Distribution of Pathogenic Races and Changes in Virulence of Rice Blast Fungus, *Pyricularia oryzae* Cav., in Yunnan Province, China

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A blast disease caused by *Pyricularia oryzae* Cav. is most destructive in growing rice in Yunnan Province, China. Use of resistant varieties is the most effective and economical countermeasure to protect rice plants from the disease. At present, programs for breeding of resistant varieties are actively undertaken by several institutes in Yunnan.

The mechanism of blast resistance implicitly divided into the following types; qualitative resistance and quantitative resistance. The former is characterized by its specificity to each race of the blast fungus and occasionally called vertical resistance, while the latter is non-specific, being called horizontal resistance. Since breakdowns of the highly resistant varieties dependent on vertical resistance genes took place frequently in Japan[6], Korea[8], Egypt[3] and China[1,10], breeding programs in these countries envisage to develop a high level of the horizontal rather than the vertical resistance.

In order to establish an effective breeding program for durable resistance to the blast disease, it is very necessary to look in more details into the pathogenic specialization of the blast fungus and thereby classify rice varieties on the basis of their reactions to various races. This is the report of the study which was conducted to investigate pathogenic specialization and changes in virulence of blast fungus in Yunnan Province.

This paper is the report of the joint study on the rice breeding in China undertaken by the Yunnan Academy of Agricultural Sciences, China and the Tropical Agriculture Research Center, Japan during the period 1984 to 1989.

Materials and method

Rice samples, including leaves and panicles, infested by rice blast were collected in various districts of Yunnan. Each sample was incubated in a petri dish for a period 20 to 24 hr at the temperature of 24–26°C under the satulated humidity condition. A fresh spore was isolated from each sample by the single-spore isolated method. The isolates were cultured on a PSA slant, and stored under the room temperature condition.

In differentiating blast races, an approach comprising 9 differential varieties proposed by Yamada et al.[7] was employed. The differentials consist of cultivars such as Shin 2 (with a resistance gene *Pi-k*^{s}), Aichiasahi (*Pi-a*), Ishikarishiroke (*Pi-i*), Kanto 51 (*Pi-k*), Tsuyuake (*Pi-k*^{m}), Fukunishiki (*Pi-z*), Yashiromochi (*Pi-ta*), Pi No. 4 (*Pi-ta*^{2}) and Toride 1 (*Pi-z*^{t}). Each race number refers to the designation after Yamada et al.[7], and Yamada[9]. Two additional varieties, or BL 1 and K 59, were also used in part of the ex-

periments. These varieties have a resistance gene *Pi-b* and *Pi-t*, respectively, both of which are not included in the above differential varieties. In this paper, the races virulent to BL 1 and K 59 were characterized by b^+ and t^+ such as $001b^+$, $033b^+$, $017t^+$.

Rice plants were grown in plastic nursery boxes of approximately 5 × 15 cm and 10 cm in depth to mimic upland conditions in a greenhouse with a full supply of nitrogen fertilizer. One box contained 9 or 11 varieties with 5 seeds for each. The plants were inoculated at the four-leaf stage by spraying a spore suspension. The spores for inoculation were produced on the oatmeal decoction sucrose agar.

Results and discussions

1) *Blast race distribution in Yunnan Province*

(1) Race distribution at the provincial level

Two hundred and forty isolates were collected in the paddy fields of 14 counties in Yunnan Province in 1988. Results of the race identification of those isolates are presented in Table 1. The isolates tested were divided into 3 race groups. The races in Group I are virulent only to resistance genes *Pi-k*^{s}, *Pi-a* and *Pi-i*, which are all associated with Japanese native varieties. The races in Group II are virulent to *Pi-k* and *Pi-k*^{m}, both of which are introduced from Chinese varieties, as well as to *Pi-z* relating to the US variety, or Zenith. The races in Group III are virulent to *Pi-ta*, *Pi-ta*^{2} and *Pi-z*^{t}, which were all introduced from indica rice varieties. The races in Groups I, II and III were further classified into 6, 8 and 13 races, respectively. The Group I races were most predominant, and the relative frequency of the isolates was 51.7%. The III and II group races followed, and their relative frequencies were 23.0% and 25.4%, respectively.

