Centro Internacional de Agricultura Tropical

Trabajos presentados por

VARIABILITY OF PYRICULARIA ORYZAE AND ITS RELATION TO VARIETAL RESISTANCE

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SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz, Palmira, Valle del Cauca - 1971

Octubre 8-12, 1971

been referred to as "vertical resistance","specific resistance", or "major gene resistance". Another type of resistance is stable; it is unaffected by variation in pathogenicity of races. This type of resistance has been called "horizontal resistance", "field resistance", "general resistance", "race non-specific resistance", as well as by other terms. Many genes are usually involved in controlling this type of resistance (Van der Plank, 1963;Caldwell, 1968; Robinson, 1969).

"Vertical resistance"against a specific race will break down because when a new virulent race multiplies, the population increases and all individuals are pathogenic to the variety, i.e., breed true to the new race. If, however, the new race does not breed true and produces other races in its progeny and if the variety has a strong gene or genes for resistance or a broad spectrum of resistance against most of the races developed,severe outbreak will not occur because few of the original pathogenic races occur in the progeny. This seems to happen for the blast fungus, Pyricularia oryzae, against varieties that have a broad spectrum of resistance. The fungus seems to be extremely variable in pathogenicity. Even with pathogenic races, the resistance of the varieties does not seem to break down because the fungus has changed. The resistance seems to be stable (Ou et

al., 1971). It could be called "horizontal resistance" but it does not agree with the definition of the term by Robinson (1969).

Variability in nature and identification of broad spectrum resistance through the International Blast Nurseries

During the half century, numerous tests have been made in several countries to identify blast resistant varieties and use them for breeding. The success in these breeding programs has been limited. The new varieties were resistant only for a few years, possibly because the variability of the fungus was underestimated. Varietal reaction varies from locality to locality as well as from season to season in the same locality while the work of testing varietal resistance in the past was limited to a relatively small number of varieties, a few seasons, and a limited number of geographic areas. The resistant varieties selected as donors of resistance have not been exposed to many pathogenic races and consequently they did not have a very broad base of resistance.

Some of the work done in the Philippines illustrates the change of varietal reaction by localities and by seasons. From 1962 to 1964, 8,214 varieties of the world collection of the International Rice Research Institute (IRRI) were tested in a

blast nursery. Of these, 1,457 were found highly resistant in the first test. These resistant varieties were tested seven additional times in the same blast nursery. Only 450 remained resistant. The 450 varieties were tested in seven stations in different regions of the Philippines and after a few repeated tests only 75 remained that showed resistant reaction in all tests at all stations.

In closer examination of changes of races in a blast nursery during a 21-month period (Quamaruzzaman and Ou, 1970) (Fig. 1), we found that both the composition (different races) and frequency (population of each race) are different each month. Of the 363 samples tested, 60 races were identified. Though the number of samples is small in comparison with the actual number of conidia and races that might have been present in the nursery, it nevertheless illustrates the changes of races in the same blast nursery. It is conceivable that such changes also occur in the field and this may explain the observations that certain varieties, while resistant in seedling stage, are susceptible to neck blast.

To identify material that has a broad spectrum of resistance, blast resistance must be tested repeatedly in many geographic regions. Thus, an international program is essential. The International Uniform Blast Nurseries (IBN) was started in 1963. Testing materials included 258 leading commercial varieties and the varieties used by three countries for differentiating races. In

1966 another 321 resistant varieties selected from the IRRI blast nursery were added to constitute group II of testing varieties. In 1969 most of the susceptible varieties were deleted, a few other varieties were added and the two groups were consolidated to form one group of 356 varieties. Up to 1970, more than 200 test results were obtained from 50 stations in 26 countries, mostly in Asia, some in Latin America and Africa. Detailed data are reported biannually in International Rice Commission Newsletters: 1964, 1966, 1968, and 1970.

The results of the IBN showed that many rice varieties which are resistant in one station, one country or in a region of several countries are susceptible to other stations, countries, or regions because of the existence of different prevailing races. Many of the varieties tested in a new region are resistant, at least initially, i.e.,many japonicas are resistant in tropical Asia while many indicas are resistant in Japan and Korea. The blast fungus apparently is capable of producing new races all the time. But the new races can only survive when there are susceptible host varieties. Thus, after a long period of time, prevailing races in Japan or Korea are virulent to japonicas while the races in the tropics are virulent to indicas.

The most valuable information obtained from the IBN is the identification of many varieties that have a broad spectrum of

resistance, although no variety was resistant in all tests (Ou et al., 1970). Some of the most resistant varieties are shown in Table 1. Varieties such as Tetep are consistently more resistant than others. It is resistant in 97.5 percent of the tests made. Fanny, a susceptible variety, was resistant in only 19 percent of the tests.

Variability among conidia from single lesions and from single conidial cultures.

Strains of P. oryzae differing in pathogenicity were first noticed by Sasaki (1922). Latterell et al. (1954) first identified physiologic races in the United States. During the last decade, such identification of races was carried out in many countries using different sets of differential varieties. Many races were identified in each country (Ou and Jennings, 1969). Atkins et al. (1967) recommended an international set of differentials and Ling and Ou (1969) suggested the standardization of the international race numbers.

In all the above studies, a pure culture was obtained by a single conidium from a sample. The inoculum was prepared from the pure culture for testing pathogenicity. Ou and Ayad (1968) tested 56 monoconidial cultures from one leaf lesion on the Philippine set of differentials and found 14 races. The 44 monoconidial cultures from another lesion were differentiated into eight races. They also found that 25 monoconidial subcultures each from two single conidial pure cultures were differentiated into nine and

ten races (Table 2).

Giatgong and Frederiksen (1969) found that 20 monoconidial lines were separated into four to seven races by testing on four varieties. In three consecutive generations, the monoconidial lines continued to change into new races in each generation.

These studies indicated that the conventional method of race identification by use of only one conidium presents only a partial and transitory picture of pathogenicity.

It is known that plant pathogenic fungi do change, but once changed, the new races are generally stable. P. oryzae, as shown in the above study, changes in each generation, and if more varieties were used as differentials, each of the conidia would have a different pathogenicity. This was shown early in our study on races of P. oryzae (Bandong and Ou, 1966). Of the 50 monoconidial isolates none had the same pathogenicity on the 110 varieties selected as candidates for differentials. P. oryzae seems to have a new dimension of variability.

The cause of such great variability is still uncertain. Suzuki (1965) reported that the conidia, appresoria, and mycelial cells are in a "persistent" heterokaryotic state, that anastomosis is common, and that each of these cells contains three to seven nuclei. Yamasaki and Niizeki (1965), however, reported on the contrary that most of the cells are uninucleate, though in certain

strains 13 to 20 percent of the cells were multinucleate, containing from two to six nuclei. Anastomosis and migration of the nucleus were observed, and nuclei had apparently fused to form diploids. Other studies also showed that most cells are uninucleate (Wu, 1967; Giatgong and Frederiksen, 1969). Kiyosawa (1967) reported that the frequency of spore mutation from avirulent to virulent to a variety may be as high as 26.3 percent in certain strains. A cytological study by Giatgong and Frederiksen (1969) concluded that variation could have derived from mutation, sexual hybridization, the parasexual cycle, or heterokaryosis. The perfect stage of P. oryzae is not known nor is any evidence of any of these genetic changes known.

Variability in relation to varietal resistance

As a result of constant change in pathogenicity, numerous races are present in the field and the blast nursery. This was substantiated by isolating 363 single conidia from a blast nursery from which 60 races were identified as mentioned above (Quamaruzzaman and Ou, 1970).

The variability of P. oryzae also extends the range of the host varieties. The group of varieties with a broad spectrum of resistance identified by the international blast nurseries (Ou et al., 1970), while usually free from infection, occasionally showed susceptible reaction (Table 1). According to conventional thought, new races have developed and the resistant varieties will break

down when the population of the new races increases.

These varieties have also been tested in our blast nurseries over 40 times during the last 8 years. Under epiphytotic conditions, a few large susceptible type lesions occasionally appeared. This gives us the opportunity to study the fungus races and to find whether these varieties will break down or maintain their level of resistance by producing only few lesions.

The possible reasons why these varieties produce few lesions in the blast nurseries are: (1) conidia population of the pathogenic races specific to these varieties are low, and (2) genetically controlled interaction between the fungus and the host variety. To determine this, the pathogenic races on Tetep, one of the most resistant varieties, were isolated, cultured and inoculated back to Tetep and another resistant variety, Carreon. The races were also reisolated and inoculated. A very susceptible variety, Khao-teh Haeng 17 (KTH), was used as control.

The results of 37 such inoculations show that Tetep consistently produced a few susceptible type lesions while there were many on KTH (Table 3). The average number of lesions per seedling on Tetep was 2.2 and on KTH, 32.7. One inoculation produced 14.1 lesions on Tetep and another produced 16.1 lesions. Several isolates produced no lesions on Tetep. These results indicated that the few lesions produced on Tetep are not due to the low conidial

population of the pathogenic races inoculated.

The small number of lesions on Tetep and the large number on KTH in the same inoculations suggest that many of the conidia failed to infect Tetep even though the fungus was isolated from Tetep. To determine this, many single-conidium subcultures were made from six of the pathogenic isolates from Tetep: 160 from isolate FR-1, 48 from FR-1-138 (single-conidial reisolate from FR-1), 45 from FR-78, 100 from FR-78-16 (the most pathogenic single-conidial reisolate from FR-78; it produced 16.1 lesions on Tetep), 52 from FR-79, and 45 from FR-80. All these subcultures were inoculated to Tetep, Carreon, the 12 Philippine differential varieties (Bandong and Ou, 1966) and eight international differentials (Atkins et al., 1967). The number of susceptible and intermediate types of lesions on Tetep, Carreon and KTH were counted in each inoculation. Lesions of intermediate type were not included in the data as they produce a relatively small number of conidia and are unimportant epidemiologically.

By the Philippine differentials, the 160 single-conidial subcultures of FR-1 were separated into 28 pathogenic races; 48 of FR-1-138 into 12 races, 45 of FR-78 into eight races, 100 of FR-78-16 into 51 races, 52 of FR-79 into 19 races, and 45 of FR-80 into seven races (Table 4). Altogether, 78 different races were identified among the 450 single-conidial subcultures of the six isolates. These races differ greatly in their pathogenicity.

Some infect only one or two varieties, others infect 11 or all the 12 differential varieties. Based upon the number of the Philippine differential varieties infected by these races,they were grouped as shown in Table 5. The distribution of subcultures among the races developed varies. Usually a few races have a larger number of subcultures.

The numbers of races separated by the international differentials and combination of the two sets are shown in Table 6. When more differentials are used, more races are differentiated.

The number of races and number of subcultures that infect Tetep, Carreon, and KTH,and the number of susceptible type of lesions on these three varieties are shown in Table 7. Many of the races and many of the subcultures originally isolated from Tetep failed to infect Tetep. The numbers of lesions on Tetep and Carreon were consistently and significantly smaller than that on KTH. Even if the races or the subcultures are considered pathogenic, the numbers of lesions produced on Tetep and Carreon are small.

Tetep and Carreon were planted in several blocks, each of 10 rows, in our blast nursery and a susceptible variety,Tjeremas, was planted as control after every two rows of either Tetep or Carreon. Before the appearance of any lesion on young seedlings they were inoculated with an isolate from Tetep, FR-78-16. The

number of lesions on 100 seedlings was counted every other day, about a week after inoculation. Tetep and Carreon showed only a small number of lesions in comparison with Tjeremas (Fig. 2). The results agree very well with greenhouse inoculations.

The pathogenic fungus races from the few lesions on other resistant varieties are being studied in the same manner. Preliminary results show they behaved similarly to those from Tetep.

The above experiments suggest that the few lesions produced on Tetep and other resistant varieties are probably the result of a genetically controlled reaction between the fungus and the host variety. The original pathogenic fungus races separate into a great number of races in each generation of multiplication and the broad spectrum resistance of the host operates against most of the races developed.

Discussion

The above studies showed the extreme variability in pathogenicity of Pyricularia oryzae. Many races are present in nature and are produced from single lesions and single conidial cultures. They vary greatly in pathogenicity. This phenomenon is unusual, but not unique. Snyder (1933), in studying the variability in Fusarium, said "All evidence from studies upon variation in fungi illustrate the hazard of using single-spore culture in the study of a species exhibiting variation, unless large numbers of

monoconidial cultures are employed." Moreover, "... within a given monoconidial line it was possible to assemble, through the phenomenon of dissociation, a group of cultures almost representative of the range in colony types and virulence exhibited by the entire group of strains. Thus a monoconidial parent has been shown in certain instances by its dissociates to possess the potentialities of most of the type of colony character and virulence of the 15 strains studied." Snyder and Hansen (1954) stated "Although the principle (variability of fungi) is recognized and accepted, the significance of variability is not yet fully appreciated, nor is it widely utilized." It is well illustrated by the pathogenic variability of P. oryzae. Such variability may also exist in some other fungus pathogens.

Stakman (1954), after the outbreak of race 15B of Puccinia graminis tritici, wrote: "Concepts regarding the dynamics of rust must be broadened and deepened by extensive and intensive investigation." And, "The number of biotypes of P. graminis tritici appears to be comparable to Ustilago zeae and Helminthosporium sativum. At least 15,000 biotypes of U. zeae and at least 1,000 of H. sativum are present in Minnesota and there is no visible limit to numbers."

The great variability of P. oryzae enables the fungus to extend the range of host varieties. On the other hand, a particular pathogenic race cannot build up rapidly, they become sep-

arated into many races. The population of original races present in the progeny is small or nil, as indicated by some isolates (Table 3). Since the varieties possess a broad spectrum of resistance, most of the races developed cannot infect the varieties. Thus at most a few lesions developed. These varieties are therefore not broken down by the presence of new pathogenic races.

Tetep and other varieties seem to be stable in resistance to blast but they are not "race non-specific" nor "horizontal" as defined by Robinson (1969). They react differently to different races. They are resistant to most races, but susceptible to a few, at least in a qualitative sense, though they have few lesions. Such a pathogen-host relationship resulting in a stable resistance is seemingly new.

The level of resistance in varieties such as Tetep depends on how broad the spectrum of resistance is. The more races the varieties are able to resist, the fewer lesions that develop. As shown in Table 3, Carreon is resistant to the isolates from Tetep. It may be possible to combine the resistance of such varieties to further broaden the spectrum of resistance. The degree of resistance to blast may be measured by the percentage of all races, potential or in existence, to which a variety is resistant.

Sakuri and Toriyama (1967) and Yunoku et al. (1970) reported

varieties St 1 and Chugoku 31 have "field resistance." By greenhouse and blast nursery tests, both varieties produced a small number of lesions. It may be specualted that a similar genetic mechanism, as described above, is involved, though they did not study the fungus in detail.

The genetics of resistance in Tetep and other varieties are not known. It would be most interesting to learn whether few strong genes or many genes are involved. Because of the lack of genetic information, extensive and intensive tests must be used to select a genotype with broad spectrum resistance in breeding programs.

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Table 1. The most resistant varieties selected from the International Uniform Blast Nurseries.

Variety	1964-65		1966-67		1968-69		1970		Total		%
	No. Tests	No. Suscept.	No. Tests	No. Suscept.	No. Tests	No. Suscept.	No. Tests	No. Suscept.	No. Tests	No. Suscept.	Resis-tant
From Group I varieties											
Tetep	22	2	59	0	62	2	23	1	199	5	97.5
Nang chet cuc	39	2	49	2	63	3	23	0	176	7	96.0
Tadukan	56	3	57	2	63	5	23	0	201	10	95.0
R 67	51	4	51	2	63	1	21	3	188	10	94.7
C46-15	56	2	60	3	63	4	22	4	203	13	93.6
CI 7787	50	4	59	2	63	4	20	2	194	12	93.9
Pah Leuad 29-8-11	47	4	59	4	59	4	20	2	187	14	92.5
D25-4	31	3	60	6	64	3	23	2	180	14	92.3
Trang Cut L. 11	27	4	50	5	64	2	23	0	166	11	94.0
Pah Leuad 111	18	2	55	5	63	3	22	2	160	13	91.9
Fanny (susceptible)	54	51	49	34	47	32	23	23	173	140	19.0
From Group II varieties											
Mamoriaka			32	0	62	0	22	1	117	1	99.1
Huan-sen-goo			32	1	63	1	21	0	116	2	98.4
Dissi Hatif (DH-2)			32	0	63	1	22	2	117	3	97.5
Carreon			31	0	62	3	22	0	115	3	97.4
Pah Leuad 29-8-11			31	1	61	0	22	2	114	3	97.3
Ram Tulasi			32	0	60	1	22	2	114	3	97.3
C46-15			30	1	61	1	23	1	112	3	97.3
Ram Tulasi (sel)			33	0	60	2	19	1	112	3	97.3
Ca 435/6/5/1			31	1	62	2	22	1	115	4	97.4
DNJ-60			29	1	62	1	22	2	113	4	96.5

TABLE 2.- Pathogenic races of *Pyricularia oryzae* among monoconidial cultures from lesion No. 1, lesion No. 2, and monoconidial subcultures of cultures L-1-43 and L-1-49 based upon reactions on the Philippine differential varieties (Ou & Ayad, 1967)

Differential variety	Reaction																												
Kataktara DA2	R	Rs[a]	R	R	R	R	R	R	R	R	R	R	R	Rs	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CI5309	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Chokoto	R	Rs	R	R	R	R	R	Rs	R	R	R	R	Rs	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
CO 75	S	R	R	Rs	R	Rs	R	R	R	R	S	S	S	S	Rs	R	R	R	S	R	S	Rs	Rs	R	Rs	S	S	R	R	Rs
Waewaz	R	S	S	Rs	R	R	Rs	S	R	Rs	S	R	Rs	R	Rs	R	S	S	Rs	S	R	S	S	R	S	R	S	R	S	S
Pai-kan-tao	S	Rs	S	Rs	R	R	R	Rs	R	R	R	R	R	R	R	R	S	R	R	R	R	Ms	S	R	R	R	R	R	R	R
Peta	S	S	S	S	S	Rs	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	?	S
Ruminad Str 3	S	S	S	S	S	Rs	S	Rs	Rs	S	S	S	S	S	S	S	S	S	Rs	Rs	S	S	Rs	S	S	S	S	S	Rs	S
Taichung T-C-W-C	S	S	S	Rs	S	R	Rs	S	R	S	S	S	Rs	R	R	R	S	Rs	R	S	R	Rs	S	R	S	Rs	R	S	Rs	S
Lacrosse	S	S	S	Rs	S	R	S	S	R	S	S	R	S	Rs	Rs	R	Rs	S	Rs	Rs	S	Rs	S	S	S	R	Rs	R	S	Rs
Sha-tiau-tsao (S)	S	S	S	S	S	R	S	S	R	Rs	S	S	S	S	Rs	Rs	R	S	R	S	R	S	S	Rs	Rs	Rs	S	Rs	Rs	S
Khao-tah-haeng 17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Philippine race designation	P 6	P 8	P 12	P 16	P 17	P 26	P 30	P 36	P 58	P 47	P 52	P 54	P 55	P 56	P 57	P 58	P 59	P 62	P 63	P 64	P 69	P 70	P 71	P 73	P 76	P 77	P 78	P 79	P 90	P 81
Lesion No. 1																														
No. of monoconidial cultures per race				12		4	8		1		2	1	4	4	13	3	1				1	1		1						
Lesion No. 2																														
No. of monoconidial cultures per race	1	24	11					2										2	1	2			1							
Culture L-1-43																														
No. of monoconidial subcultures per race		6		4	3		4			1					2			1				3						1	1
Culture L-1-49																														
No. of monoconidial subcultures per race				9			7																	1		1				

[a] Where two or more cultures were inoculated, this column include R reaction

Table 3. Lesion development on varieties Tetep, Carreon, and Khao-teh-haeng 17 inoculated at the same time with isolates and reisolates of P. oryzae from Tetep.

Isolates and reisolates from Tetep	Average number of lesions per seedling[a/]		
	Carreon	Tetep	KTH
FR-1	0	0.0	63.4
FR-4A10	0	14.1	53.3
FR-13-141	0	0.1	67.3
FR-13-1a	0	0.3	42.5
FR-28	0	0.0	39.2
FR-30A2	0	0.4	20.3
-30A3	0	2.5	26.0
-30A5	0	5.8	44.5
-30A6	0	2.6	43.0
-30A7	0	2.1	61.4
-30A8	0	0.2	62.8
-30A42	0	0.4	15.2
-30A43	0	0.0	15.7
-30A44	0	0.9	17.0
-30A45	0	0.5	14.6
-30-1a	0	0.1	38.4
-30B1	0	0.8	14.1
-30B2	0	0.6	29.7
-30B3	0	0.0	14.6
FR-31	0	0.1	58.3
FR-35-1b	0	0.7	38.6
FR-50-1b	0	0.3	30.3
FR-52-1b	0	0.0	24.1
FR-54-1b	0	0.4	17.7
FR-56	0	4.8	55.6
-56A2	0	2.5	15.6
-56A9	0	5.0	16.3
FR-57	0	0.3	35.5
-57-1b	0	0.2	17.2
FR-59 A1	0	8.1	34.5
-59-1b	0	0.2	20.3
FR-78	0	0.8	22.4
-78A4(1)	0	3.7	44.0
-78A4(2)	0	3.8	19.9
-78-1a	0	3.1	21.5
-78-1b	0	1.3	9.7
-78-16	-	16.1	44.6
Average	0	2.2	32.7

[a/] Counted from 20 plants.

Table 4. Pathogenic races of P. oryzae from monoconidial subcultures of six isolates from Tetep; based on the reaction of Philippine differential varieties.

Parental isolates and race	Philippine races (no. isolates)	Total no. of races	Total no. of monoconidial subcultures
FR-1 (P8)	P 8(61) P18 (1)P32 (3) P64 (2) P118(1) P149(1) P12(29) P19 (2)P36 (4) P70 (1) P125(1) P150(2) P15 (1) P21 (1)P50(14) P80 (1) P141(4) P153(1) P16 (1) P25 (1)P52 (4) P81(13) P142(2) P17 (3) P30 (2)P62 (1) P87 (2) P143(1)	28	160
FR-1-138 (P81)	P 8(19) P17 (6)P52 (1) P118(1) P 9 (1) P36 (2)P62 (1) P141(1) P12(10) P50 (4)P98 (1) P175(1)	12	48
FR-78	P87 (2) P92(33)P120(1) P131(2) P89 (1) P112(4)P123(1) P166(1)	8	45
FR-78-16 (P-92)	P 8 (1) P 26(1)P 77(1) P148(1) P179(1) P189(1) P15 (1) P 28(3)P87(11) P152(1) P180(1) P190(1) P16 (1) P 33(1)P89(2) P153(1) P182(1) P191(1) P17 (1) P 35(2)P90(1) P166(2) P183(1) P192(1) P18 (2) P 46(1)P92(14) P167(1) P184(1) P193(1) P19 (3) P 48(1)P100(1) P172(1) P185(1) P194(1) P20 (1) P 52(8)P102(4) P173(3) P186(1) P21 (1) P 66(5)P114(1) P174(1) P187(1) P25 (1) P 70(1)P120(2) P178(2) P18[illegible](1)	51	100
FR-79	P 8(10) P 35(4)P102(5) P168(4) P12 (1) P 81(4)P120(2) P169(1) P16 (1) P 90(2)P165(1) P170(1) P17 (5) P 92(4)P166(1) P177(1) P18 (2) P100(2)P167(1)	19	92
FR-80	P 8(37) P 17(2)P 52(1) P117(1) P12 (2) P 50(1)P 62(1)	7	45

Table 5. Pathogenic races derived from isolates FR-1, FR-1-138, FR-78, FR-78-16, FR-79, and FR-80 grouped by the number of the Philippine differential varieties infected.

No. of differential varieties infected	FR-1		FR-1-138		FR-78		FR-78-16		FR-79		FR-80	
	No. Races	No. Subc.	No. Races	No. Subc.	No. Races	No. Subc.	No. Races	No. Subc.	No. Races	No. Subc.	No. Races	No. Subc.
1							1	1				
2	1	1					1	1				
3	1	1					6	6				
4	4	4					7	9	5	12		
5	7	15	2	2			5	7	1	1		
6	5	22	5	11			7	10	5	17	2	3
7	1	61	1	19			6	12	2	11	1	37
8	3	34	3	12	2	3	6	13	2	2	2	3
9	3	17	1	4	3	6	7	11	3	5	2	2
10					1	33	3	17	1	4		
11	2	3			2	3	2	13				
12	1	2										
Total no. of races	28		12		8		51		19		7	
Total no. of subcultures		160		48		45		100		52		45

Table 6. Number of pathogenic races differentiated from the single conidial subcultures of seven single conidial parental isolates of Pyricularia oryzae by three different sets of differential varieties.

Isolate and total no. of subcultures	By 8 international differential varieties	By 12 Philippine differential varieties	By combination of two sets and Tetep & Carreon (20 varieties)
FR-1 (160)	20	28	59
FR-1-138 (48)	6	12	22
FR-78 (45)	3	8	11
FR-78-16 (100)	23	51	63
FR-79 (52)	25	19	37
FR-80 (45)	3	7	12

Table 7. Qualitative (pathogenic races) and quantitative (no. susceptible lesions) pathogenicity of monoconidial subcultures of isolates FR-1, FR-1-138, FR-78, FR-78-16, FR-79, FR-80, isolated from Tetep when inoculated on Tetep, Carreon, and Khao-teh-haeng 17 (KTH).

Isolate	Variety	No. of races		No. subcultures		Ave. no. lesion per plant by all subcultures	Ave. no. lesion per plant by pathogenic subcultures
		Total	Pathogenic	Total	Pathogenic		
FR-1	Carreon	28	11	160	60	0.3	1.1
	Tetep	28	5	160	19	0.1	1.4
	KTH	28	28	160	160	33.9	33.9
FR-1-138	Carreon	12	6	48	15	0.8	2.8
	Tetep	12	1	48	3	0.1	6.2
	KTH	12	12	48	48	56.6	56.6
FR-78	Tetep	8	7	45	44	5.2	6.1
	KTH	8	8	45	45	22.6	22.6
FR-78-16	Carreon	51	1	100	1	0.1	8.7
	Tetep	51	17	100	43	3.6	8.9
	KTH	51	48	100	97	17.4	18.0
FR-79	Carreon	19	1	52	1	0.01	0.5
	Tetep	19	11	52	17	0.7	2.5
	KTH	19	19	52	52	46.3	46.3
FR-80	Carreon	7	3	45	7	0.6	4.1
	Tetep	7	1	45	1	0.2	7.9
	KTH	7	7	45	45	47.9	47.9
All isolates	Carreon					0.3	1.7
	Tetep					1.5	6.6
	KTH					34.6	34.8

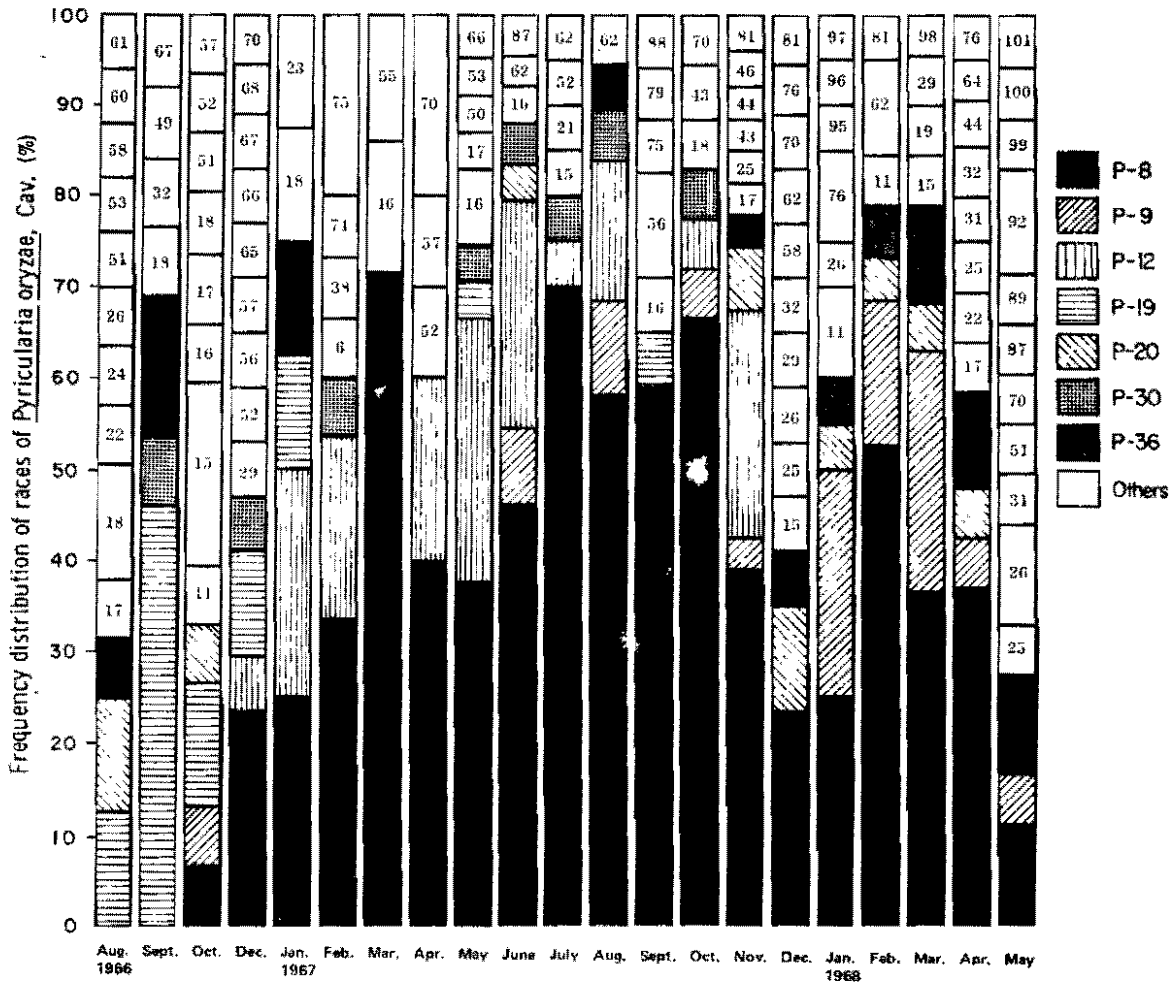

Fig. 1. Population of the Philippine race groups of *Pyricularia oryzae* Cav., sampled monthly at the blast nursery, Los Baños, Laguna, The Philippines, from August 1966 to May 1968. (Quamaruzzaman and Ou, 1970).

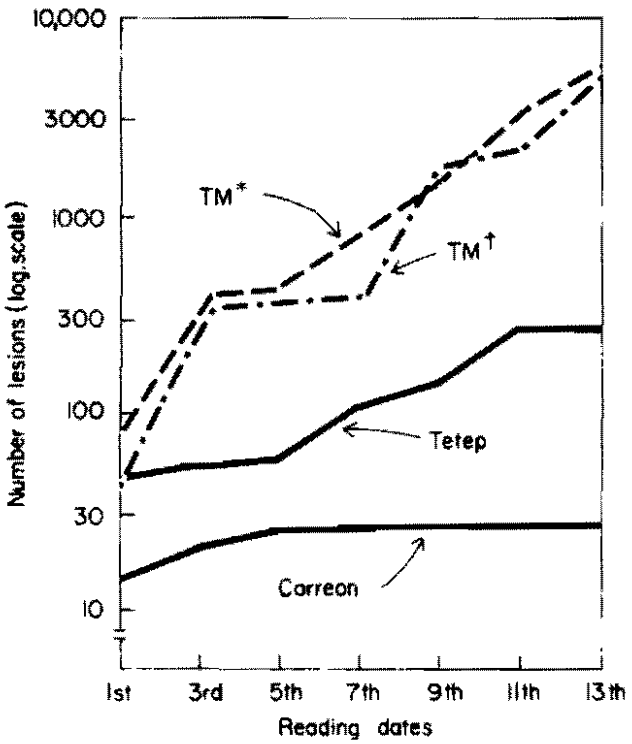

Fig. 2. Number of lesions per 100 seedlings on resistant varieties (Tetep, Carreon) and on susceptible variety Tjeremas adjacent to Tetep (TM*) and adjacent to Carreon (TM †) inoculated with isolate (FR-78-16) from Tetep in blast nursery. (Ou et al., 1971)

Centro Internacional de Agricultura Tropical

TECHNIQUES AND PHILOSOPHIES ON THE DEVELOPMENT AND USE OF PERFECT STAGES TO UNDERSTAND PATHOGEN VARIATION AND HOST RESISTANCE TO PLANT DISEASES

R. R. Nelson

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

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Plant pathogenic fungi are notorious for their ability to vary. Their capacities to generate increased or novel parasitic abilities are the principal reasons for man's continuing efforts to control plant diseases by means of host resistance. Stable resistance would be commonplace in an era of stable pathogens.

