Physiological Races of *Cercospora oryzae* in the Southern United States

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ABSTRACT

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Single-conidiospore isolates of *Cercospora oryzae* were obtained from leaf and sheath samples of rice (*Oryzae sativa*) collected from rice-growing regions of Louisiana and Arkansas, and one isolate was obtained from Texas. Eight differential cultivars (Nira, Mars, Kamrose, Rexoro, Shoemed, Zenith, Bluerose, and Fortuna) were inoculated with conidial suspensions from each isolate 4 wk after planting, then evaluated for disease reaction 3 wk later. Forty-three races in six physiologic race groups were identified among 76 isolates. The recently released cultivar Mars, which has vertical resistance to *C. oryzae*, was found to be susceptible to 18 of the 43 races. The weed form of rice known as Louisiana red rice was resistant to all 76 isolates tested.

Narrow brown leaf spot, caused by *Cercospora oryzae* Miyake, is a foliar disease of rice (*Oryza sativa* L.) prevalent worldwide wherever rice is grown. The disease was considered to be the most serious fungal disease of rice in Louisiana during the 1940s (8). Control of narrow brown leaf spot has been attempted through breeding for race-resistant cultivars. However, cultivars with complete or race-specific resistance become susceptible within a few years after their release when new physiologic races of the fungus develop (9).

Ryker (5) first reported physiologic specialization in *C. oryzae* in 1940. He initially found three races among 20 isolates tested (6) and later found five distinct races among 36 isolates tested on five differential cultivars (6). When three more differential cultivars were added to the original five, a total of 16 physiologic races were identified among 36 isolates tested. Recently, Estrada et al (2) identified 19 physiologic races in six race groups among 60 isolates from the Philippines. Because recent information on physiologic races of *C. oryzae* in the United States is lacking, this study was undertaken to reexamine the pathotypic variability in the southern United States and to use this information in varietal screening for resistance to *C. oryzae*.

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MATERIALS AND METHODS

Diseased leaf and sheath samples were collected from the Rice Research Station at Crowley, Louisiana, and from fields located in seven main rice-growing parishes: St. Landry, Evangeline, Acadia, Jefferson Davis, Vermillion, Allen, and Iberia. Also, F. Lee of the University of Arkansas provided diseased leaf samples and G. Whitney provided an isolate from Texas. Specimens of infected leaf or sheath were cut into small pieces, immersed for 20–30 sec in a 5.25% sodium hypochlorite solution, and washed three times with sterile distilled water. The specimen was air-dried for 2 min before being placed on a microscope slide maintained inside a petri dish moist chamber containing filter paper soaked in sterile distilled water. The chambers were incubated for 72 hr at 28 C in a 12-hr light (incandescent)/12-hr dark regime. Conidia from sporulating lesions were streaked onto a water agar plate for germination, and a single conidium was transferred to potato-dextrose agar (PDA) slants. All cultures were stored in a refrigerator at 4 C either as cultures grown on PDA slants or as blocks of mycelium on PDA in screw-capped test tubes containing sterile distilled water. Isolates were transferred to water agar and incubated at 24 C for 1 wk to produce conidia. Inoculum was increased by streaking these conidia onto cornmeal agar and incubating the culture for 7 days at 28 C. Conidia were washed from colonies using sterile distilled water containing Tween 20 (one drop per 100 ml of water). Concentrations were adjusted to 10^5 conidia per milliliter before inoculation.

On the basis of disease reactions

Table 1. Key to identification of physiologic race groups of *Cercospora oryzae* in the southern United States

		Predesignated race groups and reaction to *C. oryzae*[a]								
Sequence	**Cultivars**	**LA**	**LB**	**LC**	**LD**	**LE**	**LF**	**LG**	**LH**	**(LI)**
A	Nira	S								
B	Mars	R	S							
C	Kamrose	R	R	S						
D	Rexoro	R	R	R	S					
E	Shoemed	R	R	R	R	S				
F	Zenith	R	R	R	R	R	S			
G	Bluerose	R	R	R	R	R	R	S		
H	Fortuna	R	R	R	R	R	R	R	S	(R)
Races possible		128	64	32	16	8	4	2	1	1

[a] The first cultivar showing a susceptible reaction to *C. oryzae* determined the race group of an isolate, regardless of the reaction that followed. L = Louisiana, A through H = race groups arranged according to susceptibility of the key cultivar examined in the sequence, and I = race group with no susceptible reaction on any key cultivar. S = susceptible and R = resistant to *C. oryzae*.