Among the 27 races, most predominant was the race 001, the relative frequency of which was 18.8%, being collected in 10 counties. This race was followed by the race 003 with 15.0%, and the rest races comprising 007 (9.2%), 117 (8.8%), 005 (7.9%), 013 (6.7%), 017 (4.2%), 011 (2.9%), 037 (2.9%) and 107 (2.9%). Approximately 79% of the total races under testing belonged to these 10 subgroups. The resistance genes such as *Pi-a*, *Pi-i*, *Pi-k*, *Pi-ta*, *Pi-ta*^{2}, *Pi-z*^{t} are found in

Table 1. Rice blast races identified in Yunnan, 1988

Group	Race	Number of isolates	(%)	Group	Race	Number of isolates	(%)	Group	Race	Number of isolates	(%)
I	001	41	(18.8)	II	015	2	(1.3)	III	$107t^+$	7	(2.9)
	$001b^+$	3			$015t^+$	1			115	1	(0.8)
	$001t^+$	1			$017t^+$	10	(4.2)		$115t^+$	1	
	002	1	(0.4)		031	5	(2.5)		$116t^+$	1	(0.4)
	003	25	(15.0)		$031t^+$	1			117	2	(8.8)
	$003b^+$	5			033	3	(1.7)		$117t^+$	19	
	$003t^+$	6			$033t^+$	1			131	1	(0.4)
	005	13	(7.9)		036	2	(0.8)		134	2	(1.7)
	$005t^+$	6			037	1	(2.9)		$134t^+$	2	
	$006t^+$	1	(0.4)		$037t^+$	6			136	1	(0.8)
	007	13	(9.2)						$136t^+$	1	
	$007t^+$	9		III	101	3	(1.7)		137	1	(1.7)
II	011	7	(2.9)		$101t^+$	1			$137t^+$	3	
	013	8	(6.7)		103	2	(0.8)		$303b^+$	5	(2.1)
	$013t^+$	8			105	6	(2.5)				
					106	2	(0.8)				
									Total isolates	240	(100.0)

the Chinese varieties[2,5]. Since the races 001, 003 and 007 were more predominantly found than the other races in this survey, it is estimated that the varieties grown in large areas of Yunnan Province may not have any resistance genes or have resistance genes such as *Pi-k*^{s}, *Pi-a* and/or *Pi-i*.

Among the races in Group III, 12 races were virulent to Yashiromochi, and only one race (303) was virulent to Pi No. 4. All the isolates identified as the race 303 came from Xishuangbanna county, which was a region grown to indica rice varieties. Thirteen isolates identified as the race 001, 003 and 303 were virulent to the variety BL 1; out of them, 12 isolates were collected in the regions grown to indica varieties. The relative frequency of the isolates virulent to the variety K 59 was 36.2%. In Japan, however, no isolates virulent to K 59 have been found so far.

In 1980, a big number of isolates, amounting to 2,376, collected from various locations throughout Japan were identified regarding their related races. They were classified into 23 races[9]. As mentioned earlier, 240 isolates tested in this experiment were classified into 27 races. If more isolates had been collected and tested, more races might have been found. In Japan, a majority of the isolates virulent to Kanto 51 was also virulent to Tsuyuake[9].

Table 2. Fields where diseased crop samples were collected

Field	Locality	Variety	Seriousness of disease	Date of collection
A	Kunming	—	Heavy	1988. 9. 06
B	Dali	—	Middle	1988. 9. 01
C	Kunming	—	Heavy	1988. 9. 06
D	Tonghai	8126	Heavy	1988. 9. 12
E	Zuxung	Zegen 3	Severe	1988. 9. 02
F	Zaotong	Jinnuo 1	Heavy	1988. 9. 27
G	Yiliang	—	Severe	1988. 9. 15
H	Luliang	—	Heavy	1988. 9. 26

—: Not identified.