Plant pathogenic fungi effectively exercise the conventional mechanisms of genetic variation and, in addition, employ other means of variation which may be unique to certain of their members. Fungi change rapidly and dramatically by mutation and by genetic recombination through "conventional" sexual processes. Genetic reassortment via parasexuality or mitotic recombination is an acknowledged means of variation within some fungal species, as is heterocaryosis, the coordinated capacities of genetically dissimilar nuclei in vegetative or asexual systems. It is not the purpose of this paper to discuss how these mechanisms function or to document them as functional phenomena for any given fungal species.

Although mutations constitute the ultimate source of genetic diversity, the reassortment of genetic material during the sexual process probably is the most important mechanism contributing to pathogen variation within many fungal pathogens. Variation in certain parasitic attributes may be dependent upon the combined or additive effects of two or more genes. In such cases, single mutations within a population would not improve a particular attribute. Another population may incur a different mutation which again would not significantly enhance a given trait. The recombination of these two mutant genes into a single genotype during the sexual process would provide the appropriate gene combination to create a new pathogenic variant. It seems safe to conclude that species with a perfect stage should possess greater opportunities to generate new, important genotypes than species lacking a system that assures genetic recombination.

Pyricularia oryzae, the incitant of the blast disease of rice, is recognized widely for its extreme variability. Many pathogenic variants can be obtained from a single monoconidial culture. The mechanism(s) contributing to such variation are not well understood. The perfect stage of the fungus is not known, although a Ceratosphaeria ascigerous stage of Pyricularia grisea, a species which is considered to be morphologically identical to P. oryzae, has been produced in vitro. To date, isolates of P. grisea from crabgrass have not been crossed with isolates of P. oryzae from rice. The ability to detect the perfect stage of P. oryzae and

to readily induce its formation *in vitro* would provide the opportunity to analyze the genetic control of pathogenicity and virulence. An understanding of the factors influencing variation within and among populations of the fungus could be derived from studies with its perfect stage. The detection of stable resistance to any parasite is a more likely prospect when pathogen variation and pathogen potential is at least reasonably well understood. The first portion of this paper discusses some techniques, knowledge, and theories that may be helpful in discovering perfect stages of fungal pathogens. The latter portion briefly outlines and discusses the value of research with perfect stages to better understand pathogen variation and host resistance.

Certain basic generalities regarding the nature and production of fungal perfect stages probably are germane to most species, although notable and obvious exceptions are readily evident. The following remarks acknowledge these exceptions.

It is reasonable to assume that fungal species that fail to produce their perfect stages readily *in vitro* are heterothallic. Most homothallic species produce their sexual stages abundantly *in vitro* on natural or synthetic substrates when relatively few basic requirements are evaluated. Light and temperature regimes are readily monitored by the investigator. Nutritional requirements usually are less exacting for homothallic species.Exogeneous applications of specific compounds enhance reproduction of homothallic species in a quantitative manner more often than they serve as definitive,

qualitative nutrient sources.

Assuming that a particular species, such as P. oryzae, is heterothallic, certain generalities are more or less applicable to a search for perfect stages. The vast majority of species which reside in the Fungi Imperfecti and for which no perfect stage is yet known will ultimately be shown to have perfect stages in the class Ascomycetes. There are relatively few examples in which imperfects have been associated with a Basidiomycete stage and no example of an association with a Phycomycete stage, since species in the latter class largely are identified originally on vegetative and/or asexual characters.

All known heterothallic species of Ascomycetes exhibit a basic pattern of bipolar sexuality, a mechanism whereby compatibility or incompatibility between strains is conditioned by a single major gene locus. While some species are reported to have a multiple allelic system at the compatibility locus, most heterothallic species of Ascomycetes possess only two alternate states which often are cited in allelic form as + or - or A or a. Such species consist of only two kinds of potentially cross-compatible populations. From the standpoint of probabilities, there is but a 50 percent chance that any two strains of a species when paired will be of opposite compatibility types and potentially capable of producing a perfect stage.

Thus far in our theoretical approach to discover the perfect stage of an imperfect species, we can assume with a high degree of

confidence that we are seeking a heterothallic Ascomycete with but two alternate compatibility types. The two principal tasks that remain are finding compatible strains and determining the environmental and nutritional regimes that are conducive for the in vitro production of the ascigerous stage. Some of the author's experience with the detection and development of perfect stages of Helminthosporium species will be used to illustrate certain basic points.

The failure to detect the perfect stage of a heterothallic plant pathogenic fungal species in nature may be due to: 1) the presence of but one compatibility type in a given area; 2) the relative infrequency of its occurrence; and/or 3) the failure of the investigator to detect its presence. The author's research with Cochliobolus heterostrophus Drechs. (Helminthosporium maydis Nisik. and Miyake), the incitant of Southern corn leaf blight, can speak in part to these possible reasons, at least from the standpoint of probability. The fungus is a heterothallic Ascomycete with two distinct compatibility groups designated in allelic form as A - a. Its perfect stage is readily induced in vitro when compatible strains are paired on an appropriate substrate and under an appropriate environmental regime. The disease is incited exclusively by the asexual or conidial stage, from disease onset through initial and subsequent generations of disease spread. On only rare occasions have strains of both compatibility types been isolated from a single corn field. Whether this phenomenon can be accounted for by concluding that the perfect stage plays a relatively minor role in the life history of

the species is a moot point. The perfect stage of H. maydis has been detected in nature only on rare occasions and exclusively on dead or senescent plant parts. With notable exceptions, heterothallic species with perfect and imperfect stages fit this pattern, i.e., the perfect stage is not an active part of the parasitic phase, but rather serves as a means of survival during a non-parasitic stage. Perfect stages of some parasites provide inoculum for initial infection, e.g., Venturia inaequalis, the incitant of apple scab. When living host material is available for active parasitism, the impetus of the parasite tends towards continued vegetative growth or asexual reproduction. When known compatible strains of a heterothallic species are available for mating studies, a search for its perfect stage would be most profitable in an area where both mating types have been determined to exist in vivo. Furthermore, a search for perfect stages in nature should be confined largely, if not exclusively, to senescent plant material, unless the perfect stage is known to be active during the disease cycle.

The author has sought for and successfully detected perfect stages of several Helminthosporium species for almost twenty years. A number of lessons have been learned; some of them were learned the hard way. One lesson has virtually become a principle to this investigator. That lesson is to obtain a wide geographic distribution of isolates before attempting to detect the perfect stage. Precisely what constitutes a wide geographic distribution is impossible to comment on. The general principle of diversity is related

to probabilities. The probability of securing isolates of both compatibility types among 50 isolates from 50 different areas is far greater than it would be if 50 isolates were collected, for example, from an area of one square mile. Both mating types may well be present in a one square mile area or even in a single field, but the probabilities are less. The chances are further diminished if the perfect stage does not readily occur in nature. In summary, and in a sense a word of sound advice, obtain a collection of isolates from a wide geographic area.

Compatible isolates of P. grisea from crabgrass have been reported to be incompatible with isolates of P. oryzae from rice. It was suggested, and appropriately so, that the failure of isolates of P. grisea to cross with isolates of P. oryzae probably was genetically based rather than being due to different environmental or nutritional requirements. The author has reported previously on the effects of geographic origin and host association on cross-fertility between isolates of Helminthosporium species. In one study, for example, a total of 79 isolates obtained from 37 species of 31 genera of Gramineae were studied for mating behavior and degree of reproduction isolation (incompatibility). Viable ascospore progeny were produced in 464 of a possible 1155 pairings between isolates of opposite compatibility, a frequency of 40 percent. Eleven isolates exhibited complete reproductive isolation in all paired cultures. The frequency of fertile crosses between isolates from cultivated hosts was 43 percent of such crosses, while the frequency between isolates from wild hosts was 14 percent. The frequency between isolates from cultivated and wild hosts was intermediate. Ten of the 11 reproductively-isolated strains were obtained from wild species.

These results suggest that host association may be an important factor conditioning the evolution and complexity of sexual mechanisms. The intermediate frequency obtained with crosses between isolates from wild and cultivated hosts suggests that the reduced fertility between isolates from wild hosts is due in part to genetic deficiencies and inhibitors rather than to entirely opposing compatibility mechanisms.

The author has reported in several publications on the presence of genetic factors that block the sexual process in Helminthosporium species. Genetic blocks are known to inhibit perithecial, ascus, and ascospore formation in pairings of isolates of opposite mating type. Such genetic blocks are detected more frequently in isolates obtained from wild hosts. This thought, as well as the foregoing discussion of host association and cross fertility, may well serve as advice to obtain a majority of isolates that form a wide geographic source for a collection from cultivated hosts. For example, a search for the perfect stage of P. oryzae may be more profitable if many of the collected isolates were obtained from rice.

Certain general guidelines useful in studies designed to induce perfect stages of heterothallic species can be derived from a review of Kleb's postulates. His postulates, formulated in 1900, are concerned with growth and reproduction and briefly stated are: 1) growth and reproduction are life processes which all organisms depend upon under different conditions. Lower organisms are influenced to a

greater extent by their external environment; 2) reproduction does not set in as long as the external conditions necessary for growth are present. Conditions favoring reproduction in general do not favor growth; 3) working conditions are narrower for reproduction. Growth may occur even though certain factors inhibit reproduction; and 4) growth appears primarily as a preliminary for the initiation of reproduction and therefore as an inner condition for it. Other food for thought can be obtained from the reflections of Sachs, the German physiologist, who a century ago advanced the hypothesis that proper development and functioning of sexual organs in plants depend upon specific chemical compounds. Through the ensuing years, research on the physiology of sexual development in the fungi has accepted and supported this hypothesis. Research has repeatedly demonstrated that the initiation and differentiation of sexual reproduction structures are controlled by the manipulation of the chemical and physical factors of the fungus' environment, particularly its nutritional regime. Numerous attempts have been made to understand the physiological processes by which these effects are achieved but it is not clear as yet how such changes occur or what their role is in the processes that regulate sexuality. It has been concluded, nevertheless, that positive qualitative and quantitative responses resulting from exogeneous applications of specific compounds are due to the compounds functioning directly as regulators either of the sexual process or of biosynthetic pathways controlling sexual processes. However, it may be assumed with equal validity that stimulatory compounds trigger

metabolic pathways which serve as precursors to subsequent processes which may be initiated by other unrelated biosynthetic pathways.

The perfect stage of P. grisea has been induced in culture by pairing compatible strains on Sach's agar with barley grains and rice straw. Perfect stages of several heterothallic species of Helminthosporium have been induced in vitro between compatible strains by essentially the same technique. The specific substrate or the particular plant parts are probably not as important as some of the general principles that underlie their success. Sach's agar is basically a salts medium with relatively little nutritional value. Most fungal species that the author has studied exhibit sparse vegetative growth on the substrate. Conversely, most species exhibit prolific vegetative growth on potato-dextrose-agar (PDA), an example of a nutritionally rich substrate. Sexual reproduction of heterothallic Helminthosporium species is significantly more abundant in matings on Sach's agar as compared with matings of the same strains on PDA. The general principle seems to be that reproduction is more likely to occur and will occur more abundantly on substrates that are not optimum for vegetative growth. That principle probably should be respected when considering possible substrates to use in inducing sexual reproduction.

There are several possible reasons to account for the need of some plant part as a requisite to sexual development in many heterothallic species. The requirement of a plant part on which the sexual structures develop probably satisfies both a physical and a

nutritional need. It has been said that competition for oxygen is the fundamental reason for the absence of reproduction under conditions which allow abundant growth. Some stimulus apparently starts with the utilization of a stored food supply by oxygen and leads to reproduction when an organism is in a hunger state. While some of the particulars of these statements may not be directly pertinent to the present consideration, the general idea of competition for oxygen may relate to enhanced reproduction of heterothallic species when plant parts or other solid materials are used. It is possible that the lack of a direct contact with the agar substrate creates a more aerobic environment which triggers reproduction. Agar substrates are moist and moisture can contribute to an anaerobic state. In this connection, the author has found that increased perithecial production by heterothallic species of Helminthosporium is directly correlated with increased concentrations of agar. A twenty-five fold and greater increase in perithecial formation has occurred consistently on a 5 percent agar substrate as opposed to a 1 percent agar concentration. Substrates with 5 percent agar are considerably more dry than substrates with less agar. The increased dryness of a more concentrated agar substrate may permit a greater availability of oxygen. Other evidence that a physical requirement must be satisfied will be discussed later in the text.

That plant parts satisfy a nutritional need for sexual reproduction is well documented for heterothallic species of Helminthosporium and probably have a comparable pertinence to all species which require

similar mating techniques. Several general principles appear to have emerged from studies by the author and several of his students and associates. Many of these studies have utilized strains of Cochliobolus carbonum Nelson (Helminthosporium carbonum Ullstrup), a heterothallic Ascomycete parasitic to corn and other gramineous species. The sexual process is initiated and completed in a precise and consistent fashion when compatible strains are paired under an appropriate chemical and physical environment. Certain paired strains are highly cross-fertile and consistently produce a dense ridge of perithecia in the zone of mycelial contact, permitting a reliable evaluation of qualitative and quantitative responses to altered nutritional environments.

Sexual reproduction in C. carbonum occurs readily and abundantly when compatible strains are paired on opposite sides of a small disc of sterile senescent corn leaf (Zea mays L.) placed on the surface of Sach's nutrient agar in petri dishes. However, pairings of the same compatible strains on green corn leaves, agar substrates, filter paper, cellophane, or membranes fail to initiate the sexual stage. These observations suggested that: 1) paired strains of C. carbonum are not able to synthesize all of the biochemical requirements for sexual initiation and development; 2) the biochemical requirements for sexual reproduction are present in senescent corn leaves; and 3) chemical changes occurring in the transition from green to senescent tissue provide the required metabolites. Consequently, a systemic screening of compounds was

initiated and included those known to be present in senescent tissues or in differential amounts between green and senescent corn tissues and those tested previously by other workers in studies on sexual reproduction.

One early phase of the screening studies was concerned specifically with inorganic compounds. Incorporation of any zinc salt in Sach's nutrient agar enabled compatible strains to produce the sexual stage when paired on filter paper. Further studies demonstrated that perithecial production depended upon the concentration of zinc used and that zinc effects in turn were influenced by the concentration of agar utilized in the preparation of the basal medium.

Maximum perithecial development consistently occurred when crosses were made on a Sach's nutrient substrate containing 5 percent agar supplemented with 30 ppm zinc. However, the use of a 5 percent agar substrate posed considerable difficulty in keeping the filter paper sections adhered to the substrate, presumably due to a lesser amount of available free moisture. When 40 ppm or more zinc/1 liter were used, vegetative growth was retarded and perithecial initiation required several additional days. Thus, a 4 percent agar substrate and 30 ppm zinc in the form of $ZnSO_4$ were used as part of a second basal medium.

Because of the important role of zinc in many physiological processes in fungi and higher plants, the study was expanded to include those related to zinc. Compounds were tested for their

effects on reproduction in the presence and absence of zinc. The number of perithecia formed on Sach's nutrient agar with zinc was a quantitative measure of a compound's activity.

A total of 173 organic and inorganic compounds were tested at various concentrations on Sach's nutrient agar supplemented with 30 ppm zinc and without zinc for their effects on sexual reproduction in C. carbonum. Of these, 37 organic and inorganic compounds increased perithecial production in the presence of zinc, as compared to crosses paired on Sach's nutrient agar plus zinc. However, sexual reproduction did not occur when these compounds were used in the absence of zinc. The compounds included several carbohydrates, vitamins, sterols, amino acids, and fatty acids.

Eight compounds, betaine, choline, homocysteine, homocystine, lecithin, DL-methionine, D-methionine and L-methionine, increased perithecial production at most concentrations in the presence of zinc and supported perithecial development to varying extents in crosses paired on Sach's nutrient agar without zinc. Sexual reproduction in crosses on Sach's nutrient agar without zinc was sparse when betaine, choline, or lecithin was applied to the filter paper sections, while application of homocysteine, homocystine, and D-, L-, or DL-methionine resulted in perithecial development numerically similar to that occurring in check crosses paired on Sach's nutrient agar plus zinc.

The remaining 128 compounds, including all inorganic salts other than zinc compounds, were either non-stimulatory or inhibitory in the presence of zinc and totally ineffective in the absence of zinc.

With all salts of zinc tested, perithecial production occurred consistently on Sach's nutrient agar, provided the concentration of agar was at least 2 percent.

Vegetative growth was not stimulated markedly by compounds increasing perithecial production, although some increases were observed. Some inhibition of vegetative growth occurred in the presence of compounds inhibiting perithecial production, although no general correlation existed between vegetative and sexual inhibition. In general, no stimulation of ascus or ascospore development was evident when perithecial formation was increased, suggesting that the stimulatory compounds function in the early stages of reproductive ontogeny. Similarly, ascus and ascospore formation were not markedly reduced in crosses in which perithecial development was inhibited.

These results suggest that the diverse kinds of compounds that initiated or stimulated sexual reproduction in C. carbonum may have served as common sources of specific nutrilites in different biosynthetic pathways that lead to the ultimate synthesis of further compound(s) directly responsible for the regulation of the sexual process. If so, no one specific precursor biosynthetic pathway would be categorically required to initiate the sexual process.

One specific characteristic of most of the compounds which initiate or increase sexual reproduction is the presence of methyl groups. Further analysis of the molecular configurations of these compounds showed that the C- and O-methylated compounds and C- and O- compounds bearing methyl groups were effective stimulators of reproduction only

in the presence of zinc. Conversely, the N- and S-methylated compounds were active in initiating reproduction in the absence of zinc and stimulatory in the presence of zinc. The relationship of methyl groups and zinc involvement may explain the role of zinc in the demethylation of compounds and/or in the enzymatic breakdown of the stable binding of the methyl group linked to C- and O-methylated compounds. The active effect on N- and S-methylated compounds in the absence of zinc may be based on their known alkylating properties. Increased activity in the presence of zinc may be due to the effect of the ion on this reaction.

One phase of our studies has shown a complete dependency of sterol activity on sexual reproduction on the presence of zinc ions. This apparent association of zinc in the conversion of sterols may offer an opportunity to investigate the biochemical nature of sterol activity in sexual reproduction in the fungi. Preliminary studies with SK & F compound 3301-A, a known inhibitor of cholesterol biosynthesis, suggest that sterol metabolism per se is not an essential requisite for sexual reproduction. Perithecial production in C. carbonum was totally inhibited by applications of 0.03 mg/mating of the inhibitor compound. Sexual reproduction in similar crosses treated with the inhibitor was partially restored by applying .4 mg/mating of methionine. Gas chromatographic studies have shown that products of cholesterol degradation by C. carbonum have retention times similar to steroids without the side chain. These results tend to substantiate our contention that the diverse group of stimulatory compounds, including sterols, may serve primarily as sources of nutrilites.

The stimulatory effect of several concentrations of sterols was obtained consistently and without difficulty. The activity of sterols appears to be dependent upon the presence and/or length of the chain at the C_{17} position, since none of the steroids, including estrone, estriol, progesterone or testosterone, was active even in the presence of zinc.

A few compounds not bearing methyl groups were active stimulators in the presence of zinc. Some of the compounds may be involved in a process serving a regulatory function. Xylose, ribose, and some amino acids are involved in nucleic acid synthesis. Sugars and fatty acids are energy sources. Reducing agents such as the sulfites are active in the reductive fission of the S-S link in biological systems and iodoacetic acid is a well-known alkylating agent. It is possible also that these non-methylated compounds may form part of a biological methylation by fission so as to eliminate a molecule being involved in an as yet unknown one carbon fragment metabolism.

The components of agar have been studied. Components of particular interest here are agarose and agaropectin, which are products of the hydrolysis of agar. Agarose is comprised of several O-methylated sugars and may, in part, account for the increasing perithecial production with increasing agar concentrations in the presence of zinc. It is possible that methylation of the sulfur atom occurs readily, involving hydrolysis, reduction, and further methylation. Preliminary studies with different lengths of time of sterilization of Sach's nutrient agar have shown that perithecial production is

increased proportionately with increased sterilization time and thus increased hydrolysis, at least up to and including 60 minutes.

The effect of zinc and S- and N- methylated compounds, such as methionine, on sexual reproduction in C. carbonum led us to investigate the role of such activity at a metabolic level. Briefly summarized, a selective inhibition of perithecial production was obtained by certain concentrations of several azaderivatives of RNA bases, but not of DNA bases. Reproduction was partially or completely restored when various concentrations of their normal bases were applied simultaneously with inhibitory concentrations of the analogues. Significantly, sensitive applications of DL-methionine also restored sexual reproduction when applied with inhibitory concentrations of the analogues. RNA content per unit dry weight ranked from least to greatest in crosses on Sach's agar, Sach's agar plus zinc, Sach's agar plus methionine, and Sach's agar plus zinc plus methionine. RNA content increased with increasing sexual reproduction stimulated by increasing amounts of methionine. Incorporation of 14C of the methyl group of L-methionine in extractable RNA was detected in crosses on Sach's agar plus methionine and in increased amounts in the presence of zinc. The dependency of the sexual process on RNA metabolism is indicated by zinc and methionine stimulation of sexuality through a primary effect on RNA synthesis.

The biochemical requirements for the formation of perithecia, asci, and ascospores in C. carbonum are present in senescent corn leaves. A crude chloroform-methanal extract from senescent corn

leaves, an "ether extract" and a "methanol extract" from the original crude extract were shown to stimulate perithecial production when aliquots were applied to filter paper. No concentration of the extracts stimulated ascus and ascospore formation. Perithecial production increased only when extracts were applied prior to the physiological time of perithecial formation, which, under the conditions of our studies, was 6-7 days after pairing.

Gas chromatographic analyses of sterol and fatty acid fractions of each of the three extracts revealed no qualitative and no quantitative differences among the extracts. The bulk of the fatty acid fractions was made up of palmitic acid and linolenic acid. Injections of the sterol fractions produced peaks corresponding to ergosterol, beta-sitosterol and stigmasterol.

Chemically pure palmitic and linolenic acid failed to stimulate perithecial formation or reduce ascus and ascospore production when applied singly or in combination at several concentrations. Similarly, no response was observed when ergosterol, beta-sitosterol, and stigmasterol were applied to filter paper.

Relatively large and easily measurable quantities of sterols and free fatty acids were obtained from 500 mg samples of crude extract by the procedure used during preparation of sterols and fatty acids for gas chromatography. Each 500 mg sample yielded about 40 mg of sterols and 25 mg of free fatty acids, an 8 percent and 5 percent yield respectively. These two fractions were then tested for activity at known concentrations.

The 0.1-0.2 mg/pairing applications of the sterol fraction significantly increased perithecial numbers over solvent checks. The fatty acid fraction failed to stimulate perithecial formation. Combinations of the two fractions were no more active than the sterol fractions. No concentration of the sterol fraction was as effective as the 0.1 mg/pairing application of the crude extract.

Perithecial numbers increased to 6-7 fold over solvent-treated checks when the sterol fraction and a solution of water soluble materials from senescent corn leaves were applied together. At certain concentrations of the mixture perithecial development was greater than when 0.1 mg of crude extract was applied.

These studies demonstrate that the metabolites required for initiation of the sexual process in Cochliobolus carbonum can be extracted from senescent corn leaves with chloroform-methanol. Suitable concentrations applied to filter paper stimulated perithecial formation comparable to that in crosses made on the corn leaf. Materials required by the fungus during perithecial formation apparently were present in the crude extract.

Other compounds active in stimulating perithecial production may be present in the corn leaf or in the chloroform-methanol (crude) extract and were not identified during this investigation.

Time of application studies suggests that the mode of action of perithecial-stimulating chemicals extracted from senescent corn leaves is nutritional. The extracts stimulated perithecial development only when they were applied prior to the physiological time at which

perithecia form. The similar stimulation observed from applications made from 0-6 days suggests that the materials become part of a nutritional reserve pool available to the strains as they reach the physiological time for sexual differentiation.

These studies further suggest that the requirements for perithecial formation in C. carbonum are different from the requirements for ascus and ascospore formation. None of the materials which stimulated perithecial production increased the numbers of asci or ascospores formed per perithecium at any of the concentrations tested.

Concentration of materials and concentration balance between materials appear to be important considerations. Active materials had an optimum stimulatory concentration and a limited range of concentrations that resulted in increased perithecial numbers.

The nutritional studies summarized to this point have been treated in some detail to offer possible techniques and approaches that may be useful to others who seek to culture perfect stages of different species. The specific materials and methods that have proven useful in our studies with Helminthosporium species may not be similarly useful to others. The general principles which we have learned, however, should be pertinent to other species.

Our studies on the biochemical requirements for the initiation and successful completion of sexual reproduction provide an enhanced understanding of certain other phenomena. It was suggested earlier in the text that plant parts or other solid materials may satisfy a physical, as well as a nutritional, requirement for reproduction.

We may now conclude that such is the case. Sexual reproduction can be induced when compatible strains are paired on opposite sides of a section of sterile filter paper to which one of a number of chemical compounds or extracts from senescent corn leaves have been applied. No sexual development occurs when the same compounds or extracts are incorporated in or applied to agar substrates.

It was stated earlier that the perfect stage of H. maydis has never been found in nature on actively growing corn plants. We have learned that sexual reproduction in vitro does not occur when sections of green corn leaf tissue are used instead of senescent corn tissue. The reason for this quite probably is one of nutrition. Actively growing corn leaves either do not possess the necessary nutrients or not enough of them to enhance fungal reproduction or they contain metabolites that are inhibitory to the sexual process. Many biochemical changes occur as active plant growth ceases and maturation and/or senesence prevail. Sterols, for example, increase significantly with maturation. Seeds or kernels have stored compounds not present in green leaves or which are present in markedly lower quantities. It seems likely, from these observations, that the perfect stage of H. maydis cannot occur during the active growth phase of its host. The same likelihood may well exist for other heterothallic species, even if compatible strains are present in the same localized area. This prospect should give an added impetus to concentrating a search for perfect stages in nature on dead or senescent plant parts. There is always a possibility, however, that diseased tissue may be

comprised of breakdown products that may support reproduction.

If the need to use plant parts to induce sexual reproduction in paired cultures is at least theoretically acknowledged, the choice of plant parts comes under consideration. H. maydis is parasitic to a variety of gramineous hosts other than corn. We have demonstrated that the perfect stage of the fungus is induced readily on senescent leaf material of a number of gramineous species. Leaf tissue of some species induces reproduction to a greater extent than others. The point seems to be that many species of the Gramineae probably possess a variety of common biochemical constituents. Their evolutionary relationships make it understandable that they would. If a choice of host species were to be made, it may be logical to select senescent leaf tissue from plant species which serve as hosts for the fungal parasite in question. Related plant species not serving as hosts to the parasite would be a sound alternative choice.

Light requirements should be considered in any attempt to induce perfect stages of heterothallic species. Sexual reproduction in heterothallic species of Helminthosporium and other genera of Ascomycetes is retarded and can be totally inhibited when paired strains are cultured under continuous light. While this phenomenon may not exist for all species, the potential influence of light is sufficiently important to culture pairings of potentially compatible strains in light and darkness.

Temperature requirements usually are not overly exacting. To be sure, maximum sexual reproduction often occurs within a relatively narrow range, e.g., + or - 5°C. However, deviations above or below

the more optimum temperature range usually restrict the abundancy of reproduction rather than to totally inhibit it. Temperatures which support satisfactory vegetative growth probably will also support sexual reproduction.

A brief summary of the techniques and philosophies that may be useful in studies designed to induce the perfect stage of a plant pathogen in artificial culture may be helpful to the reader. The assumption will be made that the search is for the perfect stage of a plant pathogen and that our potential guidelines are based on probabilities.

1) The fungus will be a heterothallic Ascomycete.
2) A basic pattern of bipolar sexuality will be present, whereby compatibility between isolates is governed by a single gene with two alternate alleles.
3) The perfect stage does not play an active role during the parasitic phase, but rather serves as a means of survival during a non-parasitic stage, if and when it does occur.
4) Isolates of both compatibility types seldom will be present in a confined localized area, e.g., a field.
5) The perfect stage will be induced most often between isolates collected from different geographic areas and will occur more often in pairings of isolates obtained from cultivated species serving as hosts to the parasite.
6) The production of the perfect stage <u>in vitro</u> will be dependent on certain physical and nutritional requirements which

include:

a) The need for some plant tissue or solid material on which the sexual structure will be produced.

b) The agar substrate will be a minimal medium which fails to support vigorous vegetative growth.

c) Senescent plant tissue may contain the biochemical requirements for sexual initiation and development; green plant tissue will not support reproduction.

d) Certain nutrients applied exogeneously to senescent plant tissue may enhance reproduction and will induce reproduction when applied to inert materials such as filter paper.

e) Certain compounds will trigger different biosynthetic pathways that will lead to the ultimate synthesis of further compounds which regulate sexuality.

f) The selection of compounds to induce perfect stages can take advantage of available knowledge concerning their potential activity and usefulness in other fungal systems.

g) Light and temperature requirements should be monitored.

The techniques and conceptual approaches pertinent to inducing fungal perfect stages in vitro may not be familiar to many of the readers who have not had the need to pursue such a venture. The potential benefits of being able to work with the sexual stage to study several genetic aspects of a parasite no doubt are well known to most readers. They are briefly cited herein and discussed only in a cryptic fashion.

Normally, different strains or populations of a fungal pathogen will vary in their ability to attach different host genotypes and/or in the extent to which they can do so. A considerable understanding as to the number of genes conditioning these qualitative and quantitative parasitic abilities can be gained by evaluating hybrid progeny obtained from crosses of isolates exhibiting different parasitic aptitudes. At least theoretically, it should be possible to develop a more effective and stable resistance in plants with some knowledge of the genetic capacities of a parasite.

One of the more significant benefits that accrue from working with the perfect stage of a plant pathogen is a genetic study designed to evaluate and/or predict the genetic potential of future races. Races of plant pathogens arise totally independent of the relative resistance or susceptibility of their hosts to existing races. To assume any degree of dependency upon their hosts would dictate an acceptance of the idea of "directed" origin for which there is no evidence. Host genotypes will influence the ultimate frequency or the sustained presence of new races, but not their origin.

The continued appearance of strains of fungus pathogens that are virulent to supposedly resistant varieties of plants has reduced many programs of breeding for disease resistance to a stop-gap basis. Knowledge of the number of genes conditioning pathogenicity, the mechanisms controlling their inheritance, and the pathogenic response of genes in the fungus to genes for resistance in the host should permit a partial evaluation of the potential pathogenicity of

the species. With such knowledge, it might be possible to develop plants with resistance to current and potential genotypes, particularly in instances where increased resistance is due to greater numbers of genes rather than to more favorable gene combinations. The term "potential" is used herein in an operational sense, in that there probably is no theoretical limit to potential resistance or pathogenicity.

The increased frequency of virulent races and the concurrent "loss" of resistance of a host variety usually is associated with cases in which varieties have been developed with resistance to a specific race. Resistance of this type commonly is referred to as race-specific resistance or vertical resistance (VR). While VR is effective against a certain race or races it is equally ineffective against other races; plants lacking VR to a race usually are highly susceptible. Genes for VR function against epidemic development of plant diseases by reducing the initial amount of inoculum available for disease onset. Races lacking virulence genes to match VR genes are essentially disqualified from epidemic involvement. Genes for VR are considered to have no influence on epidemic increase of disease by races with patching genes for virulence. From a genetic standpoint, race-specific VR is usually conditioned by a single gene.

A race which increases in distribution and frequency among populations of a plant pathogen must exhibit two fundamental assets. The race must, of course, possess the necessary gene(s) for virulence which negates a particular gene for VR. It must also possess

the ability to become a more dominant member of the species. These abilities can be characterized as "fitness attributes" and include: 1) the ability to attack its host under the different environmental regimes in which the host is grown; 2) the ability to become disseminated over wide areas; and 3) the ability to persist or survive in the absence of its primary host.

Other attributes may not directly affect the ultimate frequency of a race but influence the rate at which that frequency is attained. Races able to cause a greater amount of disease in less time and able to produce greater amounts of inoculum would assume a more dominant position among populations in a shorter period.