obtained in 1983 field experiments, 15 cultivars or lines were selected for final evaluation under greenhouse conditions. Eight cultivars—Nira, Mars, Kamrose, Rexoro, Shoemed, Zenith, Bluerose, and Fortuna—were selected because of their differential reactions to preliminary inoculation with 10 isolates. With the exception of Mars and Kamrose, these cultivars had been used as differentials in earlier studies (6). By including the old cultivars, we could compare the current race composition with those reported in the 1940s. All eight cultivars were planted side by side in a predetermined sequence in an aluminum tray (0.37 × 0.6 m) containing two parts organic soil and one part sand. Four-week-old plants were inoculated by uniformly spraying a conidial suspension over the leaf surfaces. The plants were incubated for 7 days inside a plastic-covered moist chamber built onto a greenhouse bench. The chamber temperature ranged from 16 to 20 C at night and from 27 to 32 C in the daytime, and relative humidity ranged between 65 and 100%. Twenty-one days after inoculation, the plastic was removed and plants were rated for disease reaction on a 0–9 scale, with 0 = no symptoms, 1 = less than 1%, 3 = less than 5%, and 9 = more than 50% leaf area diseased (3). Cultivars rated as 0 were considered resistant, those rated 1–3 were intermediate, and those rated higher than 3 were susceptible.

The race classification of 76 isolates was determined by the method described for *Pyricularia oryzae* Cav. (4). On the basis of susceptible or resistant reactions on the eight differential cultivars, a theoretical maximum of 256 races can be differentiated. The 256 races consist of 128, 64, 32, 16, 8, 4, 2, 1, and 1 in the race groups LA, LB, LC, LD, LE, LF, LG, LH, and LI, respectively. The letter "L" denotes Louisiana, and A, B, C, D, E, F, G, and H denote the susceptibility reaction of the key cultivar when all the cultivars were examined in the same sequence as listed in Table 1. The letter "I" was assigned to a race group if all key cultivars showed a resistant reaction. Races were identified using predesignated pathogenicity patterns of all possible races and a dichotomous arrangement of susceptible and resistant reactions of the differential cultivars. A lowercase letter—a, b, c, d, e, f, g, or h—was assigned to the race number to indicate a susceptible intermediate reaction on that differential cultivar. Susceptibility of the cultivars Labelle, Lebonnet, and Lemont

Table 2. Reaction of differential rice cultivars to physiologic races of *Cercospora oryzae* in the southern United States

Race group[a]	Race[b]	Isolate number[c]	Reaction of host range cultivars[d]							
			A	B	C	D	E	F	G	H
LA	LA-2g	20	S	S	S	S	S	S	S(I)	R
	LA-5bc	23	S	S(I)	S(I)	S	S	R	S	S
	LA-7bc	2	S	S(I)	S(I)	S	S	R	R	S
	LA-8bc	1	S	S(I)	S(I)	S	S	R	R	R
	LA-34g	33	S	S	R	S	S	S	S(I)	R
	LA-35b	24	S	S(I)	R	S	S	S	R	S
	LA-36	51	S	S	R	S	S	S	R	R
	LA-40	16	S	S	R	S	S	R	R	R
	LA-44abf	19	S(I)	S(I)	R	S	R	S(I)	R	R
	LA-50	17	S	S	R	R	S	S	S	R
	LA-54g	26	S	S	R	R	S	R	S(I)	R
	LA-55b	31	S	S(I)	R	R	S	R	R	S
	LA-56b	3,56	S	S	R	R	S	R	R	R
	LA-96	15	S	R	S	R	R	R	R	R
	LA-98	12,73	S	R	R	S	S	S	S	R
	LA-99	8	S	R	R	S	S	S	R	S
	LA-100f	22,25,53	S	R	R	S	S	S(I)	R	R
	LA-101h	37,43	S	R	R	S	S	R	S	S(I)
	LA-103h	36	S	R	R	S	S	R	R	S(I)
	LA-104	6,13,42,46	S	R	R	S	S	R	R	R
	LA-116	4,44,61,68	S	R	R	R	S	S	R	R
	LA-118	38,41	S	R	R	R	S	R	S	R
	LA-119h	40,76	S	R	R	R	S	R	R	S(I)
	LA-120	35,59,60,62,75	S	R	R	R	S	R	R	R
	LA-128	18,63,71	S	R	R	R	R	R	R	R
LB	LB-44b	9	R	S(I)	R	S	R	S	R	R
	LB-51	48	R	S	R	R	S	S	R	S
	LB-52	28	R	S	R	R	S	S	R	R
	LB-56	5	R	S	R	R	S	R	R	R
	LB-62	27	R	S	R	R	R	R	S	R
LD	LD-1h	72	R	R	R	S	S	S	S	S(I)
	LD-2g	55,70	R	R	R	S	S	S	S(I)	R
	LD-4f	11	R	R	R	S	S	S(I)	R	R
	LD-5dh	29,66	R	R	R	S(I)	S	R	S	S(I)
	LD-7dh	50,67	R	R	R	S(I)	S	R	R	S(I)
	LD-8	32,58	R	R	R	S	S	R	R	R
	LD-14	69	R	R	R	S	R	R	S	R
LE	LE-1fg	57	R	R	R	R	S	S(I)	S(I)	S
	LE-2f	65	R	R	R	R	S	S(I)	S	R
	LE-6	21,45	R	R	R	R	S	R	S	R
	LE-8	7,10,14,30,34,39,47,49,54,64	R	R	R	R	S	R	R	R
LF	LF-4	74	R	R	R	R	R	S	R	R
LI	LI-1	52	R	R	R	R	R	R	R	R