Table 3. Race composition in a paddy field in different districts of Yunnan Province, 1988

	Race						
Field A	117t$^+$	137t$^+$					
	52* (96. 3)**	2 (3. 7)					
Field B	Race						
	007	107					
	25 (96. 2)	1 (3. 8)					
Field C	Race						
	001	003	007	005			
	42 (80. 8)	5 (9. 6)	4 (7. 7)	1 (1. 9)			
Field D	Race						
	005	007	001	103			
	37 (68. 4)	13 (24. 1)	2 (3. 7)	2 (3. 7)			
Field E	Race						
	001	003	103	101	303		
	28 (52. 8)	11 (20. 8)	8 (15. 1)	4 (7. 5)	2 (3. 8)		
Field F	Race						
	033	137t$^+$	005	037t$^+$	117t$^+$		
	42 (77. 8)	9 (16. 7)	1 (1. 9)	1 (1. 9)	1 (1. 9)		
Field G	Race						
	001	003	011	013	005	007	
	43 (84. 4)	2 (3. 9)	2 (3. 9)	2 (3. 9)	1 (2. 0)	1 (2. 0)	
Field H	Race						
	005	007	001	115	117t$^+$	015	017t$^+$
	25 (54. 3)	8 (17. 4)	6 (13. 0)	3 (6. 5)	2 (4. 3)	1 (2. 2)	1 (2. 2)

* Number of isolates.
** Relative frequency (%) of each race.

Table 4. Race composition in a field at the Yunnan Academy of Agricultural Sciences

Host variety	Year	Race								
		001	001b$^+$	003	005	015	101t$^+$	105t$^+$	131t$^+$	317t$^+$
Shin 2 (*Pi-k^s*)	1987	9	2	2	1	2	2	3	0	3
	1988	40	0	1	2	0	0	0	1	0

However, in Yunnan Province, 67% of the isolates virulent to Kanto 51 were avirulent to Tsuyuake.

(2) Race composition in a paddy field

With the purpose of identifying a pathogenic race composition in a paddy field, in 1988, a number of diseased panicles were collected from 8 fields, as listed in Table 2. To avoid biased sampling, the samples were collected at a regular space from each field, size of which varied, though.

Results of the race identification are presented in Table 3. As the race 001 was isolated from five fields such as C, D, E, G and H, it is concluded that the varieties planted to these fields had either only a *Pi-k^s* gene, or otherwise no resistance gene.

Two races were isolated from the fields A and B, 4 races from C and D, 5 races from E. Five races were isolated from F, and one isolate classified into the race 033 was virulent to the variety BL 1. Six races were isolated from G, and one isolate classified into the race 001 was virulent to BL 1. Seven races were isolated from the H field. A predominant race existed in each field, although it was very likely that multiple races were present in a paddy field.

In order to compare yearly differences of race composition in a paddy field, in 1987 and 1988, rice samples infected by blast were collected from the differential variety, Shin 2, grown in the same paddy field at the Yunnan Academy of Agricultural Sciences. The race compositions in the two subsequent years are shown in Table 4. Twenty-four isolates collected in 1987 were classified into 8 races, among which the race 001 was most predominant; its relative frequency was 38%. Forty-four isolates collected in 1988 were classified into 4 races, 91% of which belonged to the race 001. This result indicated that the race composition in that field varied from year to year. If the level of blast resistance of a variety or varieties with various resistance genes is to be evaluated at a field level or among different locations, the race composition in the test field or locations has to be carefully identified.

2) Change in virulence of some isolates in Yunnan Province

A survey was carried out to analyze the variation in virulence of blast fungus in Yunnan Province. Thirty-five isolates collected from different locations of Yunnan Province were identified in their races in 1984. Each isolate was maintained on a PSA slant and stored under the room condition. In 1986, the race of each isolate was re-identified. Results of the analysis are presented in Table 5.