In constrast with race-specific or vertical resistance, some resistance mechanisms are effective to some extent against all races. Resistance of this type often is called non-specific. Non-specific resistance functions by reducing the amount of disease and the rate of disease development. Such resistance mechanisms may retard penetration, increase the incubation period, restrict lesion size and reduce the amount of sporulation and the period in which sporulation occurs. Some or all of the effects are imposed upon all races of a pathogen in a similar although not equal manner.

From a genetic standpoint, non-specific resistance is usually polygenic in inheritance. More often than not, non-specific resistance has remained stable and effective for long periods of time. It is likely that a race would have to acquire several new genetic abilities to overcome resistance conditioned by several genes. Each

genetic improvement in the pathogen occurs independently of other improvements needed to overcome polygenic resistance. The stability of polygenic resistance, then, seems based on probabilities of sequential events occurring in the pathogen.

The need to detect the occurrence of new races before they cause serious problems is imperative, whether the accent is on race-specific or non-specific resistance. Anticipating genetic potentials need not be a theoretical exercise in wishful thinking. The author has demonstrated that genetic studies with several Helminthosporium species can reveal new and potent genetic capacities among strains obtained by crossing compatible strains. If new genotypes can be "manufactured" in vitro they most certainly can occur in nature by similar means. Whether new strains can arise to overcome non-specific resistance is only a matter of probabilities. Investigating these probabilities by genetic analysis of recombinant strains may provide considerable insight into this matter.

Some recent studies by the author on Southern corn leaf blight suggest that virulent races can acquire epidemiological attributes over time. The 1970 epidemic of Southern corn leaf blight in the United States was incited by race T of the pathogen, with an unprecedented virulence to corn hybrids in male sterile cytoplasm. Studies with isolates of the pathogen collected in previous years demonstrated that race T has been in existence in the United States

at least since 1955. Comparative studies with an isolate of race T collected in 1955 and preserved in limbo in leaf tissue since that time and an isolate of race T collected in 1970 revealed that the 1970 isolate possesses up to 15 times greater sporulation capacities and colonizes susceptible tissue more rapidly than the 1955 isolate. Either of these attributes could contribute significantly to the increased frequency of the race. Studies are currently in progress to evaluate the genetic control of such improved fitness and serve as another example of the value of working with the perfect stage of a plant pathogen.

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Centro Internacional de Agricultura Tropical

THE RICE BLAST DISEASE IN PERU

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SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

THE RICE BLAST DISEASE IN PERU

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1. Area, production and importance of rice in Peru

Rice is one of the main basic cereal crops grown in Peru and in the world. About 1.5 percent of the total American area planted with rice is in Peru. This area contributes 3 percent of the total American production. Peru ranks sixth among the highest yield per area countries in the world and second in America after the United States. The national average rice yield is 4,098 kg/ha (Contreras & Giron, 1969).

The importance of this crop in Peru is economical and social mainly because of the habitual consumption among the population. Almost 77 percent of the cultivated area and 80 percent of the production is concentrated in the north coast. The jungle region plants 19 percent of the national rice area and supplies 15 percent of national production (Contreras & Giron, 1969).

2. Main characteristics of the rice lands in Peru

The main characteristics of the rice lands in Peru are (Velasquez, Huerta & Sanchez, 1970):

Coast Alluvial soils, plains and variable texture; low organic matter content, nitrogen fertilizer response. Desertic subtropical climate, high solar radiation. The relative humidity average is low although with high dew accumulation on plants during the night. Rice is commonly planted under intermittent flooding conditions.

High Jungle (Zones of Jaen, Bagua and Huallaga Central.) This area has alluvial and vertizolic soils with satisfactory fertility and with good response to nitrogen and phosphorous fertilization. Its climate is classified as dry and tropical high pluvial precipitation, high temperature and good solar radiation. In the Jaen and Bagua zones, rice is sown under lowland conditions while in Huallaga Central zone it is sown under upland conditions.

Low Jungle Its climate is humid tropical forest; studies indicate the predominance of ultisols including those formerly known as red-yellow podzolics and ground water laterites. There is a good possibility of increasing the cultivated areas in this region.Direct sowing method is practiced under upland conditions.

3. Predominant disease in each region

Coast Blast disease is the most important disease, mainly in the years when there is high dew accumulation on the plant surfaces. Apparently the amount and duration of the dew formed, between 10 p.m. and 9 a.m., is considered the main factor in the spread of blast. Another factor which has increased this

disease has been the high nitrogen fertilization used recently. It is common to find blast at seedling stage and then from panicle initiation stage to prematurity. Rice blast disease is not general in the coast; it is only found in certain areas, probably because of the microclimate conditions present in specific areas, type of soils or amount of nitrogen used or plant pathogens distribution — all apparently governed by an epidemiologic factor still unknown to us.

Stem rot incited by Lepthosphaeria salvinii and brown spot incited by Helminthosporium oryzae are considered as secondary diseases.

High and Low Jungle As on the coast, blast is the most important disease here. It is largely helped by rainfall, which is very common. In other geographical areas, brown spot disease is the main trouble. Other diseases found in a minor scale, although certain rice varieties are seriously affected by them, are leaf scald (Rhynchosporium oryzae), false smut (Ustilaginoidea virens) and linear spot (Cercospora oryzae).

4. Creation of National Rice Program in Peru

Since its creation in September, 1968, the National Rice Program was integrated by researchers from "Pedro Ruiz Gallo University", the Ministry of Agriculture and the North Carolina State University Agricultural Mission to Peru. Among the different groups of work, the plant pathology staff is comprised of 17

members who are carrying out research works in the main rice production zones in Peru. It is advised by Dr. Teddy T. Hebert, Plant Pathology Professor at North Carolina State University.

Great successes have been obtained in all branches studied, and results were published in National Rice Program Technical Reports. As a consequence, our supply now meets the national demand in rice; many other factors have also contributed to this self-sufficiency.

The main targets of the plant pathology group can be summarized as follows:

a) Evaluation of rice varieties and lines resistant to main diseases.

b) Determination and preponderance of physiological races of Pyricularia oryzae in each season and each rice land area.

c) Study of some epidemiologic aspects from the most important diseases in each rice area.

d) Control of the main diseases with fungicides.

5. Resistance to Blast

5.1 The international blast nurseries

The methods used in these tests were the ones suggested by Ou (1965) and have been used since January, 1969. At first they were started in the jungle (Yurimaguas, Tarapoto and Tingo Maria). At present we have installed other ones in Bagua (jungle)

and the coast (Tumbes and Lambayeque). These results will help us to have a wider view and clearer idea of the performance in these areas of the traditional rice varieties as well as the selections made by national rice program breeders.

From the beginning we had rice varieties and lines resistant to blast from national rice selections and from the world collection as well as from the Philippines, Colombia and U.S.A. collections. We obtained them by interchanging material and information with the International Rice Research Institute (IRRI), the Philippines; from the Centro Internacional de Agricultura Tropical (CIAT), Colombia; and from the United States Department of Agriculture from Dr. J.G. Atkins of Beaumont, Texas.

In Table 1 it is possible to see the number of groups and lines of rice evaluated in each rice zone. The majority of the tests were carried out in the tropical regions, where the climatic conditions were the best.

At the beginning, we tried to test the higher number of rice varieties and lines in order to know their reaction to rice blast disease in Peru. Our first evaluations began in the 1969/1970 growing season, and we introduced collections from a different origin and selection (Table 2). The collections were: World Collection (CI); Agricultural Experimental Station Collection from Lambayeque (CEL), the International Blast Nursery for the

Americas (IBNA) and a group from IR lines, brought from the Philippines (IRRI) and Colombia (CIAT). From all these collections we obtained a major number of rices from IR type with resistant and intermediate reaction (Table 2).

From the tests made in the 1969-1970 growing season, lines with resistance or intermediate reaction were selected; they showed important agronomic characteristics. Those varieties formed the I-PNA group that was evaluated during the 1970/1971 season. This group, together with other national and international collections, formed the groups to be evaluated the next season. Origin, number of lines in each group, number of evaluations for each one, the percentage of resistant, intermediate and susceptible varieties are shown in Table 3. PNA IV was the group which showed the highest number of resistant varieties; it mainly had IR lines such as IR 790; IR 1147; IR 1093; IR 1416; IR 667; IR 828; IR 498; IR 1006; IR 1163; IR 825; IR 854; IR 822; IR 879; IR 1157; IR 841; IR 1112; IR 844; IR 835; IR 790; IR 930; IR 1154; IR 1170; etc.

However, in other evaluations a lot of the resistant varieties became susceptible under our conditions (Huerta,1971). It will be necessary to do other trials to corroborate these results.

I-PNA and V-PNA groups did not show any resistant lines in the evaluations done (Table 3). The V-PNA group showed a high

number of varieties with intermediate reaction. This was expected because these varieties belonged to blast moderately resistant varieties (III-IRRI group), and were sent by Dr. Ou in the Philippines.

Table 1. Number of sets and lines tested in several Peruvian rice zones. 1971.

Places	No. of sets	No. of lines
Yurimaguas	20	4,173
Tingo Maria	15	1,960
Tarapoto	10	2,316
Bagua	7	1,243
Tumbes	1	511
Lambayeque	1	511

Table 2. Blast reaction types by groups tested in Peru from 1969 to 1970

Groups*	No. of lines	Reactions types in % Resistant	Intermediate	Susceptible
CI	259	3	10	87
CEL	508	1	20	79
IBNA	178	14	5	81
IR	292	41	36	23

* CI = International Collection; CEL = Lambayeque Experimental Station Collection; IBNA = International Blast Nursery for the Americas, U.S.A.; IR = IRRI, the Philippines and CIAT, Colombia collections.

From I-PNA group, 31 percent of the lines showed intermediate reaction (Table 3) in more than 30 evaluations made in different rice areas since 1969. Table 4 shows a list with selected lines from that group. Selection was made because, in most of the evaluations, lines had a resistant reaction and, in a few evaluations, intermediate reaction was found with type 3 lesions according to the Ou (1965) scale. From those varieties studied, IR 480-5-9-2 has been considered by the National Rice Program as a promising variety for the jungle, while Tetep is considered as potential genetic material.

The last IRRI Annual Report (1970) displays a rice varieties list, selected for their permanent resistance to blast on International Nurseries in different countries from 1964 to 1970. The reactions of these varieties in Peru are shown in Table 5. Except CI 7787, which was susceptible, all had resistant reaction or intermediate reaction.

5.2 Identification of races

Identification of physiological races of P. oryzae was initiated in Peru in 1970. In general, international differential varieties have been used (Atkins, et al., 1967). Later Philippine differential varieties were included. From the different identified races, the most common one was IB-1 race. Other identified races were IA-65 ab; IB-5c; IB-38; and IC-1i. Although the number of

Table 3. Blast reaction types by groups tested in Peru from 1970 to 1971.

Groups PNA*	No. of lines	No. of sets	Reactions types in %		
			Resistant	Intermediate	Susceptible
I	511	30	--	31	69
II	356	3	31	41	28
III	251	2	20	26	54
IV	923	1	69	27	4
V	150	3	--	68	32
VI	484	1	30	68	2

* I = Lines IR selected in Peru; II = Rice varieties selected for International Partially Resistant blast nursery, IRRI; III = International Partially Resistant blast nursery, CIAT; IV = International Yield Trials, IRRI; V = Blast Moderately Resistant Varieties, Group III, IRRI; VI = Pedigree II-CRIAN, Lambayeque, Lines F_6: IR 8 x F_5 (Fortuna x Minagra).

Table 4. The most resistant varieties selected from the International Blast Nurseries from 1969 to 1971 in more than 30 trials in Peru.

Variety	Variety
IR 4-114-3-2-1	IR 593-1-34-1-3-3
IR 4-114-3-2-2-3	IR 661-17-2-1
IR 5-114-3-1	IR 662-2-7-2-2
IR 480-5-9-2	IR 662-2-7-2-2
IR 503-1-103-3	IR 665-4-4-5
IR 503-1-104	IR 667-112-3-3-3
IR 532-1-144	IR 667-113-1-1
Tetep	IR 682-23-2
IR 586-13-2-1	IR 822-432-2
IR 589-56-2-2	IR 822-432-4
IR 589-57-1	IR 822-432-5
IR 589-65-6-1	IR 848-44-1
IR 589-66-2-1	

trials were few, it is possible to demonstrate that Peruvian races are different from Colombian (Galvez & Lozano, 1968) and Filipino races (IRRI, 1967).

Our present target is the identification of more races of P. oryzae from samples brought from different places, varieties and parts of plants.

Another aspect faced was the study of some factors that would be favorable for the sporulation of P. oryzae growing on artificial mediums. Diaz (1970), according to those targets, evaluated different mediums, photoperiod cycles, and incubation periods using several monosporic cultures from pathogens of different origin. His conclusions showed that a better sporulation is gotten with potato-dextrose-agar plus coconut water and with B-Takahashi medium, made up from rice leaf extract. The best photoperiod was when the plates were incubated for the first seven days in darkness and the other seven days under uninterrupted white light. At the same time the skills to produce spores of the P. oryzae isolations were different. In a general sense, the photoperiod of incubation did not affect the sporulation as much as the interaction medium x photoperiod did.

6. Chemical control

As Peru does not yet have a rice variety with good agronomic and commercial characteristics and at the same time is resistant to blast disease, we have decided to test many chemicals to control

it. Results gotten over the years as well as in the present one have been variable. They have changed with the fungicide and the places where the trials were carried out. The fungicides that seem to give relative control to blast according to Delgado et al. (1970), Jimenez & Mujica (1971), Panizo & Incio (1968) and Panizo & Hebert (1971) were: Hinosan, Duter, Bla-S, Kasumin, Conen, Benlate, Dithane M-45; and Calixin. Other chemicals sprayed on seedling rice that were effective in controlling blast (Huerta, 1971) were: Blastin, Kitazin EC, and Antracol (Propineb).

6.1 Field test on chemical control to blast

During the last 1970/71 growing season a series of trials were carried out in different parts of the country. One of them was conducted by Engineer Juan Zapata (Ministry of Agriculture, Agrarian Zone II, Bagua) and the fungicides tested were: Kitazin 17 percent granulated, 41 kg/ha; Hinosan 50 percent, 1 litro/ha; Blastin 50 percent, 1 kg/ha; Bla-S 4 percent, 1 kg/ha; Benlate 50 percent, 0.8 kg/ha; Kitazin 48 percent EC, 1 liter/ha; Dithane M-45, 80 percent (Mn ethylene bisditiocarbamate plus ions zinc), 2.5 kg/ha; Manzate-D (Maneb 80 percent with a zinc salt added), 2.5 kg/ha; Antracol 70 percent (Propylene bis-dithycarbamate of zinc), 1 kg/ha; Conen 50 percent (S-benzil- O-butil S-etil thio-phosphate), 2.5 liter/ha; P-605 (In code: Farmagro Co.),1 liter/ha; BAS 3201 F (In code: BASF Co.), 1 kg/ha; Calixin 75 percent

N-tridecil 2.6- dimetil- morfolina: Tridemorph), 0.6 liter/ha; Sclex 30 percent (3.5 dichlorophenyil = 5.5- dimethyl exazolidine-dione- 2.4 = Dichlozine), 1 kg/ha; TPTA (Tri-phenyl tin acetate), 1 kg/ha and Triazine 50 percent (2.4- Dichloro-6- 0-chloroanilino)-1, 3.5-triazine), 1 kg/ha.

A complete and detailed result of this research work will be reported in the near future by the National Rice Program in a technical bulletin. Partial results are shown in Figure 1. Kitazin 17 percent granulated has displayed the most effectiveness in controlling blast. It attained a significant difference among the chemicals tested and checked.

As a rule, part of the chemicals tested had relatively good control to blast, but their performance changes from place to place or from year to year. At present, we have not obtained conclusive results from one chemical. The only one chemical which has a very good performance is Kitazin P 17 percent granulated.

6.2 Damage caused by fungicides in rice grain formation

The increase of the blast disease in almost all the rice lands, the use of chemicals in its control and the speculation raised by the salesmen and farmers about the phytotoxicity of certain chemicals, made it necessary to study the influence of them on grain production. During the growing season 1970/71

Eng. Daniel Cumpa from "Pedro Ruiz Gallo"University started a trial on the IR 8 variety. The rates used in our chemical

Table 5. The most resistant varieties selected from the International Blast Nurseries from 1964 to 1970 around the world* and their reactions in Peru

Variety	Reactions to Blast
Tetep	Resistant
Nang Chet cuc	Resistant
Takudan	Intermediate
R 67	Intermediate
C 46-15	Resistant
CI 7787	Susceptible
Pah Leuad 29-8-11	Resistant
D 25-4	Resistant
Trang cut L.11	Resistant
Pah Leuad 111	Resistant
Mamoriaka	Resistant
Huan-sen-goo	Resistant
Dissi Hatif (DH 2)	Intermediate
Carreon	Resistant
Ram Tulasi	Resistant
Ram Tulasi Sel	Resistant
Ca 435/B/5/1	Intermediate
DNJ 60	Intermediate

* IRRI, Annual Report for 1970.

FIGURA Nº 1 *Result of behavior of 17 fungicides on the control of the rice blast disease: Evaluated in per-cent of Leaf área, Leaf-base, Stem blast and Neck blast affected (Reported by J. Zapata - Unpublished) Bagua 1971*

control test and the doses of the fungicides used were given by the manufacturer. Each chemical was sprayed on a predetermined rice plant at booting stage, one-third, two-thirds, and three-thirds of the panicle emergence, with two different volumes of water. The water rates were 50 and 300 liters/ha corresponding to airplane and knapsack mist blower sprayers, respectively. The phytotoxicity effects were evaluated by percent of unfilled grains and deformed grains at harvest. Complete results will be reported in the future in a National Rice Program technical bulletin.

Apparently there was no damage from the fungicides when they were sprayed in all of the heading stages (Table 6). However, Bla-S in two water rates has shown the highest (number- percent) of grain damaged. A mercurial compound Granosan, used as a check, has also displayed certain damage, but only when it was sprayed at the rate of 50 liters/ha of water. Exact results will be reported after the statistic analysis.

7. Wild host of P. oryzae

Laberry & Jimenez (1971) have found that P. oryzae was capable of infecting Stenotaphrum secundatum (American grass) and that Pyricularia sp., found normally on S. secundatum in Piura (coast), was a rice pathogen too. Of another 35 weeds inoculated with P. oryzae, none displayed reaction. However, Panicum repens L., Sorghum vulgare, Cynodon dactylon, Paspalum sp., and other Echino-

chloa species reported as hosts of P. oryzae by Asuyama, 1965, Malagutti et al., 1951 and Revilla, 1953, were not infected.

Table 6. Evaluation of damage from 13 fungicides on the formation of rice seeds on IR 8 variety (reported by D. Cumpa, unpublished U.N.P.R.G., Lambayeque, 1971).

Fungicides*	Deformed grains in %		Unfilled grains in %	
	50 L/Ha**	300 L/Ha	50 L/Ha	300 L/Ha
Kasumin	3.0(0.2)***	3.1(0.3)	14.5(15.1)	14.5(11.4)
Blastin	1.6(0.4)	4.8(0.3)	8.9(6.6)	12.7(13.2)
Bla-S	1.5(5.6)	1.1(1.6)	12.8(7.1)	12.8(7.3)
Kitasin EC	4.2(5.9)	2.3(0.8)	11.5(15.6)	13.0(7.5)
BAS 3201-F	2.3(2.1)	2.0(2.9)	8.2(18.3)	9.9(6.7)
Benlate	1.2(1.2)	0.6(2.6)	7.4(6.2)	8.1(6.2)
Antracol	1.4(1.7)	3.8(0.7)	9.0(12.8)	8.9(7.4)
Dithane M-45	1.2(1.6)	1.5(2.1)	8.5(6.1)	13.5(17.7)
Manzate D	1.4(1.6)	1.0(0.0)	8.8(4.8)	7.9(11.9)
Conen	1.5(0.0)	3.2(2.0)	8.7(19.8)	8.7(6.6)
P- 605	2.4(1.8)	2.1(2.4)	8.4(8.3)	9.5(11.5)
Calixin	5.6(4.1)	1.0(2.0)	13.0(9.4)	8.0(25.4)
Granosan	2.6(2.3)	1.8(4.5)	16.4(9.5)	7.7(7.0)

* Doses used are found in the text.
** The volume of water used per hectare.
*** The numbers in parenthesis are the percent of damage obtained on the controls.

8. Summary

Blast is the most important rice disease in Peru, especially in the tropical areas. Research works on blast disease control began with the creation of the National Rice Program in September, 1968.

Now, seventeen workers are engaged in the pathology group of the program. Efforts were concentrated on International Blast Nurseries to obtain resistant rice lines; about 4,000 lines have been evaluated recently, after more than 30 trials carried out in different places and seasons. Only 25 resistant lines have been selected; among them, IR 480-5-9-2 and Tetep are the best ones. Chemical control trials consisted of evaluation of 20 fungicides. Kitazin P 17 percent granulated, 41 kg/ha was the best treatment studied. Rice grain formation was not influenced by the fungicide activity. Among 36 weeds artificially inoculated, only Stenotaphrum secundatum showed positive for the reaction to blast pathogen in the trials for the purpose of finding out weeds which act as a host for Pyricularia oryzae.

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Centro Internacional de Agricultura Tropical

PATHOGENIC VARIABILITY AND CYTOLOGY OF MONOCONIDIAL SUBCULTURES OF PYRICULARIA ORYZAE

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SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

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Many plant pathogenic fungi lack a known sexual cycle or in the absence of their sexual stage undergo numerous changes in pathogenicity. Some observed variability for pathogenicity is not explained on the basis of conventional mechanisms.

The tremendous economic significance of plant pathogens and the problems that pathogenic variability introduce into disease control programs are closely related. Previous research in this laboratory has demonstrated that the fungus pathogen causing rice blast may provide a model system for studies on the mechanisms of variability.

Recent reports and studies indicate that there is an unusually high rate of pathogenic variability in Pyricularia oryzae Cav. (6,7,9,11). These reports agree on the rate, type and significance of the pathogenic variability. The variation in pathogenicity is evident on the international set of differential rice varieties (7,13) used for identifying physiologic races of the fungus (5).

The genetic origin of the variability is not known. Based on Suzuki's (12) and Chu's (4) observations, Ou and Awad (11) believed that heterokaryosis is responsible, but they regognized that others have found cells of P. oryzae to be uninucleate (15).

Giatgong (7,9) observed a high frequency of pathogenic variability in single conidial isolates of P. oryzae (Tables 1, 2 and 3). His work indicated that heterokaryosis was probably not responsible since mycelial and conidial

Table 1. Disease reaction of twenty first-generation monoconidial lines of Pyricularia oryzae, U.S. race 1, tested on four differential rice varieties.

Variety or C.I. Number	Reaction Class [a/]							
	Parental Isolate	Monoconidial Isolates						
Zenith	S	S	S	S	R [b/]			
C.I. 8970-P	R	R	S	R	S			
C.I. 8970-S	S	S	S	R	S			
P.I. 180061	S	S	S	S	R			
	Frequency	13	4	2	1			
	Reaction of Second-Generation							
	Parental Isolate	Monoconidial Isolates						
Zenith	R	R	R	R [b/]	S	S	R	S
C.I. 8970-P	S	S	R	S	R	S	R	S
C.I. 8970-S	S	S	S	S	S	S	R	S
P.I. 180061	R	R	R	S	S	S	R	R
	Frequency	7	5	3	2	1	1	1
	Reaction of Third-Generation							
	Parental Isolate	Monoconidial Isolates						
Zenith	R	R	R	R	R			
C.I. 8970-P	S	S	S	R	S			
C.I. 8970-S	S	S	S	R	R			
P.I. 180061	S	S	R	R	R			
	Frequency	15	2	2	1			

a/ R = Resistant, S = Susceptible.
b/ Isolate selected for testing in the next generation.

Table 2. Disease reaction of twenty first-generation monoconidial lines of Pyricularia oryzae, U.S. race 3, tested on four differential rice varieties.

Variety or C.I. Number	Reaction Class [a]			
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R [b]	R	R
C.I. 8970-P	S	S	R	S
C.I. 8970-S	S	S	S	R
P.I. 180061	R	R	R	R
	Frequency	18	1	1
	Reaction of Second-Generation			
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R	R [b]	R
C.I. 8970-P	S	S	S	R
C.I. 8970-S	S	S	R	R
P.I. 180061	R	R	R	R
	Frequency	17	2	1
	Reaction of Third-Generation			
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R	R	R
C.I. 8970-P	S	S	S	R
C.I. 8970-S	R	R	S	R
P.I. 180061	R	R	R	R
	Frequency	15	3	2

a/ R = Resistant, S = Susceptible.
b/ Isolate selected for testing in the next generation.

Table 3. Disease reactions of twenty monoconidial lines that originated from Pyricularia oryzae, U.S. race 1, subculture 12 on the international differential varieties.

Race, Group and Number	Frequency
IB-5	2
IB-21	2 a/
IB-29	1
IB-33	1
IB-37	2
IB-41	1
IB-45	2
IB-53	3
IB-61	4
IB-62	2
IB-64	1

a/ Parental source.

cells of P. oryzae used in his studies were uninucleate. However, Giatgong was unable, cytologically or genetically, to determine the mechanism which caused the variability. He also observed that the concentration of inoculum (Table 4) and the temperature (Table 5) during the infection process did not contribute to the conidial variability. Mutation rate for virulence of one isolate on one differential host was estimated to be 3.2×10^3. This rate of mutation is unusually high for most plant pathogenic fungi, and is probably due to some mechanism other than gene mutation or heterokaryosis.

In preliminary work, Giatgong (6) observed some changes in the rates of variability by exposing P. oryzae to mutagens, high temperature, and by prolonged periods of culture storage prior to inoculation. However, from the relatively small populations of pathogenic isolates that were tested, he was unable to explain the observed variation.

High rates of hereditary variability are known for many fungi: Phytophthora infestans (2), Aphanomyces euteiches (1), Ustilago maydis (3) and Aspergillus nidulans (14) are but a few examples. Many workers report high levels of cultural and pathogenic variation, but have not completed the work necessary to define the mode or mechanisms of this variability. In other words, the variability problem is not unique to P. oryzae, but it is even more difficult to explain the basis for the variability in a mononucleate fungus without a sexual stage, such as P. oryzae.

Our previous observations and evidence (6,8,9) do not support heterokaryosis as a basis for hereditary variability in P. oryzae, and we have no bona fide evidence for sexuality or parasexuality. However, since there was continual segregation or mutation for pathogenicity in some serial single-conidial subcultures, and because the nuclei in conidia of P. oryzae are each

Table 4. Effect of conidial concentration on host reaction (race IG-2).

	Concentrations			
	6.0×10^5	2.8×10^5	1.6×10^5	7.5×10^4
Zenith	R	R	R	R
C.I. 8970-P	S^{+++}	S^{++}	S^{++}	S^{+}
C.I. 8970-S	S^{+++}	S^{++}	S^{++}	S^{+}
P.I. 180061	R	R	R	R

R = low reaction
S = high reaction

Table 5. Reaction of twenty second-generation monoconidial isolates, U.S. race 1, subculture 3 at three different controlled temperatures.

Variety or C.I. Number	Reaction Class at 20C [a/]				
	Parental Isolate	Monoconidial Isolates			
Zenith	S	S	R	S	R
C.I. 8970-P	R	R	R	R	R
C.I. 8970-S	S	S	S	R	R
P.I. 180061	S	S	S	S	S
	Frequency	17	1	1	1
	Reaction Class at 25C				
	Parental Isolate	Monoconidial Isolates			
Zenith	S	S	R	S	R
C.I. 8970-P	R	R	R	R	R
C.I. 8970-S	R	S	R	R	R
P.I. 180061	S	S	S	S	S
	Frequency	17	1	1	1
	Reaction Class at 30C				
	Parental Isolate	Monoconidial Isolates			
Zenith	R	R	R	R	R
C.I. 8970-P	R	R	R	S	R
C.I. 8970-S	R	R	S	R	S
P.I. 180061	S	R	R	R	S
	Frequency	10	4	3	3

a/ R = Resistant, S = Susceptible.

derived from a single-nucleated spore mother-cell, it is suggested that extra-chromosomal or cytoplasmic inheritance is involved.

The possible role of extra-chromosomal inheritance in P. oryzae, if any, must be clearly differentiated from that of gene mutation and heterokaryosis before the mechanism of pathogenic variability can be studied and understood.

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Centro Internacional de Agricultura Tropical

INDICATIONS OF PARTIAL RESISTANCE OF RICE TO THE FUNGUS PYRICULARIA ORYZAE CAV.

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and
Guillermo E. Galvez E.

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

INDICATIONS OF PARTIAL RESISTANCE OF RICE TO THE FUNGUS *Pyricularia oryzae* Cav.

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The rice (*Oryza sativa* L.) blast disease caused by *Pyricularia oryzae* Cav. is one of the limiting factors in rice production in the tropics. Many efforts have been made to obtain resistance varieties to this pathogen in several countries throughout the world, but without much success.

The use of "major gene resistant" ("vertical resistance" or "specific resistance") has failed in the control of certain diseases due to the great pathogenic variability of some plant pathogens. Because of this fact, researchers are looking for a different type of resistance usually controlled by minor genes, that here will be referred to as "partial resistance" ("horizontal resistance", gen-

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This article is based on the material for a thesis that the main author will present to the Graduate Program ICA-UN as a requirement for his M.S. degree.

eral resistance", "race non-specific resistance", "stable resistance", etc.). Plants showing partial resistance may develop low disease levels, and they should not be affected by specific races (2, 19).

The fungus P. oryzae has a great variability, as shown by the identification of several races from monoconidial subcultures. It suggests frequent genetic changes of the pathogen (4). The genetic variability is even more complex when one considers that Ou and Ayad (12) were able to identify 14 races from 56 monoconidial cultures obtained from just one lesion.

The pathogenic variability is still not understood, as the perfect stage of the fungus has not been found. Nevertheless, Hebert (7) has obtained artificially the perfect stage of P. grisea (Cke.) Sacc., Ceratosphaeria grisea n.sp., a species very similar to P. oryzae.

Type 3 lesions, producing few conidia per day (15-50) in short periods of time, and few numbers of type 4 lesions, have been considered as an indication of partial, stable, field, horizontal, or polygenic resistance in the rice blast disease work (13, 16, 17).

Ou and his co-workers (13) have suggested that the varieties Carreon and Tetep have horizontal resistance because they showed few lesions of any type per plant. These varieties showed either highly resistant or susceptible type lesions depending on the race. However, type 4 lesion number (susceptible) was always small in these varieties (11).

Likewise, in searching for partial resistance in Colombia, it has been observed that certain varieties have shown a high and broad

spectrum of resistance including Carreon, Tetep, and Colombia 3 after 20 continuous plantings under highly epiphytotic field conditions in los Llanos Orientales (3). These varieties and others have also been constantly resistant in Brazil, Panama and Peru.

Size and color of the lesions, amount of sporulation, and speed of penetration of the fungus in the host have been used to evaluate resistance or susceptibility. Suzuki (15) considers that plants showing pin-point and dark brown lesions are highly resistant, those having lesions of an intermediate size and brown color are moderately resistant, whereas those showing lesions of large size with white, purple, or green-gray color are susceptible.

The amount of sporulation per lesion is an important factor in considering varietal resistance to a plant pathogen. In the case of P. oryzae the amount of sporulation has been related to the type of lesion: the highest number of spores has been found in lesions showing a gray central zone and purple to dark brown borders (9). However, a high relative humidity, particularly during the night, is necessary for the occurrence of this type of lesion (1).

This paper reports the study of some factors that might be involved in the partial resistance of rice to P. oryzae.

Materials and methods

These studies were carried out in the greenhouses and laboratories of the Centro Nacional de Investigaciones Agropecuarias, "Tibaitata", of the Instituto Colombiano Agropecuario, ICA. The varieties used (Table 1) were selected for their high, intermediate and suscep-

tible reaction to the races of P. oryzae present in the beds of the C.N.I.A., La Libertad, Villavicencio, in los Llanos Orientales, where the disease is endemic and epiphytotic throughout the year.

Rice seeds were planted in plastic pots (10 cms diam.,and 10 cms high), containing loamy-sand soil. The plants were grown in a greenhouse for 20 to 25 days at a temperature of 20-30°C, a photoperiod of 12 hours, and 80 percent relative humidity before they were used.

IB-1, IC-1, IG-1 and ID-8 of P. oryzae from Peru, Colombia (Llanos Orientales and Cauca Valley), and Brazil, respectively, were used. They were grown in rice-polish-agar (RPA), and oats-agar (OA) media, at 2 percent of each ingredient. The cultures were kept at 4°C to stop sporulation. The inoculum consisted of an aqueous suspension of conidia (30 to 40 conidia per ml) from a fungus culture grown during 12 days in an incubator at 25°C and a 12 hour daily fluorescent light exposure. Gelatine 0.25 percent was added to the inoculum suspension as a spreader.

