been frequently discussed in the literature, which led Vanderplank (22) to postulate that natural selection tends to eliminate pathogen races with unnecessary genes for virulence.

The results also revealed a large difference in the frequencies with which the isolates were able to produce compatible reactions on the host differentials carrying various genes for resistance. For example, when tested on indica isolates, very small proportions (10% or less) of the isolates can cause compatible reactions on NILs carrying *Pi1* or *Pi2*, but a large proportion (41.5%) of the isolates can overcome the resistance of the NIL carrying *Pi3*. Similar though less dramatic differences were also found among the reactions of the isolates tested on japonica host differentials. Two possibilities may be responsible for the different frequencies of the pathogen isolates that were able to attack the various genes for resistance. The first possibility is related to the use of resistant genes in rice cultivars grown in these areas. A high frequency with which the isolates can produce compatible reaction with the NIL carrying the gene for resistance (e.g., *Pi3*) may be an indication that this gene may have been extensively used in the past and hence lost its resistance. Similarly, a low frequency may indicate that this gene (e.g., *Pi2*) has not been used very much in rice production in the areas. An alternative possibility is that a cultivar with the resistance gene that is susceptible to a smaller proportion of the pathogen may, by itself, have a wider spectrum of resistance or be more adaptive than other genes. However, it is difficult to differentiate one possibility from the other without detailed knowledge of the genes carried by the large number of cultivars grown in such vast areas in the past.

Another observation is the resistance of the recurrent parents used in this study. It is clear from Tables 3 and 4 that both CO39, the recurrent parent of the indica NILs, and LXHG, the recurrent parent of the japonica NILs, showed resistance to some pathotypes. This indicates that these susceptible checks also contain genes for blast resistance.

The results also have some implications for the manipulation of resistance genes for genetic improvement of rice. The complexity of the pathotype compositions of the pathogen populations clearly suggests that diversifying the resistance genes in various rice breeding programs may be a useful strategy. However, the most viable approach may be to combine multiple resistance genes into the most widely grown hybrids or cultivars. For example, a cultivar containing both *Pi1* and *Pi2* would be susceptible only to 2.0% of the isolates, and adding one of the *Pi4* genes would make this proportion even smaller (Table 3). However, caution should be taken in how the resistance genes are deployed. Some of the pathotypes produced compatible reactions with many of the NILs and, hence, are virulent to multiple resistance genes. Strong selection against some of the pathotypes that would be imposed by combining some of the resistance genes may lead to the increase of the pathotypes able to overcome multiple resistance genes. Thus, combined use of several approaches may be a good strategy for combating rice blast.

In summary, the results clearly showed that the pathotype composition of the fungal pathogen populations in southern and central China was very complex. The data have provided very useful information for developing strategies for the deployment of the resistance genes in rice breeding programs.

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