Among the 35 isolates tested, 13 isolates showed the same reaction pattern to the differentials in those two years, while the rest 22 isolates showed different reactions, as shown in Table 5. Out of the 22 isolates, 10 isolates were avirulent and three were virulent to Kanto 51, respectively. Four isolates were avirulent to Tsuyuake. Seven isolates were avirulent and one was virulent to Yashiromochi. It deserves to note that the changes in virulence of the isolates under testing took place exclusively on the following 3 rice varieties; Kanto 51, Tsuyuake and Yashiromochi.

A similar analysis was also undertaken in 1987. Out of the 19 isolates collected in 1986, ten showed the same reaction pattern to the differentials, and the rest 9 isolates presented in Table 6 changed their reactions in 1987.

The virulence of some isolates obtained

Table 5. Change in virulence of blast fungus in Yunnan (1)

Isolate	Race		Differential variety which showed different reaction		
	1984	1986	Kanto 51	Tsuyuake	Yashiromochi
Y84- 01	107	117	+		
- 16	037	017	—		
- 29	107	117	+		
- 37	117	007	—		—
- 40	013	003	—		
- 41	013	003	—		
- 42	013	003	—		
- 44	013	003	—		
- 45	013	003	—		
- 46	037	017	—		
- 48	013	003	—		
- 52	033	013		—	
- 55	013	113			+
- 93	107	007			—
- 94	137	017		—	—
- 95	037	017		—	
- 96	105	115	+		
-101	107	007			—
-102	137	017		—	—
-104	107	007			—
-105	107	007			—
-106	013	003	—		
Isolates which showed the same pattern of reaction to the differentials in both years, 1984 and 1986			Y84-09 (007), -26 (007), -27 (007), -28 (007), -35 (003), -36 (003), -39 (007), -43 (013), -49 (037), -50 (013), -51 (017), -53 (017), -57 (007).		

+: Resistant reaction in 1984 and susceptiple reaction in 1986,
—: Susceptible reaction in 1984 and resistant reaction in 1986.

Table 6. Change in virulence of blast fungus in Yunnan (2)

Isolate	Race		Differential variety which showed different reaction			
	1986	1987	Aichiasahi	Kanto 51	Tsuyuake	Yashiromochi
Y86- 07	013	033			+	
- 42	017	007		—		
-258	007	017		+		
-272	017	137			+	+
-284	037	017			—	
-289	007	017		+		
-334	007	037		+	+	
-377	003	001	—			
-380	003	013		+		
Isolates which showed the same pattern of reaction to the differentials in both years, 1986 and 1987			Y86- 35 (115), - 37 (007), -144 (007), -228 (033), -243 (033), -253 (033), -262 (016), -324 (017), -339 (136), -394 (013).			

+: Resistant reaction in 1986 and susceptible reaction in 1987,
—: Susceptible reaction in 1986 and resistant reaction in 1987.

from single lesions in 1984 was tested in 1986. The results are presented in Table 7. Three isolates obtained from lesion A were identified as race 107 in 1984, while in 1986, they were identified as race 117 on the basis of their virulence to Kanto 51. Four isolates from lesion B changed their reactions in Tsuyuake from virulence in 1984 to avirulence in 1986, when they were identified as race 017. Among the 3 isolates from lesion C, one isolate stably showed the reaction of 007, but the other 2 isolates differed in virulence between the two years, 1984 and 1986.

From the results of the identification tested of blast races, the isolates classified into such races as 013, 017, 031, 033 or 037, which were virulent to Kanto 51 or Tsuyuake, are divided into 2 groups in respect to the forma-

Table 7. Change in virulence of blast fungus isolated from single lesions

Lesion-isolate	Race		Differential variety which showed different reaction		
	1984	1986	Kanto 51	Tsuyuake	Yashiromochi
A-1	107	117	+		
A-2	107	117	+		
A-3	107	117	+		
B-1	037	017		−	
B-2	037	017		−	
B-3	037	017		−	
B-4	037	017		−	
C-1	007	017	+		
C-2	007	007			
C-3	107	007			−

+: Resistant reaction in 1984 and susceptible reaction in 1986,
−: Susceptible reaction in 1984 and resistant reaction in 1986.