In the laboratory, Hsu and Ou (8) technique was used for inoculating four leaf portions of 6-7 cm long, previously disinfested by immersing them in a 50:50 solution of alcohol (95 percent) and sodium hypochlorite (5.25 percent) for one minute. Then they were immediately washed in distilled water for 15 minutes.

The leaves were placed in Petri dishes on filter paper moistened in an aqueous solution of monosodic phosphate at 0.01 m. Then the inoculum was sprayed using a manual atomizer De Vilviss No.14 at 20 cm

Fanny and IR8 leaves were sprayed only with an aqueous solution of gelatine (0.25 percent) as absolute checks.

Twenty plants/variety in 4 pots were inoculated in the greenhouse. The inoculum was prepared to contain 30-40 conidia per 100X-microscope field, and sprayed at 10 p.s.i. by a De Vilbiss No. 15 atomizer. Fanny and IR8 were sprayed only with a gelatine (0.25 percent) solution as checks. The plants were kept in the dark for 24 hours in a growth chamber at 20-30 C and 100 percent relative humidity. Then they were supplied with 12 hour photoperiod for an additional 72 hours under the same conditions.

Lesion types

The evaluation of lesion types was made according to the international scale (10). The readings were taken 8 days after the inoculation.

Lesion size

The lesions in each variety were measured 8 days after inoculation. The width and length of 8 lesions at random were measured, and the results were averaged in the laboratory tests. The lesions, taken at random from each of the 4 replications, were measured for each variety in the greenhouse.

Lesion color

The lesion color from the center to the borders was determined under a stereo microscope 8 days after inoculation, using Ridgway's scale (14).

Sporulation time

From the third day after the inoculation the sporulation time was daily determined under a light microscope at 100X. Individual lesions of leaves maintained in Petri dishes were observed daily for 10 days.

Number of conidia per lesion type

The number of conidia per lesion type was determined from the 5 best visible lesions in each variety after 10 days of the inoculation. The lesion was cut and placed in one-ml aqueous gelatine solution (0.25 percent) in an test tube. After one minute of shaking, aliquots were examined in an hemacytometer to determine the number of conidia per ml.

In the greenhouse tests, the leaves were cut 8 days after the inoculation and placed on moistened filter paper for 2 days before examination.

Influence of the relative humidity in the development of the lesion type

Race IB-1 was used in these studies. The leaves were placed as usual in Petri dishes and sprayed with a conidia suspension containing 30-40 conidia per 100X microscope field.

The inoculated leaves were kept at 25°C in the dark. IR8 and Fanny were sprayed only with 0.25 percent gelatine. The excess inoculum was dried by hot air after 0, 6, 8, 10, 12 and 14 hours of exposure at 100 percent relative humidity. To avoid moist condensation on the leaves, a dry filter paper was placed at the top of the Petri

dish.

The influence of the relative humidity in the development of the lesion type under greenhouse conditions was determined by inoculating 30 plants per variety with a conidial suspension of 30 to 40 spores per 100X microscope field. Groups of 5 plants per variety were exposed to 0, 6, 8, 10, 12 and 14 hours at 100 percent relative humidity and 25-30°C. The excess inoculum was dried as previously described. The lesion type was estimated 8 days after the inoculation.

Frequency of sporulation in type 3 and 4 lesions

Six varieties showing type 3 and 4 lesions under greenhouse conditions were used in this study. Leaf samples with type 3 and 4 lesions were placed on the inside top of a Petri dish that contained water agar (WA). The samples were kept in an incubator at 25°C in the dark and at 90 to 100 percent relative humidity. The conidia that fell down on the agar were counted daily under a light microscope (100X) for 18 days.

RESULTS

Lesion type

The results on lesion type are presented in Tables 2 to 6 for each variety and race. Great fluctuations were observed among varieties when they were inoculated with the different races under greenhouse and laboratory conditions. In general, type 1 lesions prevailed over the other types. Only the variety Fanny showed type 3 and 4 lesions with the race IB-1 in the greenhouse (Table 3).

Fanny was the most susceptible variety under laboratory conditions to all the races. Bluebonnet 50, Colombia 3, Fa-yiu Tsai, Perola, and Iaca Escuro showed an intermediate reaction (Type 3) only to the races IB-1 and IC-1. The rest of the varieties had a resistant reaction (Table 2).

All the varieties were more vulnerable to the pathogen under greenhouse conditions. With race IB-1, the highest number of type 1 lesions was presented by Carreon, type 2 lesions by Iaca Escuro, and type 4 lesions by Fanny. The rest of the varieties showed a fewer number of lesions (Table 3).

With race IC-1, the largest number of type 1 and 2 lesions was observed on the varieties Carreon, Iaca Escuro, Colombia 3 and Fanny. The other varieties showed few lesions (Table 4).

With race ID-8, the varieties Colombia 3 and Carreon had the highest number of type 1 lesions whereas Fanny, Bluebonnet 50 and C46-15 had type 2 and type 3 lesions. Fanny also showed type 4 lesions (Table 5).

With race IG-1, the variety Colombia 3 had the highest number of type 1 lesions whereas Fanny, Bluebonnet 50 and C46-15 had the highest number of type 2 and 3 lesions. Bluebonnet 50, Fanny, and Iaca Escuro also produced type 4 lesions. However, the latter had few type 4 lesions as compared with Fanny and Bluebonnet 50 (Table 6).

Lesion size

Fanny and Bluebonnet 50 produced the largest lesions under laboratory conditions. The varieties C46-15, Perola, Iaca Escuro and

Fa Yiu Tsai showed larger lesions than the other varieties, but never as large as those of the two susceptible ones.

The results were similar under greenhouse conditions. Fanny and Bluebonnet 50 developed the largest lesions. Iaca Escuro and C46-15 showed larger lesions than the other varieties but smaller than the susceptible ones (Table 8).

Lesion color

The results are presented in Tables 9 and 10 for the laboratory and the greenhouse studies. Type 1 lesions showed predominantly light seal-brown and seal-brown colors whereas types 2 and 3 had more variable colors. The susceptible variety Fanny always had an oil-green to a cerro-green color.

In the greenhouse, a diversity of colors was observed at the center of the lesion, particularly with types 2, 3, and 4. As will be described later, the color of the lesions was related to the number of conidia produced by the lesions.

Sporulation time

In general, the varieties with type 1 lesions seldom sporulated. In this case, the conidia production always occurred in lesions by the leaf borders. Type 3 and 4 lesions, and occasionally type 2 lesions, sporulated earlier, usually 4-5 days after inoculation in the laboratory studies (Table 11).

The varieties with intermediate and susceptible types (3 and 4) sporulated 4 to 5 days after inoculation in the greenhouse. C46-15, IR 8/2 x Zenith, Carreon, Dissi Hatif, and Colombia 3 sporulated 5

to 7 days after inoculation, having type 2 lesions which produced fewer conidia. In the other varieties, sporulation occurred 8-10 days after the inoculation (Table 12).

Conidia number per lesion type

The results are shown in Tables 13 and 14 for the laboratory and greenhouse studies, respectively. The highest sporulation occurred in lesions with oil-green, olive-gray and cerro-green centers. These lesions have borders light-seal-brown to seal-brown. Perola and C46-15 produced few conidia although they had oil-green centers. Lesions with drag-gray, marron and seal-brown colors produced few conidia or none at all.

Under greenhouse conditions the number of spores varied considerably. Lesions with cerro-green, oil-green, olive-green or drab-gray centers produced the highest number of conidia, except for the varieties C46-15 and Colombia 3. The variety Fa Yiu Tsai showed lesions with similar colors to the above, but it did not produce any conidia with race IG-1. In general, lesions showing light seal-brown and seal-brown colors did not produce any conidia.

Influence of the relative humidity in the development of the lesion type

The results are presented in Tables 15 and 16. The minimum time necessary for this kind of study and for the development of typical lesions of the fungus in rice was from 8 to 10 hours because even susceptible varieties showed only type 1 lesions when they were exposed to 100 percent relative humidity from 0 to 6 hours at 25°C.

Sporulation frequency in type 3 and 4 lesions

The results are shown in Table 17. The conidia production was abundant after the third day of inoculation, particularly in the susceptible varieties Fanny and Bluebonnet 50. In these varieties the fungus produced conidia for 10 days, whereas in Colombia 3 with type 3 lesions it sporulated for 11 days. In the varieties Fa Yiu Tsai, Perola and Iaca Escuro, P. oryzae produced conidia for 17-18 days. However, the number of conidia was lower in comparison with the other varieties.

DISCUSSION

The study of the behavior of rice varieties - susceptible, intermediate, and resistant to blast - under laboratory and greenhouse conditions indicated that the type, size, color of the lesions, and number of conidia per lesion are important factors in determining resistance to P. oryzae. Susceptible varieties showed larger size (type 4) and higher number of lesions. Sporulation took place in lesser time and the number of spores produced was considerably higher. Intermediate varieties in certain cases had type 3 lesions but they produced a higher number of conidia. It implies that lesion size is not a big enough factor in determining resistance. Large size lesions producing few conidia may be less important epiphytologically than smaller size lesions, but may also be active conidia producers. In general, type 3 lesions produced fewer conidia than type 4. Ou et al (13) found that the varieties Carreon and Tetep produced few lesions

to all the races tested, although in some cases type 4 lesions were observed on Tetep.

Sporulation was higher in the greenhouse than in the laboratory. The leaves, because yellow, died in 5 days and this might be the reason for the smaller number of conidia observed in the laboratory studies. The time from inoculation to sporulation initiation was shorter in susceptible varieties than in intermediate ones. However, the time difference was small. Type 3 lesions produced higher numbers of conidia than type 2 lesions, in general. But, in some cases,a high sporulation occurred in type 2 lesions. These observations differ from Yorinori and Thurston (18) who found no differences in conidia production between types2 and 3. These discrepancies might be due to the different varieties as well as the races of the pathogen used in both cases.

The time required for high humidity in the development of typical lesions of P. oryzae was in agreement with Hashioka (6). The laboratory studies for this factor were not valid because of the condensation present on the surface and borders of the inoculated leaves in the Petri dishes. Furthermore, this factor did not establish any difference among varieties.

Daily discharge of conidia was different for the susceptible and intermediate varieties. High sporulation occurred during the first days for type 4 lesions of Fanny and Bluebonnet 50. However, the type 4 lesions of Iaca Escuro always produced few conidia, an important epiphytological factor in the development of the disease. Colombia 3

showed type 3 lesions but they produced high numbers of conidia after the 5th day. This kind of resistance with a late discharge may be a critical factor in partial resistance of rice to P. oryzae.

In the late blight disease of potatoes (Phytophthora infestans Mont de Bary), partial resistance is considered to be present in varieties that show a low number and small size lesions, low and slow sporangia production per leaf area, late penetration in the leaf, and long time for lesion appearance per a given quantity of inoculum (2, 5). Differences in size of the lesion, number of lesions per leaf, number of conidia, and lesion color were observed consistently in these studies for the varieties selected for their reaction under field conditions. Partial resistance, or stable resistance, or general resistance, or horizontal resistance, whatever name is used to express this kind of resistance, is difficult to prove in a short period of time and under limited variability of the fungus. Nevertheless, these studies suggest its probable existence in rice varieties. A worldwide cooperation to establish a uniform partially resistant blast nursery to be tested under different conditions and under standard disease estimation procedures is urgent and necessary. Greenhouse and laboratory studies may be useful in understanding its nature. The finding of a variety with a sufficient number of genes to hold resistance to P. oryzae, and not to be broken by new races, will undoubtedly be useful to the rice plant breeders.

TABLE 1: Rice cultivars used in the studies of partial resistance to *P. oryzae*.

Cultivar Name	Origin	Resistance grade (1-7) a/
Fanny	France	7
Bluebonnet 50	U. S. A.	7
IR 8	Philippines	7
Colombia 3 (T 319 E-2M-2M-1M-5M)	Colombia	3,4
Fa Yiu Tsai	China	5
Perola	Brazil	5
Iaca Escuro	Brazil	3
IR 8/2 x Zenith IR 1154-106	Philippines	1,2
Nahng Mon S4 x TN1 IR 160-27-3-1-1-3	Philippines	5
IR 8 x (Dawn x TN1) IR 782-24	Philippines	1
Carreon	Philippines	1
Tetep	Japan	Many 1
C4615	Burma	1,3
Dissi Hatif	Senegal	3,4
Mamoriaka	Africa	3,4

a/ 1 = Highly resistant

7 = Highly susceptible

TABLE 2: Lesion types on rice cultivars inoculated with 4 races of *P. oryzae* under laboratory conditions.

Cultivar Name	Race Identification and lesion type [a]			
	IB-1	IC-1	ID-8	IG-1
Fanny	2-4	2-4	2-4	2-4
Bluebonnet 50	1-3	1-3	2	1-2
IR 8	1-2	1	1	1
Colombia 3	1-2	1-3	1-2	1
Fa Yiu Tsai	1-2	1-3	1	1
Perola	1-2	1-3	1-2	1-2
Iaca Escuro	1-3	1-3	1-2	1-2
IR 8/2 x Zenith	1	1	1-2	1
Nahng Mon S-4 x TN1	1	1-2	1-2	1
IR 8 x (Dawn x TN1)	1-2	1	1-2	1
Carreon	1	1	1	1
Tetep	1	1	1	1-2
C4615	1-2	1-2	1-2	1-2
Dissi Hatif	1	1-2	1	1
Mamoriaka	1	1	1	1

[a] Lesion type (1-4) according to the International Scale

TABLE 3: Average number of lesions due to race IB-1 of *P. Oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation [a]			
	1	2	3	4
Fanny	29.00[a]	17.25	48.75	57.00
Bluebonnet 50	117.75	34.75	46.50	4.50
IR 8	63.50	2.50	0.00	0.00
Colombia 3	179.50	4.50	1.50	0.00
Fa Yiu Tsai	90.00	13.50	15.50	0.00
Perola	94.25	3.75	2.00	0.00
Iaca Escuro	92.50	52.00	16.75	4.25
IR 8/2 x Zenith	134.25	0.25	0.00	0.00
Nahng Mon S4 x TN1	103.50	0.00	0.00	0.00
IR 8 x (Dawn x TN1)	143.00	0.00	0.00	0.00
Carreon	256.25	14.75	0.00	0.00
Tetep	24.50	0.00	0.00	0.00
C4615	182.50	6.00	0.00	0.00
Dissi Hatif	215.75	1.75	0.00	0.00
Mamoriaka	186.25	0.00	0.00	0.00

[a] Type of lesion (1-4) according to the International Scale.

TABLE 4: Average number of lesions due to race IC-1 of *P. oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation [a/]			
	1	2	3	4
Fanny	128.00 [a/]	79.25	63.50	30.00
Bluebonnet 50	206.00	38.25	49.00	7.25
IR 8	129.75	16.50	0.25	0.00
Colombia 3	296.75	68.50	15.75	0.00
Fa Yiu Tsai	395.00	69.50	33.25	1.25
Perola	376.75	43.75	31.00	2.75
Iaca Escuro	435.75	43.25	24.75	2.00
IR 8/2 x Zenith	119.00	6.00	0.00	0.00
Nahng Mon S-4 x TN1	183.00	10.00	2.00	0.00
IR 8 x (Dawn x TN1)	147.75	3.75	0.00	0.00
Carreon	831.25	9.50	0.00	0.00
Tetep	398.00	6.00	0.00	0.00
C4615	239.00	8.50	2.00	0.00
Dissi Hatif	286.00	7.00	0.00	0.00
Mamoriaka	275.50	0.00	0.00	0.00

a/ Type of lesion (1-4) according to the International Scale

TABLE 5: Average number of lesions due to race ID-8 of *P. oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation [a]			
	1	2	3	4
Fanny	246.25 [a]	58.25	87.00	17.50
Bluebonnet 50	685.50	21.00	4.50	0.00
IR 8	428.00	0.00	1.00 [b]	0.00
Colombia 3	725.25	17.00	0.00	0.00
Fa Yiu Tsai	144.75	6.00	0.25	0.00
Perola	407.25	3.75	0.00	0.00
Iaca Escuro	504.25	13.25	1.75	0.00
IR 8/2 x Zenith	93.50	1.00	0.00	0.00
Nahng Mon S-4 x TN1	220.50	0.00	0.00	0.00
IR 8 x (Dawn x TN1)	39.50	0.00	1.00	0.00
Carreon	720.75	0.00	0.00	0.00
Tetep	82.25	1.25	0.00	0.00
C4615	448.50	13.75	4.50	0.00
Dissi Hatif	383.50	2.50	0.00	0.00
Mamoriaka	533.25	0.00	0.00	0.00

a/ Type of lesion (1-4) according to the International Scale

b/ Lesions at the tip of the leaves.

TABLE 6: Average number of lesions due to race IG-1 of P. Oryzae in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation [a]			
	1	2	3	4
Fanny	94.75[a]	33.25	41.75	6.76
Bluebonnet 50	237.00	42.75	55.50	10.00
IR 8	83.50	1.50	0.00	0.00
Colombia 3	439.25	0.50	0.00	0.00
Fa Yiu Tsai	75.75	3.50	0.00	0.00
Perola	98.25	5.75	1.50	0.00
Iaca Escuro	240.25	2.75	1.75	0.25
IR 8/2 x Zenith	29.75	1.00	0.00	0.00
Nahng Mon S4 x TN1	113.75	4.00	0.00	0.00
IR 8 x (Dawn x TN1)	8.75	0.00	0.00	0.00
Carreon	273.25	0.25	0.00	0.00
Tetep	20.50	1.50	0.00	0.00
C4615	115.50	12.75	9.75	0.00
Dissi Hatif	254.00	1.75	0.00	0.00
Mamoriaka	246.75	1.25	0.00	0.00

[a] Type of lesion (1-4) according to the International Scale.

TABLE 7: Lesion size induced by *P. oryzae* 8 days after inoculation under laboratory conditions.

Cultivar Name	Lesion size in mm due to			
	R	A	C	E S
	IB-1	IC-1	ID-8	IG-1
Fanny	7.84 a/	6.92	6.25	6.35
Bluebonnet 50	3.16	4.03	2.84	1.69
IR 8	0.91	0.97	0.75	-0.50
Colombia 3	0.87	1.85	1.06	-0.50
Fa Yiu Tsai	0.50	2.04	0.69	-0.50
Perola	0.69	3.25	1.47	1.37
Iaca Escuro	1.28	2.16	1.17	1.25
IR 8/2 x Zenith	0.37	0.72	0.62	-0.50
Nahng Mon S-4 x TN1	0.50	1.03	0.69	-0.50
IR 8 x (Dawn x TN1)	0.94	0.51	0.91	-0.50
Carreon	-0.50	-0.50	-0.50	-0.50
Tetep	0.37	0.69	-0.50	0.62
C4615	1.69	1.95	0.84	1.56
Dissi Hatif	-0.50	1.16	-0.50	-0.50
Mamoriaka	-0.50	-0.50	-0.50	-0.50

a/ Average of the 8 largest lesions

TABLE 8: Lesion size induced by P. oryzae 8 days after inoculation under greenhouse conditions.

Cultivar Name	Lesion size in mm. due to			
	R	A	C	E S
	IB-1	IC-1	ID-8	IG-1
Fanny	17.90 a/	9.88	20.14	13.79
Bluebonnet 50	10.12	8.27	5.81	17.20
IR 8	0.52	1.52	- 0.50	1.20
Colombia 3	1.76	3.30	2.35	0.86
Fa Yiu Tsai	3.85	3.80	2.08	1.37
Perola	2.00	4.87	1.16	3.24
Iaca Escuro	4.65	5.32	2.74	3.53
IR 8/2 x Zenith	- 0.50	0.99	- 0.50	1.07
Nahng Mon S-4 x TN1	- 0.50	2.20	- 0.50	1.47
IR 8 x (Dawn x TN1)	- 0.50	0.92	- 0.50	- 0.50
Carreon	1.14	1.49	0.41	- 0.50
Tetep	- 0.50	1.58	0.58	- 0.50
C4615	2.31	2.68	2.77	4.83
Dissi Hatif	1.37	1.90	0.77	0.90
Mamoriaka	- 0.50	- 0.50	- 0.50	0.77

a/ Average of the largest 10 lesions/4 replications

TABLE 9: Colors presented by lesions due to different races of *P. oryzae* in rice varieties under laboratory conditions.

Cultivar Name	Color of the lesion			
	R	A	C	E	S
	IB-1	IC-1	ID-8	IG-1
Fanny	Oig, Lisb [a/]	Ceg, Lisb	Ceg, Lisb	Oig, Lisb
Bluebonnet 50	Olg, Seb	Oig, Seb	Olg, Seb	Olg, Seb
IR 8	Seb	Seb	Ma	Seb
Colombia 3	Seb	Oig, Seb	Seb	Seb
Fa Yiu Tsai	Olg, Seb	Olg, Seb	Seb	Seb
Perola	Olg, Seb	Oig, Seb	Olg, Seb	Oig, Seb
Iaca Escuro	Olg, Seb	Oig, Seb	Seb	Oig, Seb
IR 8/2 x Zenith	Seb	Seb	Seb	Seb
Nahng Mon S-4 x TN1	Lisb	Oig, Lisb	Lisb	Lisb
IR 8 x (Dawn x TN1)	Oig, Seb	Seb	Seb	Seb
Carreon	Lisb	Lisb	Lisb	Lisb
Tetep	Lisb	Lisb	Seb	Lisb
C4615	Drg, Seb	Oig, Seb	Oig, Seb	Seb
Dissi Hatif	Seb	Drg, Seb	Seb	Seb
Mamoriaka	Seb	Seb	Seb	Lisb

a/ Oig = Oil green, Lisb = Light seal brown, Olg = Olive gray, Seb = Seal brown, Drg = Drab gray, Ceg = Cerro green, Ma = Marron, following the Ridgway scale (14).

TABLE 10: Colors presented by lesions due to different races of *P. oryzae* in rice varieties under greenhouse conditions.

Cultivar Name	Color of the lesion			
	R	A	C	E	S
	IB-1		IC-1	ID-8	IG-1
Fanny	Ceg, Ma [a/]	Ceg, Lisb	Ceg, Lisb	Oig, Lisb
Bluebonnet 50	Ceg, Seb	Oig, Seb	Ceg, Seb	Ceg, Seb
IR 8	Seb	Dr, Seb	Seb	Seb
Colombia 3	Oig, Seb	Oig, Seb	Seb	Oig, Seb
Fa Yiu Tsai	Cag, Lisb	Cag, Lisb	Oig, Lisb	Oig, Lisb
Perola	Oig, Lisb	Olg, Lisb	Oig, Lisb	Olg, Lisb
Iaca Escuro	Olg, Seb	Olg, Seb	Oig, Seb	Olg, Seb
IR 8/2 x Zenith	Seb	Dr, Seb	Seb	Seb
Nahng Mon S-4 x TN1	Lisb	Olg, Lisb	Lisb	Lisb
IR 8 x (Dawn x TN1)	Seb	Seb	Dr, Seb	Seb
Carreon	Lisb	Dr, Lisb	Lisb	Lisb
Tetep	Seb	Dr, Seb	Seb	Seb
C4615	Dr, Seb	Oig, Seb	Dr, Seb	Dr, Seb
Dissi Hatif	Seb	Dr, Seb	Dr, Seb	Seb
Mamoriaka	Seb	Seb	Seb	Dr, Seb

a/ Ceg = Cerro green, Ma = Marron, Seb = Seal brown, Oig = Oil green, Cag = Calla green, Lisb = Light seal brown, Olg = Olive gray, Dr = Drab, following the Ridgway scale (14).

TABLE 11: Time of sporulation initiation of *P. oryzae* in rice leaves inoculated with 4 different races under laboratory conditions.

Cultivar Name	Beginning of sporulation after inoculation (days)			
	R	A	C	E S
	IB-1	IC-1	ID-8	IG-1
Fanny	4	4	5	4
Bluebonnet 50	5	4	4	4
IR 8	_ a/	_	7 b/	_
Colombia 3	6	5	_	_
Fa Yiu Tsai	5	5	8	_
Perola	6	5	8	5
Iaca Escuro	5	6	6	5
IR 8/2 x Zenith	_	_	9	_
Nahng Mon S-4 x TN1	8 b/	6	6	_
IR 8 x (Dawn x TN1)	_	_	_	_
Carreon	_	_	_	_
Tetep	_	_	_	6
C4615	6	6	5	6
Dissi Hatif	_	6	_	_
Mamoriaka	_	_	_	_

a/ Lesions that did not sporulate

b/ Sporulation only at the leaf borders

TABLE 12: Time of sporulation initiation of *P. oryzae* in rice leaves inoculated with 4 different races under greenhouse conditions.

Cultivar Name	Beginning of sporulation after inoculation (days)			
	R	A	C	E	S
	IB-1	IC-1	ID-8	IG-1	
Fanny	5	4	6	5
Bluebonnet 50	5	4	5	4
IR 8	7 a/	_	_	7 a/
Colombia 3	6	5	6	5
Fa Yiu Tsai	7	5	8	6
Perola	6	5	9	6
Iaca Escuro	6	5	6	5
IR 8/2 x Zenith	_ b/	5	10 a/	_
Nahng Mon S-4 x TN1	_	5	_	7
IR 8 x (Dawn x TN1)	_	_	8 a/	_
Carreon	7	5	_	_
Tetep	_	6	_	_
C4615	5	5	6	6
Dissi Hatif	6	5	7	_
Mamoriaka	_	_	_	8

a/ Lesions only at the tip of the leaves

b/ Lesions that did not sporulate.

TABLE 13: Number of conidia produced by *P. oryzae* 10 days after inoculation under laboratory conditions.

Cultivar Name	Number of conidia and prevalent type lesion			
	R	A	C	E	S
	IB-1	IC-1	ID-8	IG-1
Fanny	7.541 (4) [a]	16.833 (4)	10.100 (3)	4.833 (3)
Bluebonnet 50	2.125 (3)	9.666 (3)	2.400 (3)	3.400 (2)
IR 8	____ [b]	____	250 (2)	____
Colombia 3	733 (2)	1.833 (3)	416 (2)	____
Fa Yiu Tsai	1.291 (2)	2.666 (3)	____	____
Perola	500 (2)	3.000 (3)	333 (2)	2.333 (2)
Iaca Escuro	2.066 (3)	1.500 (3)	250 (2)	2.916 (2)
IR 8/2 x Zenith	____	____	750 (2)	____
Nahng Mon S-4 x TN1	____	500 (2)	1.083 (2)	____
IR 8 x (Dawn x TN1)	____	____	300 (2)	____
Carreon	____	____	____	____
Tetep	____	____	____	____
C4615	1.166 (2)	2.333 (2)	666 (2)	750(2)
Dissi Hatif	____	666 (2)	____	____
Mamoriaka	____	____	____	____

[a] The number in the parenthesis is the type lesion

[b] Lesion that did not sporulate

TABLE 14: Number of conidia produced by *P. oryzae* 10 days after inoculation under greenhouse conditions.

Cultivar Name	Number of conidia and prevalent type lesion							
	R		A		C		E	S
	IB-1		IC-1		ID-8			IG-1
Fanny	9.466	(4) [a]	20.300	(4)	11.633	(4)		12.700 (4)
Bluebonnet 50	3.433	(4)	12.633	(4)	2.600	(3)		8.800 (4)
IR 8	____	[b]	____		____			____
Colombia 3	1.533	(3)	1.500	(3)	966	(2)		1.250 (2)
Fa Yiu Tsai	1.800	(3)	2.700	(3)	66	(2)		____
Perola	1.200	(3)	3.800	(3)	833	(2)		3.866 (3)
Iaca Escuro	3.200	(4)	4.733	(3)	466	(2)		4.800 (3)
IR 8/2 x Zenith	____		100	(2)	166	(2)		____
Nahng Mon S-4 x TN1	____		233	(2)	____			1.700 (2)
IR 8 x (Dawn x TN1)	____		____		444	(3)		____
Carreon	266	(2)	433	(2)	____			____
Tetep	____		133	(2)	____			____
C4615	1.500	(2)	1.366	(2)	1.233	(3)		4.433 (3)
Dissi Hatif	633	(2)	333	(2)	366	(2)		____
Mamoriaka	____		____		____			666 (2)

[a] The number in the parenthesis is the type lesion

[b] Lesion that did not sporulate

TABLE 15: Times of relative humidity (100%) necessary for the development of typical lesions due to race IB-1 of *P. oryzae* under laboratory conditions .

Cultivar Name	Exposure time to 100% relative humidity (hours)					
	R		A	C	E	S
	0	6	8	10	12	14
Fanny	1[a/]	1	3	3	3	3
Bluebonnet 50	1	1	3	3	3	3
IR 8	1	1	1	1	1	1
Colombia 3	1	1	1	3	3	3
Fa Yiu Tsai	1	1	1	1	1	1
Perola	1	1	1	1	2	2
Iaca Escuro	1	1	2	2	2	2
IR 8/2 x Zenith	1	1	1	1	1	1
Nahng Mon S-4 x TN1	1	1	1	1	1	1
IR 8 x (Dawn x TN1)	1	1	1	1	1	1
Carreon	1	1	1	1	1	1
Tetep	1	1	1	1	1	1
C4615	1	1	1	2	2	2
Dissi Hatif	1	1	1	1	1	1
Mamoriaka	1	1	1	1	1	1

a/ Type of lesion (1-4) according to the International Scale

TABLE 16: Times of relative humidity (100%) necessary for the development of typical lesions due to race IB-1 of P. oryzae under greenhouse conditions .

Cultivar Name	Exposure time to 100% relative humidity (hours)					
	R		A	C	E	S
	0	6	8	10	12	14
Fanny	1[a/]	1	4	4	4	4
Bluebonnet 50	1	1	1	3	3	3
IR 8	1	1	1	2	2	2
Colombia 3	1	1	1	2	2	2
Fa Yiu Tsai	1	1	1	2	2	2
Perola	1	1	1	1	3	3
Iaca Escuro	1	1	1	1	3	3
IR 8/2 x Zenith	1	1	1	1	1	1
Nahng Mon S-4 x TN1	1	1	1	1	1	1
IR 8 x (Dawn x TN1)	1	1	1	1	1	1
Garreon	1	1	1	1	1	1
Tetep	1	1	1	1	1	1
C4615	1	1	1	2	2	2
Dissi Hatif	1	1	1	1	1	1
Mamoriaka	1	1	1	1	1	1

a/ Type of lesion (1-4) according to the International Scale

TABLE 17: Average number of conidia of *P. oryzae* (Race IB-1) produced daily in lesions type 3 and 4 during 18 continuous days.

Cultivar Name	Lesion type	Number of conidia produced at day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Fanny	4	1.2[a/]	14.8	243.8	39.8	22.4	34.6	16.6	12.8	10.6	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bluebonnet 50	4	0.4	3.0	12.4	103.0	58.2	40.8	15.2	8.4	6.8	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Colombia 3	3	0.0	3.8	8.4	24.4	23.0	214.0	95.3	112.4	70.6	41.8	24.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fa Yiu Tsai	3	0.0	1.6	3.8	2.4	2.0	35.2	8.2	10.6	2.4	0.0	2.0	6.0	7.4	2.0	8.2	7.6	11.0	3.4
Perola	3	0.0	6.2	5.2	1.0	2.4	16.8	7.2	14.2	24.6	23.8	47.4	9.4	12.4	33.4	4.4	0.4	1.2	0.0
Iaca escuro	4	0.0	0.0	2.2	20.2	16.0	29.4	21.6	14.0	24.0	33.4	18.0	13.8	11.0	3.2	1.4	1.8	0.4	0.0

a/ Average of 5 microscope field countings at 100X.

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Centro Internacional de Agricultura Tropical

FACTORS WHICH MAY AFFECT GENERAL RESISTANCE IN RICE TO PYRICULARIA ORYZAE CAV.

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H. David Thurston

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

FACTORS WHICH MAY AFFECT GENERAL RESISTANCE IN RICE TO *Pyricularia oryzae* Cav.

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Rice blast, caused by *Pyricularia oryzae* Cav., is perhaps the single most destructive disease of rice. Probably half of the world's rice is grown in the tropics (8). In temperate areas where rice is grown such as Japan, chemical control has been emphasized. Although effective chemical control is available for temperate zones, rice blast control in the tropics is limited by social, economic and environmental conditions, and economic chemical control for the tropics is not yet available. Thus, the development of rice varieties resistant to *P. oryzae* is essential.