[a] L = Louisiana; A, B, D, E, and F = race groups arranged according to susceptibility of the key cultivar examined in the original A through H sequence; and I = race group with resistant reaction to all key cultivars.
[b] Lowercase letters indicate intermediate susceptible reaction on key cultivars.
[c] Cultured from leaves and leaf sheaths of rice plants grown in Texas, Louisiana, and Arkansas.
[d] A = Nira, B = Mars, C = Kamrose, D = Rexoro, E = Shoemed, F = Zenith, G = Bluerose, and H = Fortuna. R = resistant, S = susceptible, and I = intermediate reaction to *C. oryzae*.

Table 3. Susceptibility of rice cultivars to 76 isolates of *Cercospora oryzae*[a]

Cultivar	Susceptible reaction to:		
	Number of 76 isolates	Number of 43 races	Percentage of 43 races
Labelle	75	42	97.7
Shoemed	66	35	81.4
Lebonnet	60	29	67.4
Lemont	49	25	58.1
Nira	44	25	58.1
Rexoro	34	23	53.5
Zenith	26	19	44.2
Mars	19	18	41.9
Bluerose	22	15	34.9
Fortuna	17	13	30.2
Kamrose	5	5	11.6
Louisiana red rice[b]	0	0	...

[a] Results of experiments repeated twice with five plants of a cultivar per experiment.
[b] Close relative of rice and a common weed in Louisiana rice fields.

and of the weed red rice was evaluated by the procedure described for race identification.

RESULTS AND DISCUSSION

Forty-three physiologic races of *C. oryzae* were differentiated by the host range used in this study among 76 isolates from rice cultivars (Table 2). These races were distributed in six of the nine race groups: 25 in LA, five in LB, seven in LD, four in LE, and one each in LF and LI. This race grouping and numbering system was based on the nomenclature system of Ling and Ou (4) and was also used by Estrada et al (2) for identification of physiologic races of *C. oryzae* in the Philippines, although they used a different group of differential cultivars. Among the eight differential cultivars used in our study, Nira, Rexoro, Shoemed, Zenith, Bluerose, and Fortuna were used as differentials in the United States in earlier studies (1,6,7). The results indicate that *C. oryzae* is a highly variable pathogen and that cultivars with vertical resistance may become susceptible within a few years after their release because of the development of new physiologic races of the fungus. Therefore, the use of rate-reducing resistance has been advocated (9).

Screening of rice cultivars and red rice to these races indicated a wide spectrum in resistance to *C. oryzae* (Table 3). The commercially grown cultivars Labelle, Lebonnet, Mars, and Lemont were susceptible to 42, 29, 18, and 25, respectively, of the 43 races tested. The annual weed form known as red rice was resistant to all 76 isolates from commercial rice cultivars and may provide a valuable source of resistance for use in the rice breeding program. Red rice collections cross readily with commercial *O. sativa* cultivars. In studies conducted in the 1940s (1,4,6), Kamrose was found to be resistant to all 10 races and Shoemed was susceptible only to race 9. In our tests, these cultivars were susceptible to five and 35 races, respectively. Mars and Lemont, recently released in Louisiana, were found to be susceptible to 41.9 and 58.1%, respectively, of the 43 races tested. The older cultivars Labelle and Lebonnet were susceptible to 97.7 and 67.4%, respectively, of the races. This study suggests that as the popularity of a cultivar with vertical resistance increases, the number of races of *C. oryzae* that could attack the cultivar also increases. Therefore, selecting and breeding for durable resistance may provide long-term control of narrow brown leaf spot in rice. The resistance expressed by red rice in these studies should be examined further as a possible source of durable monogenic or complete resistance.

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