Table 8. Difference in number of lesions on the three differential vareities: Shin 2, Kanto 51 and Tsuyuake, by virulent races and isolates

Race	Isolate	Differential variety		
		Shin 2	Kanto 51	Tsuyuake
013	Y88- 172, - 237, -240	+++	+++	
	Y88- 128, - 138, -231	+++	±	
017	Y88- 101, -1274	+++	+++	
	Y88- 260, -1265	+++	+	
031	Y88- 142, - 150, -420	+++	+++	+++
	Y88- 288, -1316	+++	+++	±
	Y88-1363, -1364	+++	±	±
033	Y88- 181, -1354	+++	+++	+++
	Y88- 176, -1362	+++	+++	±
	Y88-1365	+++	+	±
037	Y88- 57, - 61, -210	+++	+++	+++
	Y88-1352	+++	+++	+

+++: Over 20 lesions per plant, +: 5-10 lesions per plant, ±: Less than 4 lesions per plant.

Table 9. Change in virulence during the serial transfer of blast fungus

Isolate	Race of original isolate	Race of regenerated isolates							Remark
		1st	2nd	3rd	4th	5th	6th	7th	
Y 1	001	001	001	001	001	001	001	—	*
2	001	001	001	001	001	001	001	—	*
3	001	001	001	001	001	001	001	—	*
4	003	003	003	003	003	003	003	—	*
5	007	007	007	007	007	007	007	—	*
6	007	007	007	007	007	007	007	—	*
7	007	007	007	007	007	—	007	—	*
8	101	101	101	101	101	101	101	—	*
9	017	017	007	007	007	017	007	—	
10	017	017	017	016	016	—	017	—	
11	011	011	011	011	013	011	013	011	
12	017	017	007	007	107	107	107	—	
13	013	033	013	033	033	013	033	013	
14	115	115	105	101	115	105	115	—	
15	115	115	115	—	115	105	115	—	
16	136	136	136	132	136	136	136	—	
17	303	303	303	103	303	303	303	—	

*: The virulence did not change throughout the serial transfer of blast fungus.
—: The virulence was not tested.

tion of susceptible type of lesions, as shown in Table 8. One group formed a number of lesions on each of Shin 2, Kanto 51 or Tsuyuake, while the other group formed fewer lesions on Kanto 51 or Tsuyuake as compared with those on Shin 2. No reports have so far indicated that Japanese blast fungus is classified into the latter group. When a large quantity spores avirulent to Yashiromochi were inoculated to spindly growing plants of Yashiromochi, susceptible-like type of lesions was formed on the rice plants. It may therefore be justifiable to assume that the results of race identification may vary due to the differences in number of spores inoculated and physiologic status of rice plants, as well as in environmental conditions after inoculation. The above-stated result indicates that different identification of race might be associated with the unstable nature of reaction of rice varieties such as Kanto 51, Tsuyuake and Yashiromochi.

Another type of changes in virulence was found during the serial transfer of blast fungus of the 17 isolates, which were collected from different locations in Yunnan Province in 1986 and 1987. Each isolate was successively transferred on a PSA slant at 6 or 7 times during the period 1988 to 1989. After 3 weeks of each transfer, races of the regenerated isolate lines were identified in their virulence. The results are presented in Table 9. Among the 17 isolates tested, the virulence of the cultures derived from 8 isolates coincided with the original reaction, but the rest 9 isolates varied. Such changes in virulence of the regenerated lines took place in a very few varieties, and the variants formed relatively few and small lesions on those varieties. This result would suggest those varieties. This result would suggest that the changes in virulence of the isolates during the sequential transfer be associated with the decrease of aggressiveness of fungus or with the unstable reaction of the rice plants.

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