No single rice variety has shown resistance to all races of the pathogen and, in addition, the fungus has proven to be highly variable, with a single conidium capable of producing many races (16). Nevertheless, the major sources of resistance to *P. oryzae* utilized in commercial plantings is simply or qualitatively inherited. Such specific or vertical resistance is rapidly lost with the appearance of new races. General or horizontal resistance has been shown to be stable and is not lost with the appearance of new races. Galvez *et al* (3), Nagai *et al* (14) and more recently, Ou *et al* (17), have shown that such resistance is

available in rice. If varieties can be bred with such resistance, the resistance might be maintained indefinitely and not lost with the appearance of new races of the pathogen.

This study was made during 1969-1970 and presented as a M.S. thesis in January 1971 by the senior author (21). The object of the study was to determine if consistent differences in characteristics which have been shown to be associated with general resistances in other crops such as potatoes (10) could be found in rice inoculated with P. oryzae. These characters might be useful in measuring and identifying relative levels of general resistance in rice. Characters studied were a) size of lesions, b) color of lesions, c) time for sporulation, d) number of spores produced, and e) time of ingress.

MATERIALS AND METHODS

Culture of rice plants

A serious problem in this study was growing healthy rice seedlings in the greenhouses and controlled climate chambers. Iron deficiency was a major problem and varietal susceptibility to the deficiency varied among the varieties used. Another problem, similar to what Latterell et al (14) called "winter sickness", was also common.

Numerous soil mixtures and environmental conditions were used in the attempt to grow healthy rice seedlings. The best success was obtained when seedlings were grown in a modification of the nutrient solutions of Hoagland and Arnon (5) and Tanaka et al (18) for about 10 days and then transplanted to 4-inch plastic pots containing fine white sand. The

potted seedlings were watered daily with 60 ml of nutrient solution per pot. Seedlings were grown in a greenhouse with a temperature variation of 26 to 38 C.

Culture of P. oryzae

The isolates of P. oryzae used in the study were received from Dr. J.G. Atkins, U.S.D.A., Beaumont, Texas; Dr. C.R. Adair, U.S.D.A., Beltsville, Md., and from Dr. W.N. Harnish, Niagara Chemical Division, Middleport, New York. Cultures were maintained by monthly transfers on 2 percent rice polish agar slants containing one or two pieces of rice nodes. For initial tests, inoculum was prepared using the techniques of Latterall et al (12). Of several artificial media tested, rice polish agar (12) gave the best results, but many isolates sporulated poorly and with continued transfers many isolates had a marked decrease in sporulation. An observation that profuse sporulation occurred when conidia germinated on an injured part of the rice leaf led to the idea that the fungus could be grown and inoculum produced on fresh crushed leaves after surface sterilization (Fig. 1). The youngest fully developed leaves of 30 to 40 day-old greenhouse-grown plants were cut into lengths 5 to 6 cm long. The sections were surface-sterilized by dipping into a 50:50 solution of alcohol and sodium hypochlorite for one minute, and then were washed in running water for 10-15 min in a 500 ml beaker covered with cheese cloth. Four or five leaf sections were stretched on a filter paper in a Petri dish moistened with 2 ml of 0.01 M monosodium phosphate and 70 ppm benzimidazole (6). Leaves were crushed by

rolling them with an aluminum cylinder (1.3 cm x 3.5 cm long) (Fig.1). The leaves were inoculated by taking a small piece of the fungus grown on rice polish agar and rubbing it on the leaf, after which they were placed in the dark at 24 C. Most isolates produced spores three days after inoculation, and by the fourth day sporulated profusely (Fig. 1). Spore suspensions for inoculation were prepared by cutting the sporulating leaves into sections of about one cm long, placing them in test tubes with 2 to 3 ml sodium oleate-gelatin solution (1), and dislodging the spores by shaking the test tubes with a Vortex Jr. shaker.

For subsequent transfers pieces of the sporulating leaf were rubbed on newly prepared leaves. Using this procedure, it was possible to grow the fungus continually on fresh rice leaf tissue. Leaves of the varieties Peta, Saturn, Taichung Nativel, Binato and IR5-47-2 were used as the substrate. The varieties Taichung Native 1 and IR5-47-2 were highly resistant (lesion types 1 and 2) (7) to most of the isolates we have used in tests with excised leaves or whole plant inoculation, but abundant sporulation was observed when leaves of these varieties were used for culturing the fungus. The fungus grows better on the upper two-thirds of the younger leaves. Older and basal portions of the leaves became brown to yellow within two to three days after crushing, and the fungus sporulated poorly. To obtain good sporulation it is necessary that leaves maintain a green color four days or more after crushing.

When sporulating leaves were dried 5 to 7 days after inoculation and stored at 3 C, spores were viable up to one week. All isolates had maximum sporulation by the fifth to sixth day, but beyond this period,

if leaves were kept moist, the spores fell off and germinated. For inoculation purposes spores should be harvested 4-6 days after inoculation. Stocks of fresh rice leaves were maintained in excellent condition for over 20 days in the refrigerator at 3 C. Leaves were detached, washed thoroughly in tap water, wrapped in soaked paper towels, and stored in plastic bags.

Inoculation of detached leaves

Inoculation with microdrops. The detached leaf technique, described by Hsu and Ou (6), was used with a slight modification in an attempt to find a method of inoculation whereby size, color of lesions, time of sporulation, number of spores produced, and time of ingress could be accurately measured.

In order to test as many isolates and varieties as possible at the same time, the following experimental design was adopted. One isolate was used to inoculate the twelve youngest, fully expanded leaves detached from twelve 25 to 30 day-old plants. From each leaf, a 6 cm long section was cut from the middle, widest area of the leaf, and placed in a Petri dish on moist filter paper. Six leaf sections were placed in one Petri dish.

To determine what part of the leaf is most suitable for the detached leaf inoculation method, tests were also made by inoculating the upper (A) and the lower (B) half parts of the same leaf. In this case eight leaf sections from four leaves were placed in each Petri dish, and three dishes were inoculated with one isolate.

Inoculations were made with a special microdrop pipette made by stretching a Pasteur pipette until a fine needle of 250 to 300 u in diameter was obtained (Fig. 1). A small eyedropper rubber was fitted on the other end. Inoculation was accomplished by placing three or four droplets (about 1 ul) on the upper surface of each leaf section. Thus, each variety was inoculated with 36 to 48 microdrops. Due to the high surface tension of the droplets on the waxy surface of the rice leaves the droplets tended to remain attached to the tip of the needle or roll off when too large. This was solved by coating the tip of the needle with a thin layer of vaseline, which tended to repel the droplets. Placement of droplets was also made easier by preparing the spore suspension with a solution containing Tween 20 (0.20 ml/1), or a gelatin-sodium oelate solution used as wetting agents. The later was more effective.

To reduce the variation in spore number in each droplet, due to settling, the eyedropper rubber was always partially compressed and, after three to four droplets were applied, a small amount of air was allowed to enter the pipette, thus mixing the suspension. One droplet of spore suspension was placed on each leaf section at a time. Inoculated leaves were incubated in the dark at a constant temperature of 21 (+ 0.5 C) or 24 (+ 1.0 C). Between 16 to 20 hours after inoculation, the plates were removed to the laboratory at room temperature and the droplets dried for 20 to 30 minutes under a fan after which the plates were again returned to the incubator.

Determination of lesion types

In general, the classification of lesion types was made according to the international classification, as proposed by Ou (15) at the Symposium on Rice Blast Disease. The criteria for rating resistance and susceptibility on detached leaves was slightly changed. The lesion types were classified in five scale units and were primarily based on color, relative size of lesions, and on presence or absence of a necrotic center. For standard colors, the Dictionary of Colors (13) was used. A list of colors used is given in Table 1. The necrotic center (area of collapsed cells), as mentioned in the international classification (15), corresponds to the central area of the lesions with a gray (Cu, Sb), opaline green (Opg), olive green (Olg), white (W) to pinkish (Iv, Wj) color which is surrounded by a ring of dark discolored cells. Table 2 gives the five scale units used and the characteristics of each scale.

Determination of lesion size

Lesion size was measured only once, eight days after inoculation. The criteria adopted for the measurements were as follows: six sections were chosen at random from each variety with 12 leaf sections inoculated; of these, two lesions, the largest and the smallest, were measured. The length and width of each lesion was measured and multiplied, and the result given in mm^2. The results were then presented as the average size (mm^2) of twelve lesions. Likewise, when the apical (A) and the basal (B) portions of the same leaf were inoculated, twelve leaf sections of each leaf position were inoculated. Recordings of size were made as described above, first, separately for each leaf portion (A and

B), and second, as the average between both.

Determination of time of sporulation

Observations on the time of sporulation were made daily, from the third day after inoculation, and up to the tenth day. Notes were taken on the first appearance of conidia using a microscope at 100X. After each observation the plates were put back into the incubator.

Determination of number of spores produced

The number of spores produced was determined 10 days after inoculation. Great variation in lesion types within and between varieties was observed. Some varieties had no sporulating lesions, while others had few to almost all of the lesions producing spores. To measure spore production, the five best sporulating lesions of each variety were selected using an 80X dissecting microscope. In some cases only two or three lesions had sporulated and spore production could be counted directly under a 100X microscope.

The five lesions were cut from the leaf with a scalpel, washed in a test tube with 1 ml of gelatin-sodium oleate solution, and the spores dislodged by shaking the test tubes with a Vortex Jr. shaker. The numbers of spores were given as the average of six aliquots taken with a Pasteur pipette and counted with the Spencer "bright line" hemacytometer. Results are given as the average of five lesions.

Inoculation of greenhouse plants

Greenhouse-grown plants were also inoculated in order to compare the reactions of inoculations of detached leaves with the reactions on plants in pots. The main objective of this work was to test as many

varieties and isolates as possible and then select those varieties showing lesion types 3 or 4 for use in tests with the detached leaf technique. However, due to the difficulties in growing rice plants, both tests had to be made simultaneously whenever healthy leaves were available.

Observations were also made on lesion types, lesion color, time of sporulation, number of spores produced and time of ingress.

For inoculation tests of greenhouse plants, seedlings were grown for 25 to 30 days under the conditions previously described. Six or eight pots, each representing one variety and containing seven seedlings, were sprayed with 20 ml suspension of conidia of one isolate. Inoculum concentration was the same as previously described. When more than one isolate was used simultaneously, the concentrations were adjusted as closely as possible. Inoculations were always made between 8:30 to 9:30 p.m., when greenhouse temperatures were lower and the relative humidity higher.

Inoculations were made using a DeVilbiss hand atomizer No. 127, attached to a General Electric 1/6 h.p. vacuum pump, with 10 pounds pressure, and with the nozzle held about 24 to 28 cm from the plants. Before inoculation, the seedlings were sprayed with about 10 ml of distilled water. Since the inoculations were made outside of the humid chamber, this extra spraying was done to prevent drying of the inoculum droplets before the plants were taken to the incubation chamber. Check plants were sprayed only with the gelatin-sodium oleate solution. Following inoculation, the seedlings were maintained in a plastic chamber

(1.20 m x 1.82 x 1.36 m high), which was built by covering a steel frame over a greenhouse bench with plastic. The chamber was divided in three sections using plastic so that three isolates could be tested at one time. The base of the chamber was covered with a plastic sheet to hold a nutrient solution (about 0.5 cm deep), in which the pots were left for 20 to 24 hours after inoculation. After all plants were inoculated, the plastic chamber was sealed and high inside humidity was maintained for 12 to 14 hours by producing a mist with a DeVilbiss atomizer attached to the vacuum pump and set for 15 pounds pressure. Twelve to 14 hours after inoculation, before the inside temperature went above 30 C, the chamber was partially opened, so that the cooler air could circulate inside. Temperatures in the moist chamber varied from 21 C at night to 30 C during the day. Although a constant high humidity was maintained throughout the incubation period, the maximum relative humidity recorded was around 96 percent. Following the high humidity period of 12 to 14 hours, the relative humidity in the chamber varied from 54 percent during the day to 86 percent during the night. Two days after inoculation, the plants were watered daily with 60 ml/pot of nutrient solution.

Determination of lesion types

Notes on lesion types were taken eight days after inoculation using the first five scale units of the international classification (15). Since the readings of greenhouse tests were taken at a shorter time after inoculation, and because greenhouse experiments had a higher inoculum concentration, and a more favorable condition for symptom devel-

opment than tests with detached leaves, the international classification was slightly modified and adapted for the greenhouse tests. In the international classification, the scale units 5 to 7 are based on lesion number and area affected. In these tests, the scale 5 is meant to include all the susceptible reactions beyond scale 4 (Table 3). The color of the lesions given indicate only the most predominant discolorations observed, and are based on the color charts of the Dictionary of Colors (13).

Determination of lesion color

Lesion color was determined eight days after inoculation, and the method was the same as for the tests using detached leaves.

Determination of time of sporulation

On the same day when notes were taken on lesion type, infected leaves which were fully expanded at the time of inoculation were detached and placed in Petri dishes with moist filter paper. The time of sporulation was then checked, considering the time of detaching as the 0 hour, and thereafter observations were made every hour, up to 10 hours, following at 12, 14, 16, 24, 30 and 48 hours. Notes were taken on time of first emergence of conidiophores and first appearance of conidia. Observations were made at room temperature and, between each observation, the plates containing the leaves were kept in the dark at 24° C.

Determination of number of spores produced

Forty-eight hours after infected leaves were detached, spore counts

were made by dissecting out the five best sporulating lesions of each variety and washing them in 1 ml of gelatin-sodium oleate solution in a test tube. Spores were dislodged by shaking the test tube with a Vortex Jr. shaker. Six aliquots of the spore suspension were taken with a Pasteur pipette and counted with a Spencer "bright-line" hemocytometer.

The number of sporulating lesions varied greatly from one variety to another and the criterion adopted was to take up to five of the best sporulating lesions of each variety, from a population of seven plants (in one 6-inch plastic pot). Each test was made with the same number of plants for all varieties. In some cases, few lesions had developed and only two to three sporulating lesions were available. The results are given based on the average number of spores per five lesions.

RESULTS

Lesion type

On both detached leaves and whole plants inoculated in the greenhouse a considerable variation in lesion types was observed even on the same variety inoculated with a single isolate (Table 4). The most susceptible lesion type was considered as representative of the actual reaction of the variety.

Lesion color

Smaller lesions generally had darker coloration, delayed sporulation, and reduced number of spores. A relationship of lesion color with other characteristics suggested that plants with lesions having green (surf green, olive green, pea green, opaline green or acacia) to gray

(cub, sandy beige and mineral gray) centers were more susceptible, had larger lesions, more rapid sporulation, greater number of spores formed, and a larger yellow margin. White to pinkish (ivory or white jade) centers occurred in many lesions of types 3, 4 and 5 and on these light-colored areas sporulation was delayed and fewer spores were formed. Lesions with wider black or purple (graphite) to dark brown (autumn and burnt umber) and brown (chipmunk, cinnamon, spice or talavera) areas had lesions with a center with restricted size (1 to 2 mm in diameter) and usually white to pinkish color. The lesion size was restricted by the yellow margin, sporulation was delayed, and there were fewer spores produced.

Detached leaves generally had more susceptible reactions than plants inoculated in the greenhouse with the same isolates. The lesions were darker in color among the resistant varieties in the detached leaf inoculation as compared to susceptible varieties. The center of lesions on detached leaves was mostly green to gray and generally had fewer dark areas. Except for lesion type 3, no white or pinkish coloration was present in the center of lesions inoculated by the detached leaf technique while this was common in lesion types 3, 4 and 5 in the greenhouse inoculations.

Size of lesions

Lesion size was only measured on detached leaves. Lesions formed on susceptible varieties and selections had a large yellow margin that often coalesced with the neighboring lesions. In all measurements the entire colored area of the lesions was considered. Results are given

in Table 5. Lesion size of varieties and selections showing only hypersensitive reactions did not enlarge beyond the area where the microdrops of inoculum had been placed. The lesions rarely measured more than 1 mm in diameter. Variation was observed within the same lesion type on the same variety when inoculated with different isolates. This may have been due to a varying number of lesions, which were selected as being typical and taken as the basis for classifying the lesion types of each variety and selection. In general, the results indicated that the average size of lesions increased with greater susceptibility (Table 6). Analysis of variance on size of lesions made between varieties Binato, Peta and Saturn, and the five isolates shown in Table 5, indicated a highly significant difference among isolates, among varieties and in the interaction variety X isolates. Each variety had 12 lesions measured (mm^2), representing 12 replicates out of 36 or 48 lesions developed on 12 leaf sections.

Inoculations made on the apical (A) and basal (B) portions of the same leaf of different varieties and isolates to determine whether a difference existed in susceptibility, indicated no difference on the resistant varieties. Differences in lesion size among varieties and selections Padma, T-141, TKM-6, Peta and Saturn (increasing order of susceptibility), using isolates 27, 59L13 and 68 T1, are shown in Table 7. These differences were highly significant for varieties and leaf positions A and B. Size of lesions on position A was significantly smaller than on position B. The average size of lesions on both leaf positions A and B increased with an increase in susceptibility (Table 8).

Considerable variation in size of lesion was observed among isolates.

Time of sporulation

Observations of sporulation on detached leaves were made daily, starting 3 days after inoculation and continuing for 10 days. No spores were formed on lesion type 1, only rarely on type 2, but spores were always observed on lesion types 3, 4, and 5, often as early as 4 days after inoculation on types 4 and 5. Usually, sporulation took longer on types 3 and 2.

The results of sporulation on seedlings inoculated in the greenhouse are more useful. A great variation in time of sporulation was observed in the same lesion type and on the same variety. Results on time of conidiophore and conidia formation are presented in Table 9. Conidiophores and conidia appeared as early as 1 hour after infected leaves were detached from the varieties Peta and Saturn inoculated with isolate 68L4. No conidiophores were formed 14 hours after leaves were detached and the longest period to produce conidia was 30 hours. Spores were not formed on lesion types 1 and rarely on type 2.

A comparison of range and average time of conidiophore and conidia formation with lesion types showed that lesion type 5 took the least amount of time to sporulate, and was followed by lesion types 4, 3 and 2 (Table 10). When conidiophores emerged four hours after leaves were placed in the moist chamber, conidia formation generally followed in one to three hours. When conidiophores took more than five hours to emerge, conidia formation was delayed from four to 20 hours (Table 9).

Number of spores produced

The number of spores produced increased with an increase in susceptibility, both in the greenhouse and detached leaf inoculations. On lesion types 3, 4, and 5, the number of spores varied greatly within the same lesion type. The results obtained with seedlings in the greenhouse are given in Tables 11 and 12.

Time of ingress

A study of time of ingress with the detached leaf technique failed to show notable differences among varieties and isolates. Likewise, in a greenhouse test, no relationship was observed between time of ingress and the other characteristics studied in the one test made; therefore, the results of these tests are not given.

DISCUSSION

One of the most difficult problems encountered in making this study was the diversity of lesion types observed on the varieties and selections used. This occurred even when a single isolate of P. oryzae was used. Such results might be expected when one considers the results of Ou et al (16). Kobayashi and Abumiya (11) found that higher concentrations of inoculum resulted in severe damage to the leaves, and that varieties differed in the number of lesions produced with varying inoculum concentrations. It is possible that the differences in size and inoculum concentrations of the droplets on the leaves may be the explanation for the highly resistant reactions which were observed along with highly susceptible reactions on the same leaf.

Reports on the actual size of lesions and their relationship with

color, shape and sporulation was not found in the literature. References in the literature to size of lesions and their relationship to resistance or susceptibility were usually made as "small" or "large" lesions. Hashioka (4) noted that when lesions were large with typical fusiform shape and dark gray centers, sporulation was most abundant; and that a decrease in size resulted in less sporulation and increased dark color of the lesions. Similar results were obtained in this study.

It was found that the intensity and distribution of lesion colors may be a good indication of the degree of resistance of the rice plant to the blast fungus. Differences in color of lesions observed between detached leaf and greenhouse inoculations should be studied more carefully. Study should also be made on whether or not the color and other characteristics observed on detached leaves actually indicate the reaction patterns that are expressed by plants exposed to greenhouse or field inoculations.As mentioned by Kobayashi and Abumiya (11), inoculum concentration affects disease severity and may also alter the color intensity of the lesions. In future studies it would be useful to standardize, as well as possible, the concentration of inoculum in the droplets of spore suspension applied on detached leaves and also the inoculum used for field or greenhouse inoculations. The lighter color and higher susceptibility observed in detached leaf inoculations may be related to higher concentration of inoculum in each droplet.

Toyoda and Suzuki (20) observed that sporulation on greenhouse plants was more rapid and abundant on lesion type 4, less on 5 and 3, and spores were sparsely formed on 2 and were absent on type 1. The

present study indicates that the average time (hour) of sporulation on inoculated greenhouse plants was shorter on lesion type 5 with more abundant spores being formed. Also in contrast to Toyoda and Suzuki (20), the number of spores produced was highest in lesion type 5 when detached leaves were inoculated.

Sporulation was more abundant on detached leaves in Petri plates than on leaves inoculated in the greenhouse and then detached. Detached leaves also gave a more susceptible reaction. This may have been caused by the yellowing which began from the cut ends within four to five days. In many instances the leaves were entirely yellow before any symptoms developed. A more valid reaction would undoubtedly be obtained with leaves from healthy plants.

Differences in susceptibility between the apical and the basal portion of the last fully developed leaves could be related to tissue age. The leaf unfolds or emerges from the tips, thus the basal section has the youngest tissue. This observation agrees with that of Kato et al (10) that sporulation was greater when leaves were inoculated at the middle stage of expansion. Kahn and Libby (9) reported that younger upper leaves were the most susceptible, with resistance increasing upward. For future tests it would appear that only the basal half of the youngest, but most fully developed, leaf should be used for the detached leaf inoculation. No relationship was observed between time of ingress and susceptibility. The experiments made during this study were not replicated and should be repeated with additional varieties before definite conclusions can be drawn.

It should be pointed out that the present studies were conducted under conditions unfavorable for normal rice plant development. Plant chlorosis and other physiologic disorders, referred to as "winter sickness", were constant problems. Therefore, experiments should be made with healthier plants before conclusions can be made regarding the relationships among the characteristics observed. Experiments should be repeated with healthy plants, grown together under identical conditions and inoculated with the same spore suspension. Since physiological disorders in rice plants seem to be a common problem in greenhouse cultures (12), experimental results should be confirmed with healthy plants grown in the field.

No conclusions can be made as to what might represent general resistance in rice to P. oryzae from the results of these studies. In addition, the results obtained were highly variable. Nevertheless, it has been demonstrated that the characteristics considered can actually be measured, and the study may serve as a valuable source of information for further studies.

SUMMARY

A study of certain factors which may affect general resistance in rice to Pyricularia oryzae Cav. was made in the laboratory by inoculating detached leaves in Petri dishes and by inoculating potted plants in the greenhouse. The inoculation of potted plants in the greenhouse was made primarily in order to find varieties with lesion types 3 and 4. Unfortunately, plants grew poorly so that studies with detached leaves and

greenhouse inoculations had to be made simultaneously with a limited number of varieties and isolates. Inoculum of P. oryzae was initially produced on 2 percent rice polish agar, but culture on fresh, sterilized, crushed leaves gave better results and inoculum from this source was used during the rest of the study.

General resistance in rice to P. oryzae might be expressed through reduced size of lesions, delayed time of sporulation, reduced number of spores, delayed time of ingress, and specific color of lesions. Thus the factors studied as possible indicators of general resistance were: a) size of lesion, b) color of lesions, c) time of sporulation, d) number of spores produced, and e) time of ingress. Attempts were made to find relationships among these characteristics which would express general resistance. Tests were also made to determine the differences in susceptibility between the apical and basal portions of the youngest, but fully expanded leaves.

Even using a single isolate, a wide variation in lesion types occurred on the same variety in both the detached leaf and greenhouse inoculations. Reactions varied from highly resistant to highly susceptible. The size of lesions measured on detached leaves also showed extreme variation among and within the same varieties with different isolates. In general, size of lesions increased with an increase in susceptibility.

Smaller lesions generally had darker coloration, delayed sporulation, and reduced number of spores. A relationship of lesion color with

other characteristics suggested that plants with lesions having green (surf green, olive green, pea green, opaline green or acacia) to gray (cub, sandy beige and mineral gray) centers were more susceptible, had larger lesions, more rapid sporulation, greater number of spores formed, and a larger yellow margin. White to pinkish (ivory or white jade) centers occurred in many lesions of types 3, 4 and 5 and on these light-colored areas sporulation was delayed, and fewer spores were formed. Lesions with wider black or purple (graphite) to dark brown (autumn and burnt umber) and brown (chipmunk, cinnamon, spice or talavera) areas had lesions with a center with restricted size (1 to 2 mm in diameter), and usually white to pinkish color. The lesion size was restricted by the yellow margin, sporulation was delayed, and there were fewer spores produced.

Detached leaves generally had more susceptible reaction than plants inoculated in the greenhouse with the same isolates. The lesions were darker in color among the resistant varieties in the detached leaf inoculation as compared to susceptible varieties. The center of lesions on detached leaves was mostly green to gray and generally had fewer dark areas. Except for lesion type 3, no white or pinkish coloration was present in the center of lesions inoculated by the detached leaf technique, while this was common in lesion types 3, 4 and 5 in the greenhouse inoculations.

Time of conidiophore and conidia formation in the greenhouse inoculations showed variation in the same lesion type among varieties. In general, the time to produce spores increased with increased resistance.

Conidiophores were rarely formed in less than 4 hours under high humidity in susceptible to highly susceptible varieties. When conidiophores were formed within 4 to 5 hours under high humidity, conidial formation followed 1 to 7 hours later. Conidial formation was frequently delayed when conidiophores took more than 6 hours to emerge.

No differences were noted between lesion types 4 and 5 in regard to time of sporulation on detached leaves. Sporulation took longer on lesion types 3 and 2. Sporulation rarely occurred on lesion type 2.

The number of spores produced increased with an increase in susceptibility, both in the greenhouse and detached leaf inoculations. On lesion types 3, 4 and 5, the number of spores varied greatly within the same lesion type. In general, sporulation was more abundant when detached leaves were inoculated.

Inoculations of the apical and basal portions of the same leaf, with the same isolate, indicated that the basal portion was more susceptible. Lesions were much larger, sporulation was faster, and a larger number of spores were produced on the basal portions. No differences were observed among the highly resistant varieties between the apical and basal portions of the same leaf.

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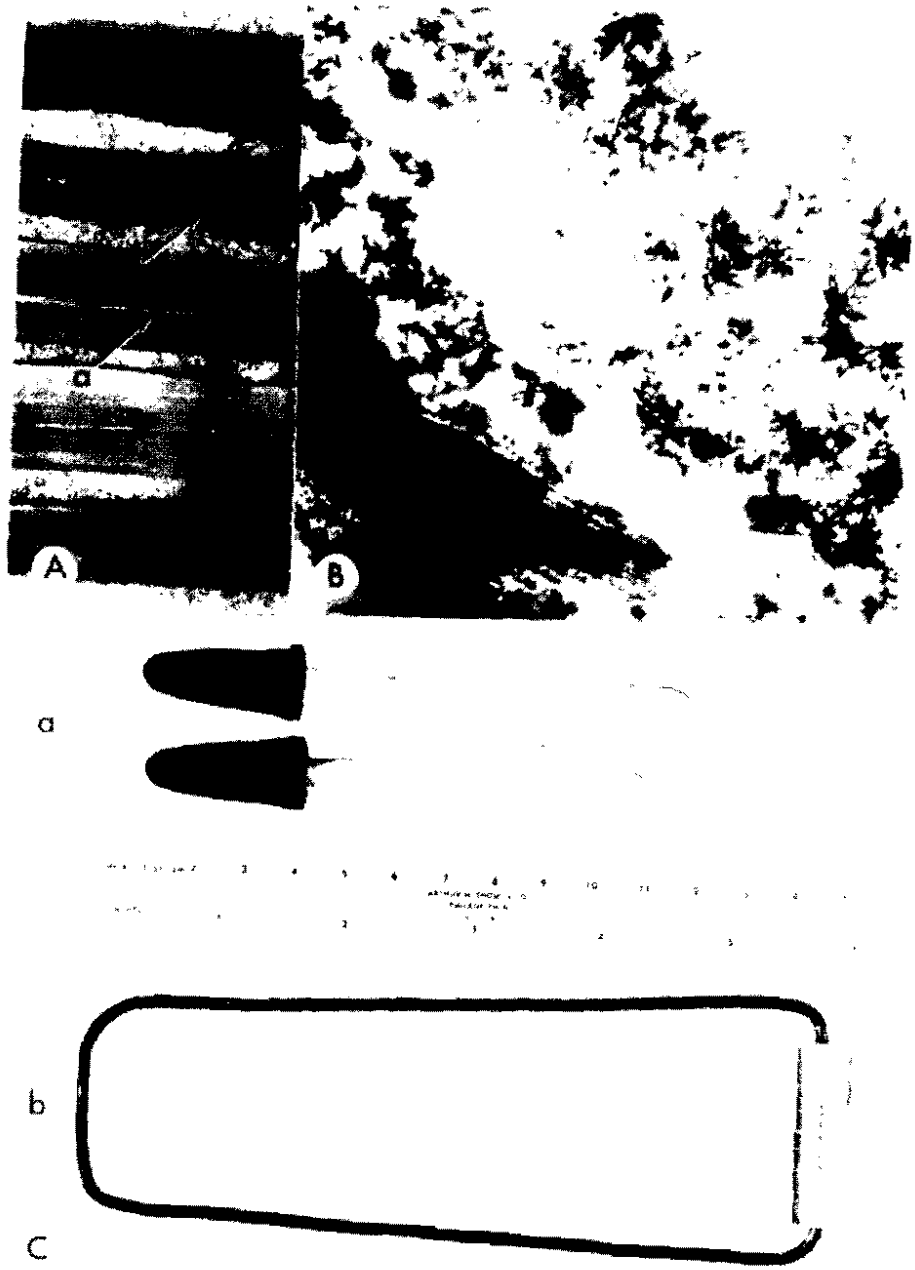

selection [illegible] inoculated with P. oryzae [illegible]
[illegible] reactions (a) and injury area where high [illegible]
[illegible] Sporulation [illegible] the injured area (b)
[illegible] used for inoculation of detach[illegible]
[illegible] used for crushing the rice leaves [illegible]

Table 1. List of colors observed on lesions caused by P. oryzae on rice leaves inoculated in Petri dishes and the greenhouse.

Symbols	Standard 1/ color	Key to the Dictionary a	b	c 3/	Common 2/ colors
Olg	Olive green	22	B	6	
Opg	Opaline green	17	A	6	
Pg	Pea green	20	G	6	Green
Sg	Surf green	20	C	7	
Fl	Flax	12	B	2	
Ac	Acacia	11	K	1	Grayish-green
Cu	Cub	15	C	1	
Sb	Sandy Beige	14	A	3	Gray
Mg	Mineral gray	20	A	2	
Wj	White jade	10	A	2	Light pink
Iv	Ivory	10	B	2	
W	White				White
Gr	Graphite	48	C	7	Black, purple
Au	Autumn	8	A	12	Dark brown
Bu	Burnt umber	15	A	12	
Ch	Chipmunk	13	L	9	
Cm	Cinnamon	12	E	7	Brown
Sp	Spice	13	A	12	
Tv	Talavera	12	A	12	
Ap	Apricot	10	F	7	Reddish-brown
Gl	Gold leaf	11	K	8	
My	Martius yellow	9	I	1	Yellow
Lcy	Light chrome yellow	10	L	4	
Sy	Spruce yellow	12	K	8	

1/ According to the Dictionary of Colors (13).

2/ Common names cited in the literature.

3/ a) Plate number, b) Column, and c) Line

Table 2. Five scale units of disease reaction on detached rice leaves used in the classification of lesion types caused by P. oryzae.

Scale units	Lesion types 1/
1	Lesions limited to the site of inoculation; graphite (Gr) to burnt umber (Bu); no necrotic center; no yellow margin formed.
2	Restricted lesion, about 2-3 mm long; graphite (Gr), autumn (Au) to burnt umber (Bu) center; spice (Sp), gold leaf (Gl) to martius yellow (My) margin; no necrotic center.
3	Lesions up to 6 mm long; small (2-3 mm) opaline green (Opg), pea green (Pg), olive green (Olg), to ivory (Iv) necrotic center; thick graphite (Gr), autumn (Au) burnt umber (Bu) to spice (Sp) zone; and gold leaf (Gl) to martius yellow (My) margin.
4	Lesions up to 10 mm long; frequently with pea green (Pg), olive green (Olg) to mineral gray (Mg) necrotic center; burnt umber (Bu) and occasionally graphite (Gr) zone; gold leaf (Gl), martius yellow (My) to light chrome yellow (Lcy) margin.
5	Lesions more than 10 mm long; pea green (Pg), opaline green (Opg) to olive green (Olg) center; rarely with burnt umber (Bu) zone; spice (Sp), gold leaf (Gl), light chrome yellow (Lcy) to martius yellow (My) margin.

1/ Color of the lesions are from the center toward the margin.

Table 3. Five scale units of disease reaction on rice plants inoculated in the greenhouse, used in the classification of lesion types caused by P. oryzae.

Scale units	Lesion types 1/
1	Only small, brown (autumn to spice) specks, few or many with no necrotic (collapsed cell) spots.
2	Slightly larger (2-3 mm in diam), graphite (Gr), autumn (Au) to spice (Sp) spots, with gold leaf (Gl) margin; no necrotic (collapsed cell) spots.
3	Small, roundish, necrotic, gray (Cu or Sb) to olive green (Olg) center (about 1-2 mm in diam); surrounded by graphite (Gr), autumn (Au) to spice (Sp) and gold leaf (Gl) margin, which is somewhat elliptical.
4	Typical blast lesion, elliptical, somewhat restricted (up to 6 mm long), with large necrotic, gray (Cu or Sb), olive green (Olg) to opaline green (Opg), and sometimes white center; graphite (Gr), autumn (Au), spice (Sp) to gold leaf (Gl) margin.
5	Lesions larger and broader than in scale 4 (more than 6 mm long); large gray (Cu or Sb), olive green (Olg), opaline green (Opg) to white center; graphite (Gr) to autumn (Au) not always present, mostly spice (Sp) to gold leaf (Gl) margin; upper portion of seedling leaves may be killed by coalescence of large lesions.

1/ Adapted from the international classification for field and nursery tests (15).

Table 4. Range of lesion types on detached leaves of 13 rice varieties inoculated with five isolates of *P. oryzae*.

Varieties and selections	Isolate number and lesion types 1/				
	US5	27	59L13	68L4	68T1
IR8	1	1	- 2/	-	-
Taichung (Native 1)	1	1	-	-	-
IR5-47-2	1 - 2	1	1 - 2	1	-
Fortuna	1 - 2	-	1 - 2	-	-
Padma	-	1 - 3	1 - 2	1 - 2	-
T-141	-	1 - 3	1	1	-
T-Km6	-	1 - 3	1 - 2	1 - 3	-
PI215-936	1 - 3	1	-	-	-
IR154-61-1	1 - 3	-	1 - 3	1 - 3	1 - 4
Bluebelle	1 - 3	1	-	-	-
Bluebonnet 50	-	-	1 - 4	1 - 4	1 - 4
Peta	1 - 4	1 - 2	1 - 3	1 - 3	1 - 2
Binato	2 - 5	1 - 3	1 - 4	1 - 4	2 - 5
Saturn	1 - 3	1	1	3 - 5	2 - 5

1/ Lesion types: 1) Highly resistant; 2) Resistant; 3) Moderately resistant; 4) Susceptible; 5) Highly susceptible.

2/ Materials not available

Table 5. Size of lesions (mm^2), measured eight days after inoculation of detached leaves of rice with P. oryzae.[1/]

Varieties and selections	Isolate no. and size of lesions (mm^2) [2/]				
	US5	27	59 L 13	68 L 4	68 T 1
Padma	-	2.87 (3)[3/]	1.07 (2)	1.06 (2)	- [4/]
T-141	-	3.78 (3)	1.00 (1)	0.77 (1)	-
TYM-6	-	5.11 (3)	1.86 (2)	7.11 (3)	-
IR154-61-1-1	9.37 (3)	-	2.77 (3)	1.45 (3)	4.22 (4)
Bluebonnet 50	-	-	13.42 (4)	14.26 (4)	13.25 (4)
Peta	5.70 (4)	3.09 (2)	11.37 (3)	6.33 (3)	1.14 (2)
Binato	25.74 (5)	6.25 (3)	8.08 (4)	12.70 (4)	16.90 (5)
Saturn	1.53 (3)	1.00 (1)	1.00 (1)	29.99 (5)	25.46 (5)

[1/] Varieties, isolates and interaction differed at 1% level (see Table 10).

[2/] Average of 12 lesions/isolate.

[3/] Number in parentheses indicates the lesion type.

[4/] Not tested.

Table 6. Range and average size of lesions (mm^2) of each lesion type on detached leaves.

Lesion type	Size of lesion (mm^2) 1/	
	Range	Average
1	0.77 - 1.00	0.99
2	1.06 - 3.09	1.84
3	1.71 - 11.37	5.07
4	4.22 - 14.26	10.22
5	16.90 - 29.99	24.52

1/Average of 12 lesions/isolate.

Table 7. Comparison between size of lesions on detached leaves, of the apical (A) and basal (B) positions of the same leaf, inoculated with P. oryzae.[1/]

Varieties and selections	Leaf pos.	Isolate number and size of lesions (mm^2) [2/]				
		US5	27	59L13	68L4	68T1
Padma	A	- [3/]	1.00	1.00	1.00	-
	B		4.66	1.14	1.12	
T-141	A	-	6.14	1.00	0.92	-
	B		1.43	1.21	0.63	
TKM-6	A	-	0.95	1.07	0.64	-
	B		9.28	2.98	14.29	
IR154-61-1-1	A	9.37 [4/]	-	1.50	1.45	1.91
	B		-	4.04		6.54
Bluebonnet 50	A	-	-	5.75	7.52	9.66
	B		-	24.50	21.00	16.85
Peta	A	5.59	1.01	6.75	4.91	1.19
	B	5.82	5.17	16.00	7.75	1.00
Binato	A	25.74	6.25	10.08	8.43	14.66
	B			6.08	16.97	19.25
Saturn	A	1.53	1.00	1.00	21.12	20.22
	B		1.00	1.00	36.87	31.70

[1/] Varieties, isolates, leaf positions and interactions differed at 1% level (see Table 12).

[2/] Average of 12 lesions/leaf position/isolate.

[3/] Not tested.

[4/] Only one leaf section/leaf was inoculated.

Table 8. Range and average size of lesions (mm^2) on detached leaves (leaf position A and B), in relation to lesion types.

Lesion type	Leaf pos.	Size of lesion (mm^2) Range	Average
1	A	0.92 - 1.00	0.98
	B	0.63 - 1.21	0.96
2	A	1.00 - 1.19	1.04
	B	1.00 - 4.66	2.67
3	A	0.64 - 6.75	3.44
	B	1.43 - 16.00	8.79
4	A	1.91 - 10.08	6.99
	B	5.82 - 24.50	13.96
5	A	14.66 - 21.12	18.76
	B	19.25 - 36.87	29.27

Table 9. Time(hour) of conidiophore and conidia formation by P. oryzae on rice leaves detached eight days after inoculation in the greenhouse and observed in moist Petri dishes for 48 hours.

Varieties and selections	Isolate number and time (hr) of conidiophore and conidia formation															
	US5		27		59L13		68A10		68A12		68A14		68L4		68T1	
	a	b [1]	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Padma	x [2]		x		4	10	4	9	10	14	5	12	x		10	14
IR154-61-1-1	6	14	6	14	- [3]		-		-		-		-		-	
PI215-936	x		x		x		x		5	14	4	12	x		5	12
T-141	x		10	14	x		8	14	14	24	x		x		4	7
Saturn	6	10	6	24	x		x		x		x		1	1	4	5
Peta	2	6	x		4	8	x		x		x		1	1	x	
Binato	10	16	12	16	x		x		x		x		x		4	5
TKM-6	-		10	14	10	30	5	9	4	7	5	10	10	14	4	7

[1] a) Time of conidiophore formation; b) Time of conidia formation.

[2] Neither conidiophores nor conidia formed.

[3] Not tested.

Table 10. Relationship of lesion types, range and average time (hour) of conidiophore and conidia formation in P. oryzae, produced on rice leaves detached eight days after inoculation in the greenhouse and kept in moist Petri dishes for 48 hours.

Lesion type	Conidiophore		Conidia	
	range hr	average hr	range hr	average hr
1	x 1/	x	x	x
2	8 - 14	11.06	14 - 24	18.00
3	4 - 12	7.16	3 - 30	14.41
4	1 - 6	4.55	1 - 24	11.00
5	1 - 5	3.43	1 - 10	5.85

1/ No spores formed.

Table 11. Number of conidia of eight isolates of P. oryzae produced on rice leaves, detached eight days after inoculation in the greenhouse and 48 hours after being placed in moist Petri dishes.

Varieties and selections	Isolate number and number of conidia produced 1/							
	US5	27	59L13	68A10	68A12	68A14	68L4	68T1
	1000X	1000X	1000X	1000X	1000X	1000X	1000X	1000X
Padma	0	0	1.66	9.33	1.33	0.66	0	2.50
IR154-61-1-1	1.80	0.55(4) 2/	-	- 3/	-	-	-	-
PI215-936	0	0	0	0	0.33	0.16	0	5.33
T-141	0	0.07(3)	-	0.07(3)	0.10	0	0	18.00
Saturn	6.20	0.26	0	0	0	0	6.14	20.20
Peta	2.00	0	1.75	0	0	0	11.80	0
Binato	0.05	0.14	0	0	0	0	0	18.33
TKM-6	-	0.03(2)	0.10	9.00	4.50	3.12	0.50	2.70

1/ Results are the average of five best sporulating lesions selected under 80X dissecting microscope.

2/ Numbers in parentheses indicate number of sporulating lesions that were available.

3/ Not tested.

Table 12. Relationship of lesion types with range and average number of conidia of eight isolates of P. oryzae produced on rice leaves, eight days after inoculation in the greenhouse and 48 hours after being placed in moist Petri dishes.

Lesion type	Number of conidia	
	Range	Average
1	0	0
2	50 - 100	73
3	30 - 2500	669
4	160 - 9330	4447
5	2000 - 20200	10878

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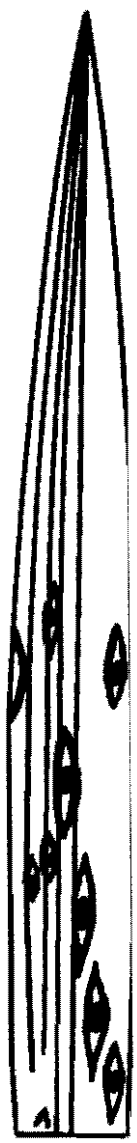

FACTORS WHICH MAY AFFECT GENERAL RESISTANCE IN RICE TO PYRICULARIA ORYZAE CAV.

Jose Tadashi Yorinori
and
H. David Thurston

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

FACTORS WHICH MAY AFFECT GENERAL RESISTANCE IN RICE TO *Pyricularia oryzae* Cav.

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Rice blast, caused by *Pyricularia oryzae* Cav., is perhaps the single most destructive disease of rice. Probably half of the world's rice is grown in the tropics (8). In temperate areas where rice is grown such as Japan, chemical control has been emphasized. Although effective chemical control is available for temperate zones, rice blast control in the tropics is limited by social, economic and environmental conditions, and economic chemical control for the tropics is not yet available. Thus, the development of rice varieties resistant to *P. oryzae* is essential.

No single rice variety has shown resistance to all races of the pathogen and, in addition, the fungus has proven to be highly variable, with a single conidium capable of producing many races (16). Nevertheless, the major sources of resistance to *P. oryzae* utilized in commercial plantings is simply or qualitatively inherited. Such specific or vertical resistance is rapidly lost with the appearance of new races. General or horizontal resistance has been shown to be stable and is not lost with the appearance of new races. Galvez *et al* (3), Nagai *et al* (14) and more recently, Ou *et al* (17), have shown that such resistance is

available in rice. If varieties can be bred with such resistance, the resistance might be maintained indefinitely and not lost with the appearance of new races of the pathogen.

This study was made during 1969-1970 and presented as a M.S. thesis in January 1971 by the senior author (21). The object of the study was to determine if consistent differences in characteristics which have been shown to be associated with general resistances in other crops such as potatoes (19) could be found in rice inoculated with P. oryzae. These characters might be useful in measuring and identifying relative levels of general resistance in rice. Characters studied were a) size of lesions, b) color of lesions, c) time for sporulation, d) number of spores produced, and e) time of ingress.

MATERIALS AND METHODS

Culture of rice plants

A serious problem in this study was growing healthy rice seedlings in the greenhouses and controlled climate chambers. Iron deficiency was a major problem and varietal susceptibility to the deficiency varied among the varieties used. Another problem, similar to what Latterell et al (12) called "winter sickness", was also common.

Numerous soil mixtures and environmental conditions were used in the attempt to grow healthy rice seedlings. The best success was obtained when seedlings were grown in a modification of the nutrient solutions of Hoagland and Arnon (5) and Tanaka et al (18) for about 10 days and then transplanted to 4-inch plastic pots containing fine white sand. The

potted seedlings were watered daily with 60 ml of nutrient solution per pot. Seedlings were grown in a greenhouse with a temperature variation of 26 to 38 C.

Culture of P. oryzae

The isolates of P. oryzae used in the study were received from Dr. J.G. Atkins, U.S.D.A., Beaumont, Texas; Dr. C.R. Adair, U.S.D.A., Beltsville, Md., and from Dr. W.N. Harnish, Niagara Chemical Division, Middleport, New York. Cultures were maintained by monthly transfers on 2 percent rice polish agar slants containing one or two pieces of rice nodes. For initial tests, inoculum was prepared using the techniques of Latterall et al (12). Of several artificial media tested, rice polish agar (12) gave the best results, but many isolates sporulated poorly and with continued transfers many isolates had a marked decrease in sporulation. An observation that profuse sporulation occurred when conidia germinated on an injured part of the rice leaf led to the idea that the fungus could be grown and inoculum produced on fresh crushed leaves after surface sterilization (Fig. 1). The youngest fully developed leaves of 30 to 40 day-old greenhouse-grown plants were cut into lengths 5 to 6 cm long. The sections were surface-sterilized by dipping into a 50:50 solution of alcohol and sodium hypochlorite for one minute, and then were washed in running water for 10-15 min in a 500 ml beaker covered with cheese cloth. Four or five leaf sections were stretched on a filter paper in a Petri dish moistened with 2 ml of 0.01 M monosodium phosphate and 70 ppm benzimidazole (6). Leaves were crushed by

rolling them with an aluminum cylinder (1.3 cm x 3.5 cm long) (Fig.1). The leaves were inoculated by taking a small piece of the fungus grown on rice polish agar and rubbing it on the leaf, after which they were placed in the dark at 24 C. Most isolates produced spores three days after inoculation, and by the fourth day sporulated profusely (Fig. 1). Spore suspensions for inoculation were prepared by cutting the sporulating leaves into sections of about one cm long, placing them in test tubes with 2 to 3 ml sodium oleate-gelatin solution (1), and dislodging the spores by shaking the test tubes with a Vortex Jr. shaker.

For subsequent transfers pieces of the sporulating leaf were rubbed on newly prepared leaves. Using this procedure, it was possible to grow the fungus continually on fresh rice leaf tissue. Leaves of the varieties Peta, Saturn, Taichung Nativel, Binato and IR5-47-2 were used as the substrate. The varieties Taichung Native 1 and IR5-47-2 were highly resistant (lesion types 1 and 2) (7) to most of the isolates we have used in tests with excised leaves or whole plant inoculation, but abundant sporulation was observed when leaves of these varieties were used for culturing the fungus. The fungus grows better on the upper two-thirds of the younger leaves. Older and basal portions of the leaves became brown to yellow within two to three days after crushing, and the fungus sporulated poorly. To obtain good sporulation it is necessary that leaves maintain a green color four days or more after crushing.

When sporulating leaves were dried 5 to 7 days after inoculation and stored at 3 C, spores were viable up to one week. All isolates had maximum sporulation by the fifth to sixth day, but beyond this period,

if leaves were kept moist, the spores fell off and germinated. For inoculation purposes spores should be harvested 4-6 days after inoculation. Stocks of fresh rice leaves were maintained in excellent condition for over 20 days in the refrigerator at 3 C. Leaves were detached, washed thoroughly in tap water, wrapped in soaked paper towels, and stored in plastic bags.

Inoculation of detached leaves

Inoculation with microdrops. The detached leaf technique, described by Hsu and Ou (6), was used with a slight modification in an attempt to find a method of inoculation whereby size, color of lesions, time of sporulation, number of spores produced, and time of ingress could be accurately measured.

In order to test as many isolates and varieties as possible at the same time, the following experimental design was adopted. One isolate was used to inoculate the twelve youngest, fully expanded leaves detached from twelve 25 to 30 day-old plants. From each leaf, a 6 cm long section was cut from the middle, widest area of the leaf, and placed in a Petri dish on moist filter paper. Six leaf sections were placed in one Petri dish.

To determine what part of the leaf is most suitable for the detached leaf inoculation method, tests were also made by inoculating the upper (A) and the lower (B) half parts of the same leaf. In this case eight leaf sections from four leaves were placed in each Petri dish, and three dishes were inoculated with one isolate.

Inoculations were made with a special microdrop pipette made by stretching a Pasteur pipette until a fine needle of 250 to 300 u in diameter was obtained (Fig. 1). A small eyedropper rubber was fitted on the other end. Inoculation was accomplished by placing three or four droplets (about 1 ul) on the upper surface of each leaf section. Thus, each variety was inoculated with 36 to 48 microdrops. Due to the high surface tension of the droplets on the waxy surface of the rice leaves the droplets tended to remain attached to the tip of the needle or roll off when too large. This was solved by coating the tip of the needle with a thin layer of vaseline, which tended to repel the droplets. Placement of droplets was also made easier by preparing the spore suspension with a solution containing Tween 20 (0.20 ml/l), or a gelatin-sodium oelate solution used as wetting agents. The later was more effective.

To reduce the variation in spore number in each droplet, due to settling, the eyedropper rubber was always partially compressed and, after three to four droplets were applied, a small amount of air was allowed to enter the pipette, thus mixing the suspension. One droplet of spore suspension was placed on each leaf section at a time. Inoculated leaves were incubated in the dark at a constant temperature of 21 (+ 0.5 C) or 24 (+ 1.0 C). Between 16 to 20 hours after inoculation, the plates were removed to the laboratory at room temperature and the droplets dried for 20 to 30 minutes under a fan after which the plates were again returned to the incubator.

Determination of lesion types

In general, the classification of lesion types was made according to the international classification, as proposed by Ou (15) at the Symposium on Rice Blast Disease. The criteria for rating resistance and susceptibility on detached leaves was slightly changed. The lesion types were classified in five scale units and were primarily based on color, relative size of lesions, and on presence or absence of a necrotic center. For standard colors, the Dictionary of Colors (13) was used. A list of colors used is given in Table 1. The necrotic center (area of collapsed cells), as mentioned in the international classification (15), corresponds to the central area of the lesions with a gray (Cu, Sb), opaline green (Opg), olive green (Olg), white (W) to pinkish (Iv, Wj) color which is surrounded by a ring of dark discolored cells. Table 2 gives the five scale units used and the characteristics of each scale.

Determination of lesion size

Lesion size was measured only once, eight days after inoculation. The criteria adopted for the measurements were as follows: six sections were chosen at random from each variety with 12 leaf sections inoculated; of these, two lesions, the largest and the smallest, were measured. The length and width of each lesion was measured and multiplied, and the result given in mm^2. The results were then presented as the average size (mm^2) of twelve lesions. Likewise, when the apical (A) and the basal (B) portions of the same leaf were inoculated, twelve leaf sections of each leaf position were inoculated. Recordings of size were made as described above, first, separately for each leaf portion (A and

B), and second, as the average between both.

Determination of time of sporulation

Observations on the time of sporulation were made daily, from the third day after inoculation, and up to the tenth day. Notes were taken on the first appearance of conidia using a microscope at 100X. After each observation the plates were put back into the incubator.

Determination of number of spores produced

The number of spores produced was determined 10 days after inoculation. Great variation in lesion types within and between varieties was observed. Some varieties had no sporulating lesions, while others had few to almost all of the lesions producing spores. To measure spore production, the five best sporulating lesions of each variety were selected using an 80X dissecting microscope. In some cases only two or three lesions had sporulated and spore production could be counted directly under a 100X microscope.

The five lesions were cut from the leaf with a scalpel, washed in a test tube with 1 ml of gelatin-sodium oleate solution, and the spores dislodged by shaking the test tubes with a Vortex Jr. shaker. The numbers of spores were given as the average of six aliquots taken with a Pasteur pipette and counted with the Spencer "bright line" hemacytometer. Results are given as the average of five lesions.

Inoculation of greenhouse plants

Greenhouse-grown plants were also inoculated in order to compare the reactions of inoculations of detached leaves with the reactions on plants in pots. The main objective of this work was to test as many

varieties and isolates as possible and then select those varieties showing lesion types 3 or 4 for use in tests with the detached leaf technique. However, due to the difficulties in growing rice plants, both tests had to be made simultaneously whenever healthy leaves were available.

Observations were also made on lesion types, lesion color, time of sporulation, number of spores produced and time of ingress.

For inoculation tests of greenhouse plants, seedlings were grown for 25 to 30 days under the conditions previously described. Six or eight pots, each representing one variety and containing seven seedlings, were sprayed with 20 ml suspension of conidia of one isolate. Inoculum concentration was the same as previously described. When more than one isolate was used simultaneously, the concentrations were adjusted as closely as possible. Inoculations were always made between 8:30 to 9:30 p.m., when greenhouse temperatures were lower and the relative humidity higher.

Inoculations were made using a DeVilbiss hand atomizer No. 127, attached to a General Electric 1/6 h.p. vacuum pump, with 10 pounds pressure, and with the nozzle held about 24 to 28 cm from the plants. Before inoculation, the seedlings were sprayed with about 10 ml of distilled water. Since the inoculations were made outside of the humid chamber, this extra spraying was done to prevent drying of the inoculum droplets before the plants were taken to the incubation chamber. Check plants were sprayed only with the gelatin-sodium oleate solution. Following inoculation, the seedlings were maintained in a plastic chamber

(1.20 m x 1.82 x 1.36 m high), which was built by covering a steel frame over a greenhouse bench with plastic. The chamber was divided in three sections using plastic so that three isolates could be tested at one time. The base of the chamber was covered with a plastic sheet to hold a nutrient solution (about 0.5 cm deep), in which the pots were left for 20 to 24 hours after inoculation. After all plants were inoculated, the plastic chamber was sealed and high inside humidity was maintained for 12 to 14 hours by producing a mist with a DeVilbiss atomizer attached to the vacuum pump and set for 15 pounds pressure. Twelve to 14 hours after inoculation, before the inside temperature went above 30 C, the chamber was partially opened, so that the cooler air could circulate inside. Temperatures in the moist chamber varied from 21 C at night to 30 C during the day. Although a constant high humidity was maintained throughout the incubation period, the maximum relative humidity recorded was around 96 percent. Following the high humidity period of 12 to 14 hours, the relative humidity in the chamber varied from 54 percent during the day to 86 percent during the night. Two days after inoculation, the plants were watered daily with 60 ml/pot of nutrient solution.

Determination of lesion types

Notes on lesion types were taken eight days after inoculation using the first five scale units of the international classification (15). Since the readings of greenhouse tests were taken at a shorter time after inoculation, and because greenhouse experiments had a higher inoculum concentration, and a more favorable condition for symptom devel-

opment than tests with detached leaves, the international classification was slightly modified and adapted for the greenhouse tests. In the international classification, the scale units 5 to 7 are based on lesion number and area affected. In these tests, the scale 5 is meant to include all the susceptible reactions beyond scale 4 (Table 3). The color of the lesions given indicate only the most predominant discolorations observed, and are based on the color charts of the Dictionary of Colors (13).

Determination of lesion color

Lesion color was determined eight days after inoculation, and the method was the same as for the tests using detached leaves.

Determination of time of sporulation

On the same day when notes were taken on lesion type, infected leaves which were fully expanded at the time of inoculation were detached and placed in Petri dishes with moist filter paper. The time of sporulation was then checked, considering the time of detaching as the 0 hour, and thereafter observations were made every hour, up to 10 hours, following at 12, 14, 16, 24, 30 and 48 hours. Notes were taken on time of first emergence of conidiophores and first appearance of conidia. Observations were made at room temperature and, between each observation, the plates containing the leaves were kept in the dark at 24^{o} C.

Determination of number of spores produced

Forty-eight hours after infected leaves were detached, spore counts

were made by dissecting out the five best sporulating lesions of each variety and washing them in 1 ml of gelatin-sodium oleate solution in a test tube. Spores were dislodged by shaking the test tube with a Vortex Jr. shaker. Six aliquots of the spore suspension were taken with a Pasteur pipette and counted with a Spencer "bright-line" hemocytometer.

The number of sporulating lesions varied greatly from one variety to another and the criterion adopted was to take up to five of the best sporulating lesions of each variety, from a population of seven plants (in one 6-inch plastic pot). Each test was made with the same number of plants for all varieties. In some cases, few lesions had developed and only two to three sporulating lesions were available. The results are given based on the average number of spores per five lesions.

RESULTS

Lesion type

On both detached leaves and whole plants inoculated in the greenhouse a considerable variation in lesion types was observed even on the same variety inoculated with a single isolate (Table 4). The most susceptible lesion type was considered as representative of the actual reaction of the variety.

Lesion color

Smaller lesions generally had darker coloration, delayed sporulation, and reduced number of spores. A relationship of lesion color with other characteristics suggested that plants with lesions having green (surf green, olive green, pea green, opaline green or acacia) to gray

(cub, sandy beige and mineral gray) centers were more susceptible, had larger lesions, more rapid sporulation, greater number of spores formed, and a larger yellow margin. White to pinkish (ivory or white jade) centers occurred in many lesions of types 3, 4 and 5 and on these light-colored areas sporulation was delayed and fewer spores were formed. Lesions with wider black or purple (graphite) to dark brown (autumn and burnt umber) and brown (chipmunk, cinnamon, spice or talavera) areas had lesions with a center with restricted size (1 to 2 mm in diameter) and usually white to pinkish color. The lesion size was restricted by the yellow margin, sporulation was delayed, and there were fewer spores produced.

Detached leaves generally had more susceptible reactions than plants inoculated in the greenhouse with the same isolates. The lesions were darker in color among the resistant varieties in the detached leaf inoculation as compared to susceptible varieties. The center of lesions on detached leaves was mostly green to gray and generally had fewer dark areas. Except for lesion type 3, no white or pinkish coloration was present in the center of lesions inoculated by the detached leaf technique while this was common in lesion types 3, 4 and 5 in the greenhouse inoculations.

Size of lesions

Lesion size was only measured on detached leaves. Lesions formed on susceptible varieties and selections had a large yellow margin that often coalesced with the neighboring lesions. In all measurements the entire colored area of the lesions was considered. Results are given

in Table 5. Lesion size of varieties and selections showing only hypersensitive reactions did not enlarge beyond the area where the microdrops of inoculum had been placed. The lesions rarely measured more than 1 mm in diameter. Variation was observed within the same lesion type on the same variety when inoculated with different isolates. This may have been due to a varying number of lesions, which were selected as being typical and taken as the basis for classifying the lesion types of each variety and selection. In general, the results indicated that the average size of lesions increased with greater susceptibility (Table 6). Analysis of variance on size of lesions made between varieties Binato, Peta and Saturn, and the five isolates shown in Table 5, indicated a highly significant difference among isolates, among varieties and in the interaction variety X isolates. Each variety had 12 lesions measured (mm^2), representing 12 replicates out of 36 or 48 lesions developed on 12 leaf sections.

Inoculations made on the apical (A) and basal (B) portions of the same leaf of different varieties and isolates to determine whether a difference existed in susceptibility, indicated no difference on the resistant varieties. Differences in lesion size among varieties and selections Padma, T-141, TKM-6, Peta and Saturn (increasing order of susceptibility), using isolates 27, 59L13 and 68 T1, are shown in Table 7. These differences were highly significant for varieties and leaf positions A and B. Size of lesions on position A was significantly smaller than on position B. The average size of lesions on both leaf positions A and B increased with an increase in susceptibility (Table 8).

Considerable variation in size of lesion was observed among isolates.

Time of sporulation

Observations of sporulation on detached leaves were made daily, starting 3 days after inoculation and continuing for 10 days. No spores were formed on lesion type 1, only rarely on type 2, but spores were always observed on lesion types 3, 4, and 5, often as early as 4 days after inoculation on types 4 and 5. Usually, sporulation took longer on types 3 and 2.

The results of sporulation on seedlings inoculated in the greenhouse are more useful. A great variation in time of sporulation was observed in the same lesion type and on the same variety. Results on time of conidiophore and conidia formation are presented in Table 9. Conidiophores and conidia appeared as early as 1 hour after infected leaves were detached from the varieties Peta and Saturn inoculated with isolate 68L4. No conidiophores were formed 14 hours after leaves were detached and the longest period to produce conidia was 30 hours. Spores were not formed on lesion types 1 and rarely on type 2.

A comparison of range and average time of conidiophore and conidia formation with lesion types showed that lesion type 5 took the least amount of time to sporulate, and was followed by lesion types 4, 3 and 2 (Table 10). When conidiophores emerged four hours after leaves were placed in the moist chamber, conidia formation generally followed in one to three hours. When conidiophores took more than five hours to emerge, conidia formation was delayed from four to 20 hours (Table 9).

Number of spores produced

The number of spores produced increased with an increase in susceptibility, both in the greenhouse and detached leaf inoculations. On lesion types 3, 4, and 5, the number of spores varied greatly within the same lesion type. The results obtained with seedlings in the greenhouse are given in Tables 11 and 12.

Time of ingress

A study of time of ingress with the detached leaf technique failed to show notable differences among varieties and isolates. Likewise, in a greenhouse test, no relationship was observed between time of ingress and the other characteristics studied in the one test made; therefore, the results of these tests are not given.

DISCUSSION

One of the most difficult problems encountered in making this study was the diversity of lesion types observed on the varieties and selections used. This occurred even when a single isolate of P. oryzae was used. Such results might be expected when one considers the results of Ou et al (16). Kobayashi and Abumiya (11) found that higher concentrations of inoculum resulted in severe damage to the leaves, and that varieties differed in the number of lesions produced with varying inoculum concentrations. It is possible that the differences in size and inoculum concentrations of the droplets on the leaves may be the explanation for the highly resistant reactions which were observed along with highly susceptible reactions on the same leaf.

Reports on the actual size of lesions and their relationship with

color, shape and sporulation was not found in the literature. References in the literature to size of lesions and their relationship to resistance or susceptibility were usually made as "small" or "large" lesions. Hashioka (4) noted that when lesions were large with typical fusiform shape and dark gray centers, sporulation was most abundant; and that a decrease in size resulted in less sporulation and increased dark color of the lesions. Similar results were obtained in this study.

It was found that the intensity and distribution of lesion colors may be a good indication of the degree of resistance of the rice plant to the blast fungus. Differences in color of lesions observed between detached leaf and greenhouse inoculations should be studied more carefully. Study should also be made on whether or not the color and other characteristics observed on detached leaves actually indicate the reaction patterns that are expressed by plants exposed to greenhouse or field inoculations.As mentioned by Kobayashi and Abumiya (11), inoculum concentration affects disease severity and may also alter the color intensity of the lesions. In future studies it would be useful to standardize, as well as possible, the concentration of inoculum in the droplets of spore suspension applied on detached leaves and also the inoculum used for field or greenhouse inoculations. The lighter color and higher susceptibility observed in detached leaf inoculations may be related to higher concentration of inoculum in each droplet.

Toyoda and Suzuki (20) observed that sporulation on greenhouse plants was more rapid and abundant on lesion type 4, less on 5 and 3, and spores were sparsely formed on 2 and were absent on type 1. The

present study indicates that the average time (hour) of sporulation on inoculated greenhouse plants was shorter on lesion type 5 with more abundant spores being formed. Also in contrast to Toyoda and Suzuki (20), the number of spores produced was highest in lesion type 5 when detached leaves were inoculated.

Sporulation was more abundant on detached leaves in Petri plates than on leaves inoculated in the greenhouse and then detached. Detached leaves also gave a more susceptible reaction. This may have been caused by the yellowing which began from the cut ends within four to five days. In many instances the leaves were entirely yellow before any symptoms developed. A more valid reaction would undoubtedly be obtained with leaves from healthy plants.

Differences in susceptibility between the apical and the basal portion of the last fully developed leaves could be related to tissue age. The leaf unfolds or emerges from the tips, thus the basal section has the youngest tissue. This observation agrees with that of Kato et al (10) that sporulation was greater when leaves were inoculated at the middle stage of expansion. Kahn and Libby (9) reported that younger upper leaves were the most susceptible, with resistance increasing upward. For future tests it would appear that only the basal half of the youngest, but most fully developed, leaf should be used for the detached leaf inoculation. No relationship was observed between time of ingress and susceptibility. The experiments made during this study were not replicated and should be repeated with additional varieties before definite conclusions can be drawn.

It should be pointed out that the present studies were conducted under conditions unfavorable for normal rice plant development. Plant chlorosis and other physiologic disorders, referred to as "winter sickness", were constant problems. Therefore, experiments should be made with healthier plants before conclusions can be made regarding the relationships among the characteristics observed. Experiments should be repeated with healthy plants, grown together under identical conditions and inoculated with the same spore suspension. Since physiological disorders in rice plants seem to be a common problem in greenhouse cultures (12), experimental results should be confirmed with healthy plants grown in the field.

No conclusions can be made as to what might represent general resistance in rice to P. oryzae from the results of these studies. In addition, the results obtained were highly variable. Nevertheless, it has been demonstrated that the characteristics considered can actually be measured, and the study may serve as a valuable source of information for further studies.

SUMMARY

A study of certain factors which may affect general resistance in rice to Pyricularia oryzae Cav. was made in the laboratory by inoculating detached leaves in Petri dishes and by inoculating potted plants in the greenhouse. The inoculation of potted plants in the greenhouse was made primarily in order to find varieties with lesion types 3 and 4. Unfortunately, plants grew poorly so that studies with detached leaves and

greenhouse inoculations had to be made simultaneously with a limited number of varieties and isolates. Inoculum of P. oryzae was initially produced on 2 percent rice polish agar, but culture on fresh, sterilized, crushed leaves gave better results and inoculum from this source was used during the rest of the study.

General resistance in rice to P. oryzae might be expressed through reduced size of lesions, delayed time of sporulation, reduced number of spores, delayed time of ingress, and specific color of lesions. Thus the factors studied as possible indicators of general resistance were: a) size of lesion, b) color of lesions, c) time of sporulation, d) number of spores produced, and e) time of ingress. Attempts were made to find relationships among these characteristics which would express general resistance. Tests were also made to determine the differences in susceptibility between the apical and basal portions of the youngest, but fully expanded leaves.

Even using a single isolate, a wide variation in lesion types occurred on the same variety in both the detached leaf and greenhouse inoculations. Reactions varied from highly resistant to highly susceptible. The size of lesions measured on detached leaves also showed extreme variation among and within the same varieties with different isolates. In general, size of lesions increased with an increase in susceptibility.

Smaller lesions generally had darker coloration, delayed sporulation, and reduced number of spores. A relationship of lesion color with

other characteristics suggested that plants with lesions having green (surf green, olive green, pea green, opaline green or acacia) to gray (cub, sandy beige and mineral gray) centers were more susceptible, had larger lesions, more rapid sporulation, greater number of spores formed, and a larger yellow margin. White to pinkish (ivory or white jade) centers occurred in many lesions of types 3, 4 and 5 and on these light-colored areas sporulation was delayed, and fewer spores were formed. Lesions with wider black or purple (graphite) to dark brown (autumn and burnt umber) and brown (chipmunk, cinnamon, spice or talavera) areas had lesions with a center with restricted size (1 to 2 mm in diameter), and usually white to pinkish color. The lesion size was restricted by the yellow margin, sporulation was delayed, and there were fewer spores produced.

Detached leaves generally had more susceptible reaction than plants inoculated in the greenhouse with the same isolates. The lesions were darker in color among the resistant varieties in the detached leaf inoculation as compared to susceptible varieties. The center of lesions on detached leaves was mostly green to gray and generally had fewer dark areas. Except for lesion type 3, no white or pinkish coloration was present in the center of lesions inoculated by the detached leaf technique, while this was common in lesion types 3, 4 and 5 in the greenhouse inoculations.

Time of conidiophore and conidia formation in the greenhouse inoculations showed variation in the same lesion type among varieties. In general, the time to produce spores increased with increased resistance.

Conidiophores were rarely formed in less than 4 hours under high humidity in susceptible to highly susceptible varieties. When conidiophores were formed within 4 to 5 hours under high humidity, conidial formation followed 1 to 7 hours later. Conidial formation was frequently delayed when conidiophores took more than 6 hours to emerge.

No differences were noted between lesion types 4 and 5 in regard to time of sporulation on detached leaves. Sporulation took longer on lesion types 3 and 2. Sporulation rarely occurred on lesion type 2.

The number of spores produced increased with an increase in susceptibility, both in the greenhouse and detached leaf inoculations. On lesion types 3, 4 and 5, the number of spores varied greatly within the same lesion type. In general, sporulation was more abundant when detached leaves were inoculated.

Inoculations of the apical and basal portions of the same leaf, with the same isolate, indicated that the basal portion was more susceptible. Lesions were much larger, sporulation was faster, and a larger number of spores were produced on the basal portions. No differences were observed among the highly resistant varieties between the apical and basal portions of the same leaf.

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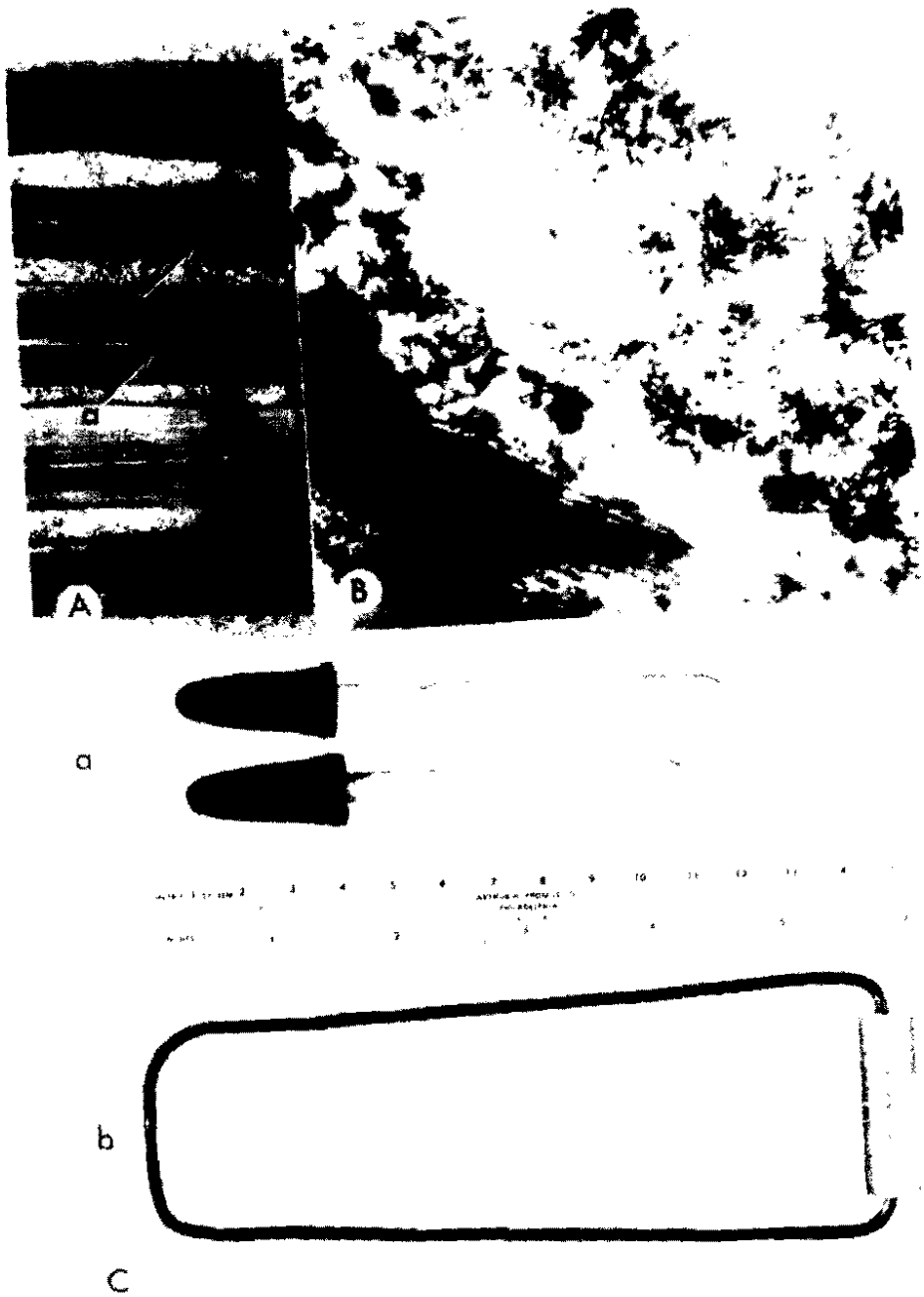

Fig. 4. a) Diseased leaves of selection 128 inoculated with P. oryzae [illegible] ness; hypersensitive reactions (a) on injured area where high [illegible] lation [illegible]. b) Sporulation on the injured area (a). c) Microtrop injector used for inoculation of detached [illegible] and aluminum roller used for crushing the rice leaves [illegible] the fungus (b).

Table 1. List of colors observed on lesions caused by P. oryzae on rice leaves inoculated in Petri dishes and the greenhouse.

Symbols	Standard color 1/	Key to the Dictionary a	b	c 3/	Common colors 2/	
Olg	Olive green	22	B	6		
Opg	Opaline green	17	A	6		
Pg	Pea green	20	G	6	Green	
Sg	Surf green	20	C	7		
Fl	Flax	12	B	2		
Ac	Acacia	11	K	1		Grayish-green
Cu	Cub	15	C	1		
Sb	Sandy Beige	14	A	3	Gray	
Mg	Mineral gray	20	A	2		
Wj	White jade	10	A	2	Light pink	
Iv	Ivory	10	B	2		
W	White				White	
Gr	Graphite	48	C	7	Black, purple	
Au	Autumn	8	A	12	Dark brown	
Bu	Burnt umber	15	A	12		
Ch	Chipmunk	13	L	9		
Cm	Cinnamon	12	E	7	Brown	
Sp	Spice	13	A	12		
Tv	Talavera	12	A	12		
Ap	Apricot	10	F	7	Reddish-brown	
Gl	Gold leaf	11	K	8		
My	Martius yellow	9	I	1	Yellow	
Lcy	Light chrome yellow	10	L	4		
Sy	Spruce yellow	12	K	8		

1/ According to the Dictionary of Colors (13).

2/ Common names cited in the literature.

3/ a) Plate number, b) Column, and c) Line

Table 2. Five scale units of disease reaction on detached rice leaves used in the classification of lesion types caused by P. oryzae.

Scale units	Lesion types 1/
1	Lesions limited to the site of inoculation; graphite (Gr) to burnt umber (Bu); no necrotic center; no yellow margin formed.
2	Restricted lesion, about 2-3 mm long; graphite (Gr), autumn (Au) to burnt umber (Bu) center; spice (Sp), gold leaf (Gl) to martius yellow (My) margin; no necrotic center.
3	Lesions up to 6 mm long; small (2-3 mm) opaline green (Opg), pea green (Pg), olive green (Olg), to ivory (Iv) necrotic center; thick graphite (Gr), autumn (Au) burnt umber (Bu) to spice (Sp) zone; and gold leaf (Gl) to martius yellow (My) margin.
4	Lesions up to 10 mm long; frequently with pea green (Pg), olive green (Olg) to mineral gray (Mg) necrotic center; burnt umber (Bu) and occasionally graphite (Gr) zone; gold leaf (Gl), martius yellow (My) to light chrome yellow (Lcy) margin.
5	Lesions more than 10 mm long; pea green (Pg), opaline green (Opg) to olive green (Olg) center; rarely with burnt umber (Bu) zone; spice (Sp), gold leaf (Gl), light chrome yellow (Lcy) to martius yellow (My) margin.

1/ Color of the lesions are from the center toward the margin.

Table 3. Five scale units of disease reaction on rice plants inoculated in the greenhouse, used in the classification of lesion types caused by P. oryzae.

Scale units	Lesion types 1/
1	Only small, brown (autumn to spice) specks, few or many with no necrotic (collapsed cell) spots.
2	Slightly larger (2-3 mm in diam), graphite (Gr), autumn (Au) to spice (Sp) spots, with gold leaf (Gl) margin; no necrotic (collapsed cell) spots.
3	Small, roundish, necrotic, gray (Cu or Sb) to olive green (Olg) center (about 1-2 mm in diam); surrounded by graphite (Gr), autumn (Au) to spice (Sp) and gold leaf (Gl) margin, which is somewhat elliptical.
4	Typical blast lesion, elliptical, somewhat restricted (up to 6 mm long), with large necrotic, gray (Cu or Sb), olive green (Olg) to opaline green (Opg), and sometimes white center; graphite (Gr), autumn (Au), spice (Sp) to gold leaf (Gl) margin.
5	Lesions larger and broader than in scale 4 (more than 6 mm long); large gray (Cu or Sb), olive green (Olg), opaline green (Opg) to white center; graphite (Gr) to autumn (Au) not always present, mostly spice (Sp) to gold leaf (Gl) margin; upper portion of seedling leaves may be killed by coalescence of large lesions.

1/ Adapted from the international classification for field and nursery tests (15).

Table 4. Range of lesion types on detached leaves of 13 rice varieties inoculated with five isolates of *P. oryzae*.

Varieties and selections	Isolate number and lesion types 1/				
	US5	27	59L13	68L4	68T1
IR8	1	1	- 2/	-	-
Taichung (Native 1)	1	1	-	-	-
IR5-47-2	1 - 2	1	1 - 2	1	-
Fortuna	1 - 2	-	1 - 2	-	-
Padma	-	1 - 3	1 - 2	1 - 2	-
T-141	-	1 - 3	1	1	-
T-Km6	-	1 - 3	1 - 2	1 - 3	-
PI215-936	1 - 3	1	-	-	-
IR154-61-1	1 - 3	-	1 - 3	1 - 3	1 - 4
Bluebelle	1 - 3	1	-	-	-
Bluebonnet 50	-	-	1 - 4	1 - 4	1 - 4
Peta	1 - 4	1 - 2	1 - 3	1 - 3	1 - 2
Binato	2 - 5	1 - 3	1 - 4	1 - 4	2 - 5
Saturn	1 - 3	1	1	3 - 5	2 - 5

1/ Lesion types: 1) Highly resistant; 2) Resistant; 3) Moderately resistant; 4) Susceptible; 5) Highly susceptible.

2/ Materials not available

Table 5. Size of lesions (mm^2), measured eight days after inoculation of detached leaves of rice with P. oryzae.[1/]

Varieties and selections	Isolate no. and size of lesions (mm^2) [2/]				
	US5	27	59 L 13	68 L 4	68 T 1
Padma	-	2.87 (3)[3/]	1.07 (2)	1.06 (2)	- [4/]
T-141	-	3.78 (3)	1.00 (1)	0.77 (1)	-
TKM-6	-	5.11 (3)	1.86 (2)	7.11 (3)	-
IR154-61-1-1	9.37 (3)	-	2.77 (3)	1.45 (3)	4.22 (4)
Bluebonnet 50	-	-	13.42 (4)	14.26 (4)	13.25 (4)
Peta	5.70 (4)	3.09 (2)	11.37 (3)	6.33 (3)	1.14 (2)
Binato	25.74 (5)	6.25 (3)	8.08 (4)	12.70 (4)	16.90 (5)
Saturn	1.53 (3)	1.00 (1)	1.00 (1)	29.99 (5)	25.46 (5)

[1/] Varieties, isolates and interaction differed at 1% level (see Table 10).

[2/] Average of 12 lesions/isolate.

[3/] Number in parentheses indicates the lesion type.

[4/] Not tested.

Table 6. Range and average size of lesions (mm^2) of each lesion type on detached leaves.

Lesion type	Size of lesion (mm^2) 1/	
	Range	Average
1	0.77 - 1.00	0.99
2	1.06 - 3.09	1.84
3	1.71 - 11.37	5.07
4	4.22 - 14.26	10.22
5	16.90 - 29.99	24.52

1/Average of 12 lesions/isolate.

Table 7. Comparison between size of lesions on detached leaves, of the apical (A) and basal (B) positions of the same leaf, inoculated with P. oryzae.[1/]

Varieties and selections	Leaf pos.	Isolate number and size of lesions (mm^2) [2/]				
		US5	27	59L13	68L4	68T1
Padma	A	- [3/]	1.00	1.00	1.00	-
	B		4.66	1.14	1.12	
T-141	A	-	6.14	1.00	0.92	-
	B		1.43	1.21	0.63	
TKM-6	A	-	0.95	1.07	0.64	-
	B		9.28	2.98	14.29	
IR154-61-1-1	A	9.37 [4/]	-	1.50	1.45	1.91
	B		-	4.04		6.54
Bluebonnet 50	A	-	-	5.75	7.52	9.66
	B		-	24.50	21.00	16.85
Peta	A	5.59	1.01	6.75	4.91	1.19
	B	5.82	5.17	16.00	7.75	1.00
Binato	A	25.74	6.25	10.08	8.43	14.66
	B			6.08	16.97	19.25
Saturn	A	1.53	1.00	1.00	21.12	20.22
	B		1.00	1.00	36.87	31.70

[1/] Varieties, isolates, leaf positions and interactions differed at 1% level (see Table 12).

[2/] Average of 12 lesions/leaf position/isolate.

[3/] Not tested.

[4/] Only one leaf section/leaf was inoculated.

Table 8. Range and average size of lesions (mm^2) on detached leaves (leaf position A and B), in relation to lesion types.

Lesion type	Leaf pos.	Size of lesion (mm^2) Range	Average
1	A	0.92 - 1.00	0.98
	B	0.63 - 1.21	0.96
2	A	1.00 - 1.19	1.04
	B	1.00 - 4.66	2.67
3	A	0.64 - 6.75	3.44
	B	1.43 - 16.00	8.79
4	A	1.91 - 10.08	6.99
	B	5.82 - 24.50	13.96
5	A	14.66 - 21.12	18.76
	B	19.25 - 36.87	29.27

Table 9. Time(hour) of conidiophore and conidia formation by P. oryzae on rice leaves detached eight days after inoculation in the greenhouse and observed in moist Petri dishes for 48 hours.

Varieties and selections	Isolate number and time (hr) of conidiophore and conidia formation															
	US5		27		59L13		68A10		68A12		68A14		68L4		68T1	
	a	b [1]	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Padma	x [2]		x		4	10	4	9	10	14	5	12	x		10	14
IR154-61-1-1	6	14	6	14	- [3]		-		-		-		-		-	
PI215-936	x		x		x		x		5	14	4	12	x		5	12
T-141	x		10	14	x		8	14	14	24	x		x		4	7
Saturn	6	10	6	24	x		x		x		x		1	1	4	5
Peta	2	6	x		4	8	x		x		x		1	1	x	
Binato	10	16	12	16	x		x		x		x		x		4	5
TKM-6	-		10	14	10	30	5	9	4	7	5	10	10	14	4	7

[1] a) Time of conidiophore formation; b) Time of conidia formation.

[2] Neither conidiophores nor conidia formed.

[3] Not tested.

Table 10. Relationship of lesion types, range and average time (hour) of conidiophore and conidia formation in P. oryzae, produced on rice leaves detached eight days after inoculation in the greenhouse and kept in moist Petri dishes for 48 hours.

Lesion type	Conidiophore		Conidia	
	range hr	average hr	range hr	average hr
1	x 1/	x	x	x
2	8 - 14	11.06	14 - 24	18.00
3	4 - 12	7.16	3 - 30	14.41
4	1 - 6	4.55	1 - 24	11.00
5	1 - 5	3.43	1 - 10	5.85

1/ No spores formed.

Table 11. Number of conidia of eight isolates of P. oryzae produced on rice leaves, detached eight days after inoculation in the greenhouse and 48 hours after being placed in moist Petri dishes.

Varieties and selections	Isolate number and number of conidia produced 1/							
	US5	27	59L13	68A10	68A12	68A14	68L4	68T1
	1000X	1000X	1000X	1000X	1000X	1000X	1000X	1000X
Padma	0	0	1.66	9.33	1.33	0.66	0	2.50
IR154-61-1-1	1.80	0.55(4) 2/	-	- 3/	-	-	-	-
PI215-936	0	0	0	0	0.33	0.16	0	5.33
T-141	0	0.07(3)	-	0.07(3)	0.10	0	0	18.00
Saturn	6.20	0.26	0	0	0	0	6.14	20.20
Peta	2.00	0	1.75	0	0	0	11.80	0
Binato	0.05	0.14	0	0	0	0	0	18.33
TKM-6	-	0.03(2)	0.10	9.00	4.50	3.12	0.50	2.70

1/ Results are the average of five best sporulating lesions selected under 80X dissecting microscope.

2/ Numbers in parentheses indicate number of sporulating lesions that were available.

3/ Not tested.

Table 12. Relationship of lesion types with range and average number of conidia of eight isolates of P. oryzae produced on rice leaves, eight days after inoculation in the greenhouse and 48 hours after being placed in moist Petri dishes.

Lesion type	Number of conidia	
	Range	Average
1	0	0
2	50 - 100	73
3	30 - 2500	669
4	160 - 9330	4447
5	2000 - 20200	10878

Centro Internacional de Agricultura Tropical

RICE BLAST DISEASE IN BRAZIL

Regina E. de Mello Amaral

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

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INTRODUCTION

Brazil occupies the sixth place in the world as a rice-producing country, with a cultivated area of approximately 5 million hectares, of which about 35 per cent is irrigated and about 65 per cent unirrigated, with a total yield of about 7 million tons/year. The main producers are the States of Goias, Rio Grande do Sul, Sao Paulo, Minas Gerais and Maranhao (Map 1).

In the State of Sao Paulo 78.8 per cent of the cultivated area is on unirrigated soil and only 2.6 per cent is on irrigated soil; the remaining 19.1 per cent is cultivated on humid soils without irrigation. In the State of Rio Grande do Sul, the situation is the opposite, 98 per cent of the cultivated area is irrigated.

The main rice diseases found in the growing areas of Brazil are caused by fungi, and their occurrence depends on several factors, mostly climatic conditions (humidity, temperature and sunlight) which together with defective agricultural practices, favour the appearance, development and dissemination of the causal agents and increase the susceptibility of the plants.

Rice blast disease

Rice blast caused by Pyricularia oryzae, Cav., is the most common and important rice disease in Brazil. Considerable damage to the crops, limiting national average rice fields, is due to blast.

According to the available literature the first findings were made in 1912 (3) and 1920 (9) in the State of Sao Paulo, in 1930 (18) in the State of Minas Gerais, in 1935 (20) in Rio Grande do Sul, in 1946 (4) in Bahia and the northern States, particularly in the Sao Francisco River Valley.

The incidence of rice blast has been increasing year after year in the main producing areas. When the environmental conditions are favourable to the development of the disease, it becomes epiphytotic, causing large losses in either irrigated or unirrigated areas (1, 7, 10, 11, 15, 16, 17, 19, 21, 23, 25, 26, 27, 29, 30).

The symptoms of the disease are usually found on leaves, culms, branches of the panicle and floral structures, but in Rio Grande do Sul one of the most commonly observed symptoms is the "rotten-neck"(5, 23).

Research work on rice blast disease has been carried out mainly at the Instituto Biologico, Sao Paulo, in the State of Sao Paulo, at the Instituto Riograndense do Arroz, Porto Alegre, and Instituto de Pesquisas Agropecuarias do Sul, Pelotas, both in the State of Rio Grande do Sul, according to the following lines:

I - Laboratory studies: biology and morphology of the causal fungus Pyricularia oryzae.

II - Identification of the physiologic races of P. oryzae in Brazil.

III - Varietal resistance.

IV.- Control.

I - Laboratory studies on the biology and morphology of the fungus Pyricularia oryzae.

1.1 - Sao Paulo State

The results obtained indicated that:

a) In PDA culture medium the optimum growth temperature lies between 27 and 30°C (24).

b) Optimum temperature for spore germination is 27°C at pH 5.5 to 6.0 (1).

c) Isolates can be classified in three morphlogical groups according to the spore length: 1) 23,2 to 26.2 micra; 2) 17.2 to 20.1 micra; 3) intermediate measures (24).

II - Identification of the physiologic races of P. oryzae

For the identification of the physiologic races, the international methodology proposed by Atkins et al (2) has been used.

The race group and classification number was based on the Table proposed by International Rice Research Institute, IRRI (28).

The results obtained showed that:

II.1 - Sao Paulo State.

a) Physiologic races may occur in large number since a

high pathogenic variability has been detected. Four races: ID-13, IA-65, IB-1 and IC-5 were identified in a group of 5 isolates obtained from upland rice plants collected at Itu, Sao Joao da Boa Vista, Lins, Monte Aprazivel and Ribeirao Preto municipalities. In another group of 4 isolates from Bebedouro, Santa Barbara do Rio Pardo, Ituverava and Campinas municipalities, three other races will certainly be identified, depending on Raminad Str.3 reaction, which has not yet been observed (1).

b) Four isolates of P. oryzae, obtained from the basal node of the panicle, did not affect the leaves of any of the varieties included in the International Differential Group, nor of a set of 42 Brazilian cultivars: Dourado-peludo, Dourado-agulha, Cateto-branco, Jaguari, Iguape-peludo, 7-V-10, 4 meses, Matao Branco, Espinho, Perola, Cateto-dourado, Iguape-agulha, Bico-branco, Goiano, Pindorama, Guedes, Birigui- Santa America, Agulha ESALQ-12, Ponta-Preta IAMG, Terra Roxa, Paulinia, Dourado-cearense, 3 meses, Dourado-precoce, 7-V-4, 7-V-8, Veranopolis, IAC-8, IAC-9, IAC-4, Come-cru, Chatao, Aviao, Guarapiranga, Guaiba, IAC-120, IAC-435, IAC-1246, IAC-2091, Pratao-precoce and Pratao comercial (1).

c) The races IA65 and IB1 were found to be the most pathogenic on 42 Brazilian cultivars, since 35.7 per cent and 33.3 per cent of these cultivars were respectively infected by the two races. Race IC-5 was less pathogenic on these cultivars

since it affected only 11.8 per cent of them (1).

II.2 - Rio Grande do Sul State

a) Fourteen races: IA_1, IA_5, IA_{69}, IA_{85}, IB_5, IB_{21}, IB_{37} IC_5, IC_{21}, IE_5, IG_1, IG_2, IH_1, II_1 were identified in a group of 63 isolates obtained from rice plants cultivated on irrigated soil, located at the Coastal area, SE Slopes, Central Lowland and SE Hills. This result shows that there is a high pathogenic variability in the fungus population in the Rio Grande do Sul State (22-23).

b) It has been possible to identify several sub-races by inoculating the above mentioned races onto a group of 6 local cultivars: Stirpe Sel.Pelotas, EEA 404, EEA 405, EEA 406, Sel.IPEAS 2169 and EEA 201 (22).

c) Many races, although different, caused similar reactions on these 6 cultivars. The Stirpe Sel. Pelotas cultivar proved to be the most resistant and Caloro the most susceptible.

d) In Rio Grande do Sul the predominant races are: IG_1 (19.61 per cent of the isolates) less pathogenic to the local cultivars; IA_5 and IB_5 (13.74 and 11.77 per cent of the isolates, respectively) which, with the races IA_1 and IB_2, are highly pathogenic to the cultivars generally grown in the State, i.e., EEA-404 and others resulting from interbreeding Zenith and Maravilha (22).

e) A set of 145 cultivars was inoculated with the prevailing races IA_5 and IG_1, and with a mixture of both; a decrease of pathogenicity of the race IA-5 was observed when the mixture was inoculated (23).

III - Varietal resistance

III.1 - Sao Paulo State

a) The tests for blast resistance under field conditions on unirrigated soil began in the agricultural year 1964/65 with 519 cultivars (14). In the subsequent years another 36 cultivars (hybrids from the Instituto Agronomico of Campinas, and newly introduced ones) were added to these tests.

Two experimental fields were annually established in Campinas, one at Mario D'Apice Experimental Station of the Instituto Biologico, and the other at the Theodureto de Camargo Experimental Station of the Instituto Agronomico.

In the 1964/65 mentioned field, seeds of each cultivar were sown in one 2 meter long line with a spacing of 0.5 m. In the successive tests two close lines of 2 meters were sown with each cultivar, with two replications. The border lines as well as every tenth line in the field were sown with susceptible cultivars: 7-V-10, IAC-162 and Dourado-precoce. At the tillering stage some of the rice plants from the border lines were inoculated as follows: in the 1964/65 test,inoculum of the fungus was put inside the still uprolled newest leaf; in the subsequent tests, spore suspension of each of the races IC-5, IA-65, IB-1, ID-13

and isolates number G-138 and G-765 were sprayed on all the border lines plants.

The reading of the incidence of blast on leaves and panicles was made according to the type of lesions (R, MR, M, MS, S) and the percentage of the affected area ("t (trace), 5, 10, 20 ... 100). (14).

The same reading method was used for estimating the incidence of other diseases which occurred by natural infection, such as: helminthosporiosis-Helminthosporium oryzae; cercosporiosis - Cercospora oryzae;"mulata"or bronzing of the stem (unknown cause); "lista parda" or brown stripe - a well marked brown-red longitudinal stripe which, beginning at the basis of the sheath, extends to the end of the leaf (unknown cause), although some microorganisms have been consistently isolated from the lesions (a fungus of the genus Fusarium and a bacteria of the genus Xanthomonas) (1).

After successive screenings, always eliminating the cultivars which showed susceptibility over 10 S (= 10 per cent of the leaf area affected), only 31 out of the 555 tested cultivars were used in the 1970/71 fields.

As a result 20 of them were found to be resistant to blast. From these only 4 were resistant to all the above mentioned diseases: Binicol, H-4, Down and Hsedni-56 (Table 1).

Six cultivars (64/75 = (7-V-4 x 59/90), 64/47 = (IAC-1246 x 1391), IAC-435, IAC-5032, IAC-2091 and Bico-ganga) which showed resistance in the 1970/71 test had been previously found susceptible at the Theodureto de Camargo Experimental Station in 1969/70,

where the incidence of blast disease was much more severe than at the Mario D'Apice Experimental Station. Therefore these six cultivars must be included in the next field test in 1971/72 (1).

The tests at the Theodureto de Camargo Experiment Station in 1968/69 and 1970/71 were lost due to drought.

b) At the bed-test carried out in 1969/70 with the IAC-435, IAC-1246, IAC-120, Batatais, IAC-106, IAC-146, Pratao-precose, Dourado-precoce and IR-8 cultivars, inoculated with blast infected rice leaves, the cultivars IR-8, IAC-106, and Pratao-precoce were the most resistant. In the 1970/71 bed-test the cultivars IAC-435, IAC-1246, IAC-47, IAC-120, IAC-68, IAC-162 and Batatais were inoculated by spraying a spore suspension of races ID-13, IA-65, IB-33, IC-5 and isolate number G-765, the cultivars IAC-120, IAC-1246 and IAC-47 showed to be higher resistant (1).

III.2 - Rio Grande do Sul State

a) Field and greenhouse research work for blast resistance started several years ago (6, 8, 12). The field selection of resistant cultivars was carried out by natural infection under normal conditions of spacing between lines, fertilization and irrigation, Under these conditions, allied to ecological factors, the incidence of the disease was not always favoured. In spite of this defective method some cultivars with good blast resistance were selected: Stirpe Sel.Pelotas, EEA 404 and EEA 201 (23).

b) In the subsequent tests the reaction to blast incidence on leaves was read using the international scale proposed by the Symposium of the Rice Blast Disease (IRRI-1963). For the evaluation

of the panicle basal node infection, the percentage of damaged panicles on each plant was calculated.

During the agricultural year 1968/69, 850 rice cultivars were tested for blast resistance, 90 of which were rated as resistant (13). In the tests carried out in 1969/70 and 1970/71, 49 and 30 cultivars, respectively, were tested, and 16 cultivars were found to be resistant: Suwon n°152, Taichung 65, CI 5309, Down, Tainan 3, Kaoshuing 21, Kaoshuing 24, Chokoto 14, Kanto 106, Kanto 51, Norin 20, Norin 22, Stirpe Sel. Pelotas, IR.532-1-171, MO-R-500 x Nato and Swon n°158 (23).

c) In 1969/70, a set of 256 cultivars from The International Uniform Blast Nurseries was submitted to bed-tests (Ou system). Up to now three of these bed-tests have been completed. As a result several of those cultivars showed horizontal resistance under the prevailing conditions in the State of Rio Grande do Sul although some of them did not flower. Considering the group of the most resistant cultivars in the world, the following ones showed to be susceptible: Pah Leaud 29-9-11, Zenith, Ram Tulasi and Ram Tulasi (sel.); among the resistant cultivars only the following ones flowered: R-67, CI-7787, E-425, Down, KPE 6, Kataktara DA 2, Mamoriaka, NP 130, Ca/902/b/2/2, Amritsari HR 22, DT.10, DNJ-60, 370 Basmati, Pusur, T , Ca /435/6/5/1, 268 b/Pr/[illegible]/1/1, E.L. Gopher, Rajbohog N-22 and DV-75 (23).

Local cultivars and some introductions were included in all the tests mentioned above.

The cultivars that presented the greatest number of resistant reactions are mentioned below; the ones which flowered under Rio Grande do Sul conditions are underlined:

a) Susceptibility 0/3:

Tep-pep, Tadukan, C-46-15, D-25-4, H-4, H-5, H-105, H-501, Murungakayan, Jae Keun (Suwon n°152), Radin Ebos 33, Radin China 4, Lembu Basah, K.P.F.6, E.L., B-E-3, FB-86, Milbuer 5, Chianung-Yu 280, Taiwan 3, Lewang 28-1-14, Nang Chet Cuc, Trang cut L.11, Doc Phung, O Tre, Nang Quot (floating), Nang Chol, CP 231 x HO 12 (Dawn), Remadja, Sigadis, Ta-poo-cho-z, N-302, Ram Tulasi (sel)*, Ram Tulasi*, Unblatuzi Val.Sugar Co., Mamoriaka, Sorna Vari, Ramgarh, T_3, NP-97, Milketan - 20, J-519, Laka, Huan-sen-goo, Taichung line 47720, Badshabag, Jhug Paddy n°7, Acheh Puteh, Leter 08, Kataktara DA 2, Rajbhog N.22, Thava lakkannan Ptb 9, T9, T1, T23, Basmati T3, Pi 4, CP 231 x HO 12 (215), CP 231 x HO 12 (216), C-46-15, S20J.K.W., S.39.J.K.W., 268b/Pr/8/1/1, Pusur, DF-1, DN-J-60, DNJ-52, DZ-78*, DZ-74, DL-5, DL-8*, DL-9*, DD-63, DD-80, DD-89, DD-99, DD-113, Ctg 250, Ctg 680, Ctg 1516, UCP 6, UCP 27, DM-32, DV-2, DV-12, DV-68, DV-73, DV-107, DV-109, DV-112, Samba, Murungakayan, Basmati (C 5836).

b) Susceptibility 0/2 + 1 intermediate reaction (3.0 - 4.0)

CI 5309, PI 231128, Norin 22, Taichung 65, Pai-kan-tao, C-33-18, M-302, Co25, N°K-60, Norin 22, Kongo (Br. n°1), Acheh, Hagi Haroun, Eng Katek, Padang Trengganu 22, R 67, Taichung 181, Kaoshiung 24, Kaoshiung 21, Kaoshiung-Ta-lichin-yu, Tam Vout, Samo Trang, Doc Phung Lun A, Nang Dum to (floating), Pah Leuad 111, Pah Leuad 29-8-11,

Kanto 53, = 79, T1, CI 6037-4, Basmati 370, N 12, N 32, CI 7338-5, CI 6914, Bmt 53 R 3540, Rad Shabbag (scented), Ahmee Puthe, W.R.C. n°4, 818-3 BR 9, Carreon, Madae 30My 137, Cheu Kayama Ptb 26, Chuvanna Modan Ptb 30, Ca 902/b/3/3., Ca 902/b/2/1, Tun Start, Surjamukhi DA 4, Amritsari HR 22, 370, Basmati, 46 Palman, Karia, Saraya, Pi 3, S 18 J.K.W., PI 184675-4, PI 184675-2, Donduni Kunluz, 268b/Pb/22/1/1, 268/Pb/22/2/3, Td 68, DNJ-128, DNJ-101, DJ-74*, DJ-41, DZ-192, DK-11, DL-10, DD-6, DD-54, DD-100, Ctg 1206, DV-52, DV-83, DV-101, Basmati (C5875).

c) Susceptibility 0/1 + 2 intermediate reactions (3.0 - 4.0)

Ram Tulasi (sel), Hashikalmi, Kataktara, Tan Chet Cut, Zilanica*, Pendok, Charnock (Aust), NP-130, T9, J-109, Ca 435/6/5/1, Ca 902/b/2/2, 406, 41 Mushkan, 3 month variety, E.L. Gophar, Nang Sawn, 268 b/Pb/22/1/2, 268 b/Pr/22/3/2, 268 b/Pr/50/2/2, Dissi Hatif, DZ-105*, DK-13, DL 2, DL-11*, DD-24*, DD-120, DM-30, DM-59, DM-68, DV-114, DV-150, T412 (W349), JC-170, St = 1, Columbia 1, MO-R-500 x Nato.

d) Moderate susceptibility (3 intermediate reactions = 3.0 - 4.0)

E-425, TPxB 3812A e DNJ 171.

Several new screening tests are planned to be established in the next agricultural year, in the States of Sao Paulo, Rio Grande do Sul, Parana and Para. Local cultivars will be tested in each of the mentioned States, along with introduced ones.

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* More than 50 per cent of the panicles were infected ("neck-rot")

It seems very important that standard uniform reading methods for evaluating rice blast infection be established, so as to permit the comparison of results obtained in tests carried out at different parts of the country.

Table 1 - Rice cultivars which showed resistance to blast in the 1969/70, 1970/71 tests and their reaction to other rice diseases.

	Origin	Life period	Cultivation system	Helmintospo-riosis	Cerios-poriosis	Mulata	Lista parda
Binicol	Philippines						
H - 4	Ceylon						
Down	USA						
Hsedni-56	?						
IR - 8 - 288 - 3	Philippines			30 MS			
Tremesino	Italy			30 MR		S	
Saturn	USA			70 MR		S	
IAC-1246 = (Pratão x Perola)	Brazil	130	upland		S	S	
IAC-5100 = (Pratão x Perola)	Brazil	130	upland		S		S
60/1175 = (Pratão x Perola)	Brazil	130	upland		S	S	S
64/32 = (7-V-4 x 59/442)	Brazil	130	upland		S	S	
64/9 = (6-V-1 x 3 meses)	Brazil	130	upland		S		
IAC-5544 = (Pratão x Perola)	Brazil	130	upland		S		
Taiwan n°1	Formosa					S	S
6047 Days to flower 93	British Guiana					S	
60/80 = (Palawar x Pratão)	Brazil	140-150	irrigated			S	S
IAC-68* = (Iguape-agulha x Nira)	Brazil	140-150	irrigated			S	
IAC-1300 = (Iguape-agulha x IAC-3)	Brazil	140-150	upland				S
Victory**	India					S	
BMT - 22**	Philippines						

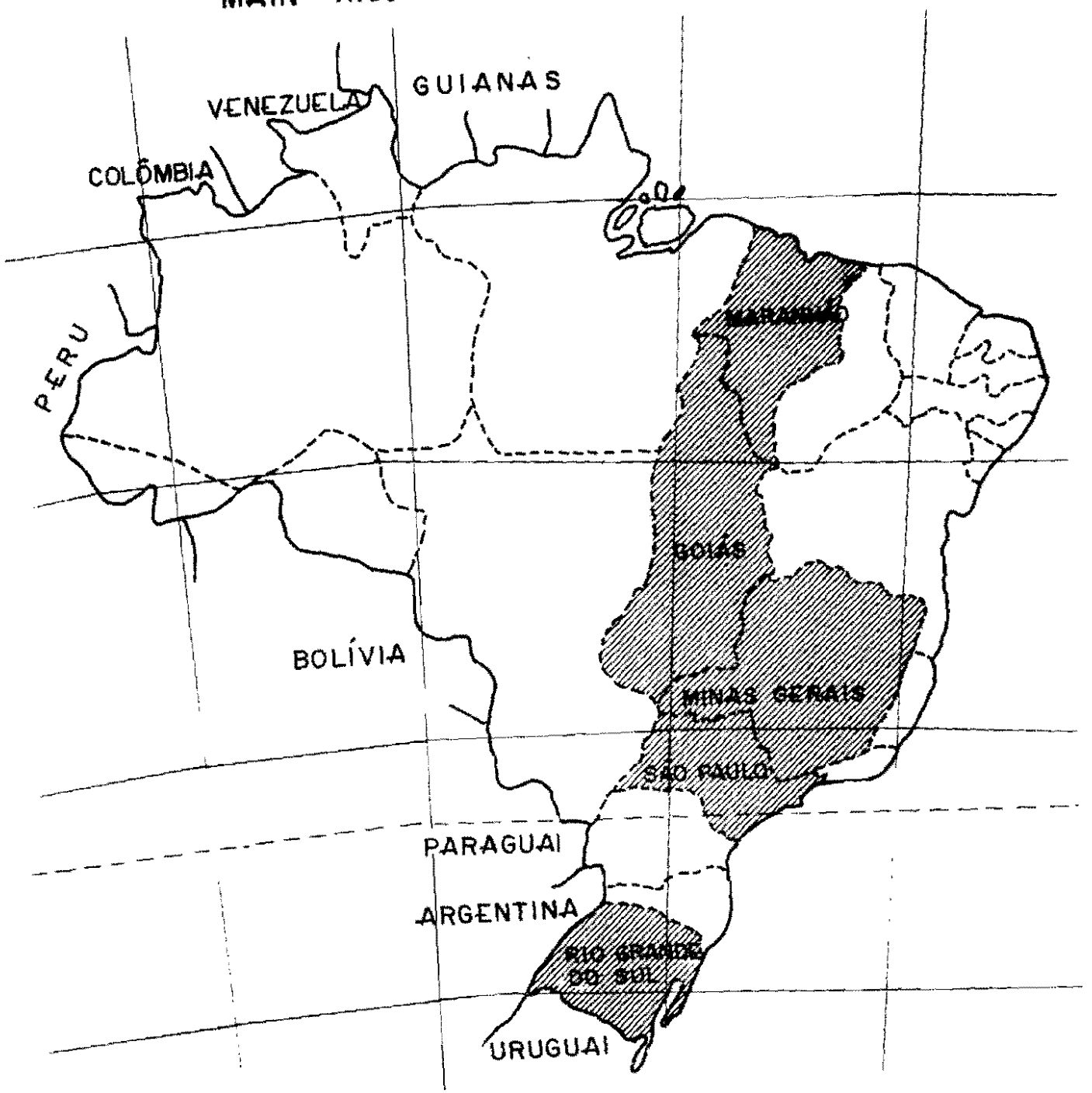
OBSERVATIONS: * Included in the 1970/71 test.
** MS Reaction to blast on the joint of the leaf sheath and leaf blade in 1970/71 test.

NOTES: Iguape-agulha = Mass selection-Instituto Agronomico of Campinas.
Perola = Mass selection-Instituto Agronomico of Campinas.
Pratão = Mass selection-Instituto Agronomico of Campinas.
3 meses = Mass selection-Instituto Agronomico of Campinas.
7-V-4 = (Nira x Dourado-agulha).
6-V-1 = (Nira x Dourado-agulha).
IAC-3 = (Jaguari x Yola).

Map. 1

BRAZIL

MAIN RICE PRODUCING STATES



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Centro Internacional de Agricultura Tropical

THE RICE BLAST DISEASE IN AFRICA

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SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

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The importance of rice in tropical Africa

At the beginning of the 1960's, annual rice consumption in Africa amounted to about 2.7 million metric tons. In the FAO Indicative World Plan the quoted estimate for annual rice consumption in Africa by 1985 is in excess of 5 million metric tons. Rice production in most tropical African countries is inadequate to meet the present consumption, and imports of rice add a heavy burden to foreign exchange balances. In the coming years, if the demand for rice is to be met, there must be either an excessive increase in rice importation or a massive increase in home rice production. The importance of rice in the kitchens and economies of the various countries of tropical Africa is shown in Table 1 (p.2). With the exception of Madagascar the East African countries are in general not so rice oriented as are many of the Central and West African countries.

Present unit yields of rice in Africa are estimated to be very low, with an average of about 1,000 kg per ha. There is obviously a great deal of improvement to be made in the technology of production on the present area cropped with rice. Also there are huge areas which have the potential for rice production which have not yet been developed. Certain West African countries (e.g., Ivory Coast, Ghana, Nigeria) have initiated programmes to increase rice production through both the expansion of the area cropped and promotion of improved technology (provision of new varieties, provision of fertilizer subsidies, etc.). However, considerable efforts will have to be made on many aspects (production, marketing, prices, supply of seed to growers, extension services, etc.) for rice production to meet present and projected demands.

Table 1: Rice production, trade and consumption data for tropical African countries*

Country	Population[1]	% in Agric.	Rice Prod Prod[2]	Rice Prod Area[3]	Rice Movements Imports[4]	Rice Movements Exports[4]	Ann. per capita consumption (kg)
ierra Leone	2514	80	400	300	300	-	115.35
iberia	1159	80	152	190	344	-	114.75
ambia	364	88	20	18	86	-	60.44
enegal	3916	75	126	89	1534	2	60.01
uinea	3988	85	330	250	187	-	58.43
vory Coast	4553	81	336	300	241	-	52.71
lali	5058	90	140	162	-	-	17.99
hana	8839	60	43	36	401	-	7.69
pper Volta	5438	86	42	50	38	-	5.70
iger	3851	91	33	12	14	-	5.97
igeria	63578	79	391	240	15	-	4.03
ogo	1863	79	32	34	27	-	1.29
ongo (K)	16353	70	120	115	450	-	7.52
had	3410	95	33	25	-	-	6.16
abon	473	84	1	1	16	-	4.24
ngola	5239	83	33	22	20	30	3.82
ent. Afr. Rep.	1459	85	7	7	1	-	3.43
ameroun	5470	84	13	13	89	1	3.11
ongo (B)	860	65	3	3	14	-	2.33
ambia	3968	81	-	-	35	-	1.01
adagascar	7224	83	1700	820	-	400	147.43
anzania	12173	95	115	110	24	4	6.33
ozambique	7124	69	74	51	-	53	6.04
ganda	7934	91	8	3	82	1	1.64
enya	9928	84	16	3	-	1	1.01
umandi	3400	95	4	3	-	-	0.88
udan	14355	77	2	1	69	-	0.56
alawi	4130	80	4	5	14	-	0.48

1. 1,000 people
2. 1,000 metric tons
3. 1,000 hectares
4. 100 metric tons

* Table prepared by Dr. D.D. Hedley, IITA Economist.

Rice culture in tropical Africa

The various types of rice culture are differentiated in terms of the way water is made available to the crops. They include:

(1) Upland rice, which is entirely dependent upon precipitation for its water supply;

(2) Swamp rice, which is grown in flood plains or poorly-drained low lying areas, and stands in surface water for much of its development, the level of which is not controllable;

(3) Rice grown in irrigated paddies where the water level is closely controllable by the farmer;

(4) Floating rice, which is grown in areas where the water level rises greatly throughout the season, and varieties are needed which are able to elongate rapidly to keep 3-5 leaves above the water level.

Upland rice accounts for over sixty-percent of rice production in West Africa (Cooper, 1970) and swamp rice makes up most of the remainder.

Rice research centres

Research into rice production has been carried out at a number of centres for many years in West and Central Africa. At the end of the second World War regional rice research bodies were set up by the various colonial powers, e.g., Centre Des Recherches Rizicoles at Koba, Guinea, whose activities covered all countries of the former French West Africa; the Rice Research stations at Rokupr (Sierra Leone) and Badeggi (Nigeria) for the Commonwealth countries; and the Yangambi Research Station of the then Belgian Congo. When the countries gained independence the research

stations met with various fates. For the Francophone countries the Institut de Recherches Agronomiques Tropicales et des cultures Vivrieres (I.R.A.T.) was created in 1960, and conducts research into rice in Madagascar, Ivory Coast, Mali, Senegal, Niger, Cameroun and the Central African Republic. In the Anglophone countries rice research is undertaken at the Rokupr Station, Sierra Leone (which has undergone several changes of administration and is now part of Njala University of Sierra Leone); Kumasi and Kpong Stations in Ghana; Badeggi Federal Rice Research Station, Nigeria; and very recently the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria. Within the last two years an attempt has been made to establish a West African Rice Development Association (WARDA) but at the present time WARDA is still very much in the planning stage.

Blast disease in Africa

The first report of blast disease in Africa was made by Small (1922a & b) who stated that the blast of rice consistently reduced yields in Uganda and was the only major disease of rice in that country. Since 1922, blast has been reported wherever rice has been grown in Africa (Anon., 1968a) and many workers cite blast as the most severe rice disease problem encountered (Bunting, 1924 and 1928; Deighton, 1936; Hansford, 1943; Anon., 1951; Lawrence, 1951; Anon., 1960; Abo-El-Dahab & Michails, 1966; Awoderu, 1970).

In Africa, as in Asia, blast can occur in the rice crop at all stages of growth. As much of the rice crop in Africa is dependent upon direct precipitation for its water supply, and as rainfall is unreliable, especially at the beginning of the wet season (Cocheme, 1971), rice can be expected to experience periods of drought stress, which according to the evidence of Suzuki (1934 & 1935) will increase its

susceptibility to blast. Lamey (1971, unpublished) noted relatively little blast in an irrigated nursery at IITA, whereas in an upland nursery nearby, several varieties (which were also present in the irrigated nursery) developed severe leaf blast. The effect of drought stress on varietal susceptibility will have to be carefully considered when techniques for the assessment of varietal reactions to blast are being developed. Periods of drought alternate with periods of heavy rains with overcast skies for many days, and during these periods the rice plants continuously have free water on their leaves and culms. Rice which is heading in these wet, overcast conditions is particularly vulnerable to the rotten-neck phase of blast. In a summary of rice research by IRAT in West Africa (Anon., 1970) it is stated that when rice is heading during heavy rains without sun, neck-blast is so severe that chemical treatment is the only way to save the crop. At IITA, varieties have been observed with very little leaf blast and extremely severe neck-blast (e.g., IR22, M7-28). Clearly, both the leaf and panicle phases of the disease need be examined in evaluation of varietal resistance to blast.

The variability within the population of Pyricularia oryzae in Africa has not been studied in any great detail. The International Blast Nursery has been tested in Sierra Leone and Nigeria and the results indicate a wide spectrum of pathogenicity within P. oryzae in these countries. The results of IBN tests in Nigeria (at the Badeggi Federal Rice Research Station and at IITA, Ibadan) reveal the presence of virulence within the local population of P. oryzae to several varieties (E425, ArmitsariHR22, DL10, DNJ-60, Rajbohog,, DV-73, CI 7787, CI 27-3, Tua Sart, 3 month variety /Entry 21/, C5561 & TD 70) that have been consistently resistant in most other parts of the world (Ou et al 1970; Tables 2 & 3). Awoderu (1970) described eleven physiologic races of P. oryzae in Nigeria, but from the

Table 2. The blast reactions at IITA of those varieties found most resistant in the International Blast Nurseries.

No.	Group I Vars.	Test 1	2	3
11	Tetep	2(4)	3	1
88	R67	2(3)	1	2
41	C46-15	1	4	1(2)
105	Nang Chet Cuc	2	1+	1
12	Tadukan	2	2(4)	1
1	C17787	3	2	3
98	Pah Leuad 29-8-11	2	1+	1+
42	D25-4	2	1+	1+
106	Trang Cut L 11	2	1	1
122	Pah Leuad 111	2	1+	1+
44	M-302	2	2	1
76	Padang Trengganu 22	2	1+	1(2)
91	E-425	5	4	3(4)
56	Ram Tulasi (SEL)	1	1	1(2)
119	Ramadja	2	1+	1
46	H-5	2	1+	1
117	Dawn	-	-	-
63	No. K-60	4	3	1(3)
123	MO-R-500 x Nato	-	-	-
79	K.P.F. 6	3(4)	3	3(4)
78	Kataktara DA-2	-	-	-
45	H4	1	2(3)	1(2)
101	Zenith	2	4	2

A minus sign following lesion type indicates very few lesions.

A plus sign following lesion type indicates very many lesions.

A figure in parenthesis following another figure, e.g., 3(4) indicates that although there were a few of the more severe reaction the predominant reaction was that given by the first figure.

Table 3: The blast reactions at IITA of those varieties found most resistant in the International Blast Nurseries.

No.	Group II Vars.	Test 1	2	3
137	Mamoriaka	2	1	2
244	Dissi Hatif (DH-3)	2(3)	1	2(3)
126	Pah Leuad 29-8-11	2	1+	1(2)
128	Ram Tulabi	2	1	1(2)
149	NP 130	2(3)	1+	1(2)
164	Huan-Sen-Goo	1	1-	1
189	Thavalakkannan Ptb 9	2	1	1
201	Ca 902/b/2/2	2	2	1(2)
203	Amritsari HR22	2	3(5)	1
127	Ram Tulabi (sel)	1	1	1(2)
291	DL-10	3(4)	5	3
217	C46-15	2	3(4)	1(2)
257	DNJ-60	2(3)	3(5)	1(2)
136	Unblatuzi Valley	2(3)	2	1(2)
147	T3	1(2)	2	1(2)
255	DB-3	4	2(3)	3
205	370 Basmati	2(3)	2	1
254	Pusur	3(4)	5	1
135	T1 (3392)	2	1	1+
265	DNJ-146	2(3)	2	3-
180	Carreon	2	1	1+
191	T1 (3294)	2	1	1(3)
196	Ca 435/b/5/1	2	1(4)	2
233	2686/Pr/8/1/1	2	1+(4)	1+
207	T23	2(3)	1(2)	1(3)
224	E.L. Gophar	2	1	2
188	Rajbohog N22	3	4(5)	3
328	DV-73	3(4)	5	3-

A minus sign following lesion type indicates very few lesions.

A plus sign following lesion type indicates very many lesions.

A figure in parenthesis following another figure, e.g. 3(4) indicates that although there were a few of the more severe reaction the predominant reaction was that given by the first figure.

evidence of Ou and Ayad (1968) on the extreme variability within monoconidial cultures of P. oryzae it is probably more valid to describe a spectrum of virulence within a local population of P. oryzae than to attempt to define distinct physiologic races. The IBN tests should be established in many more locations throughout the rice-growing areas of tropical Africa to provide comprehensive information on the spectrum of virulence genotypes of P. oryzae in the continent.

Resistance to blast has been a selection criterion in several rice-breeding programmes in West Africa for many years, and varieties have been developed with quite a high degree of what appears to be horizontal resistance (e.g., the OS6 and OS4 varieties developed at the Yangambi Research Station in the Congo). However, these varieties have a relatively low yield ceiling, with poor plant type and poor quality grain, and improved varieties are being introduced from Asia and elsewhere. Several of the IRRI varieties (IR5, IR8, IR22) have been found particularly susceptible to blast in Africa, and resistance to blast continues to be one of the major selection criteria in most of the rice-breeding programmes, in Africa.

Blast disease at IITA

The rice programme is one of the major crop improvement programmes already underway at IITA. The objectives of the rice pathology team are stated as:

(a) identify and develop broad spectrum (horizontal) resistance to the major African diseases of rice;

(b) explore methods of chemical control of rice diseases;

(c) study pathogen variability, ecology and biology, and in collaboration with other international bodies organise and stimulate interest in Pan-African blast tests (both the International Blast Nursery and horizontal resistance nurseries)

During 1970, 874 entries were planted in both upland and irrigated nurseries for comparative evaluation and seed increase. Frequent disease observations were made in both nurseries and disease encountered included blast, leaf scald (Rhynchosporium oryzae Hashioka & Yokogi), brown leaf spot (Cochliobolus miyabeanus Ito et Kuribayashi), narrow brown leaf spot (Cercospora oryzae Miyake) and false smut (Ustilagenoide virens (ckc.) Tak.). The only serious disease problem was blast. Of 539 entries assessed for leaf blast reaction in the upland nursery in 1970, 216 were highly resistant (Reaction types 1 or 2), 78 were moderately resistant (Reaction type 3 or very few type 4 lesions), 189 were moderately susceptible (Reaction type 4) and 58 were highly susceptible (Reaction type 5 and above). During 1971, 387 entries were grown in upland nurseries and again a wide spectrum of varietal reactions was observed. The varieties that consistently develop reaction type 3, or very few type 4 lesions, will be the candidate varieties for further testing for horizontal resistance.

As stated above the International Blast Nursery (IBN) was tested at IITA on three occasions, and varieties were observed to be susceptible at IITA that are resistant in most other locations where the IBN has been tested. Several varieties were highly resistant in one planting, and highly susceptible in another, indicating a shifting spectrum of virulence in the local population of P. oryzae. In the future the IBN tests will be planted more frequently at IITA, and the IITA rice team will promote the establishment of many testing locations throughout tropical Africa for both the IBN and horizontal (or partial) resistance nurseries.

Studies of horizontal resistance to blast at IITA

The programme to develop varieties of upland rice with a high degree of horizontal resistance to blast is just getting underway at IITA. Ideas on methods

for the selection of the resistance are being developed and tested. One idea is to obtain a precise measurement of those host-controlled factors that affect the rate of development of an epidemic (e.g., incubation period, sporulation capacity related to lesion size, number of lesions per unit leaf area, efficiency of spore production per unit lesion area) and compare varieties for these parameters. This would be done with controlled inoculation with a standard concentration of conidia, a standard drop size, and a controlled environment for maintenance of seedlings (the use of detached leaf segments offers the greatest precision in such an operation). However, degree of varietal susceptibility in the field will depend upon the interaction of many factors including:

(i) the complement of major resistance genes the variety possesses;

(ii) the complement of polygenes for resistance in the variety;

(iii) the complement of virulence genes in the inoculum;

(iv) the aggressiveness of the races in the inoculum;

(v) environmental conditions including soil-water relations, soil nutrient status, temperature, humidity, etc.

The questions that arise when considering precise measurement of host-controlled epidemic factors include:

(i) what nutrient levels are to be used in raising the plants;

(ii) should the plants be subjected to water stress, and if so, how much;

(iii) do seedling leaf reactions tally with the reactions of adult plant leaves;

(iv) what is the spectrum of virulence in the inoculum;

(v) even if leaf blast resistance is good, is the variety resistant to neck rot phase of the disease.

Precise measurement of the host controlled epidemic factors mentioned above will be conducted in conjuction with field testing at many locations, with several dates of planting at each location. In this way an evaluation will be made of the usefulness of the controlled precise measurement method for identification of horizontal resistance.

Chemical control of blast in Africa

Many fungicides are known that can control the blast disease organism, but their usefulness depends upon the economics of their application. In Africa, yield ceilings for rice are very low compared with those in many parts of Asia; there is no chemical industry, and pesticides are very expensive; there is a low availability of hardware for the application of pesticides and the general level of technical skill at the farm level is low. At the present time, therefore, there is little practical use of chemical control of blast in Africa. Tests are being made on the effectiveness of various chemicals for blast control at various research stations in Africa (Anon., 1968b, Anon., 1969, Anon., 1971), and at IITA in 1971 over threefold increases in yield were obtained with two applications (at 50 percent flowering and again 12 days later) of Benlate (600 g in 1000 l per ha) with the rice variety IR22. At the present time the information gained on chemical control can be usefully applied to control blast in experimental crops of rice, but it is the efforts of the geneticists and plant breeders that can be expected to have the major technological impact in the control of rice blast disease in Africa in the next decade.

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Centro Internacional de Agricultura Tropical

HORIZONTAL RESISTANCE: SIX SUGGESTED PROJECTS IN RELATION TO BLAST DISEASE OF RICE

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SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

HORIZONTAL RESISTANCE: SIX SUGGESTED PROJECTS IN RELATION TO BLAST DISEASE OF RICE

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There are two possible sorts of resistance to disease: vertical resistance and horizontal resistance. Vertical resistance is when there is a differential interaction between varieties (genotypes) of the host plants and races of the pathogen. Horizontal resistance is when there is no differential interaction, i.e.,when resistance is spread equally against all races of the pathogen.

Vertical resistance is 'lost' when a new race of the pathogen arises that can attack the host genotype. Pyricularia oryzae has shown itself to be plastic, and well able to produce the virulence genes that match and make useless the resistance genes which breeders have introduced into rice cultivars. Horizontal resistance on the other hand is not affected by the plasticity of the pathogen. The pathogen reaps no benefit from producing new races because horizontal resistance acts against all races. A horizontally resistant cultivar remains a resistant cultivar, however much the pathogen can vary. That is the advantage of horizontal resistance.

Vertical resistance can be introduced into cultivars relatively easily (if the necessary resistance genes are available), and its effects are clearly and immediately obvious. That is why rice breeders and pathologists have chosen it in the past.

Horizontal resistance is relatively difficult to introduce into cultivars, and its effects are often obscure and not immediately

apparent. Rice breeders will turn to horizontal resistance, not because it is convenient to use, but because it is necessary. As the years go by and rice becomes more and more vulnerable to blast, so will horizontal resistance become more and more necessary.

The manifestations and inheritance of vertical resistance to P. oryzae have been relatively well studied. Horizontal resistance on the other hand has been less studied in detail; and much of what we have to say of it must be inferred from what we know about other diseases.

Manifestations of vertical and horizontal resistance

If leaves of a young rice plant are infected artificially with spores of P. oryzae, the presence or absence of vertical resistance in the rice plant can be determined within a few days. If the plant responds by forming only reddish flecks or small reddish spots without differentiation into distinct zones, the plant is vertically resistant to the pathogenic race. If the plant responds by forming large spindle shaped lesions several millimetres broad, and these lesions in time bear spores, the plant is vertically susceptible. The criterion of vertical resistance is the type of lesion, not the number of lesions. (Horizontal resistance affects both the number and the type of lesion, the type of lesion reflecting the abundance of sporulation.)

Horizontal resistance manifests itself in three ways. First, the number of lesions formed in a horizontally resistant variety is less than in a susceptible variety, in the same conditions and inoculated with the same number of spores. Second, the time taken by a newly formed lesion for itself to form spores (i.e., the period between inoculation and subsequent sporulation) is longer in a resistant

variety. Third, sporulation is less abundant in lesions on a resistant variety. (This third manifestation should be subdivided to allow for the duration as well as the abundance of sporulation, but we shall ignore the distinction here.)

Horizontal resistance is determined by quantitative characters (the number of lesions produced by a given number of spores, the period needed for lesions to sporulate, and the amount of sporulation). Research projects must therefore be based on quantitative measurements in natural conditions.

Suggested projects

First: Determining horizontal resistance as field resistance

In the absence of vertical resistance, resistance is horizontal resistance. Therefore, if one can exclude all vertical resistance, one can simply compare cultivars or lines in the field, and the comparison will measure horizontal resistance alone. This is the simplest and most direct method.

When is vertical resistance absent? On available knowledge, the answer is: when the lesions are of a vertically susceptible type, i.e., when the lesions are large and normal and classed as reaction type 4 or 5 in the U.S. classification.

The method, then, is to expose lines (or cultivars) to infection in the field by virulent races to which the lines are (vertically) susceptible. The resistance that remains is horizontal resistance.

The difficulty comes in here. The lines must be exposed to a race virulent on all of them or to several races, each of which is virulent on all of them. False results are given whenever lines are exposed to a mixture of races, some of which are virulent on some

races but avirulent on other races. (Then vertical resistance enters and confuses the results.) The easiest comparisons are when all the lines are susceptible to all the local races. Otherwise special precautions are needed.

Second: The selection of lines and cultivars that are more difficult to infect

The problem here is to select the rice lines and cultivars that are the most resistant to infection. That is, if one uniformly inoculates several lines or cultivars, one wishes to select those that develop the fewest lesions per plant or per leaf or per square centimetre of leaf. The principle is easy; the practice may be difficult.

First, one needs an inoculator that gives reproducible results so that lines or cultivars can be accurately compared.

Second, one must avoid artifacts. Conditions must be natural. Plants should be of an age at which blast epidemics normally occur; one cannot assume a priori that comparisons made with young seedling plants will hold for older plants. Plants must be grown under natural conditions; one cannot assume a priori that plants raised under cover behave like plants in a rice field. Therefore, one must devise a method of growing plants in the field, bringing them to the inoculator and returning them immediately to the field without significant disturbance; or, alternatively, one must devise an inoculator that can be used in the field.

Third, the ratio of the number of lesions to the number of spores used as inoculum varies with the concentration of spores, if the concentration is high. Therefore, one should aim at spore concentrations

that give no more than an average of one lesion per square centimetre of leaf surface.

Fourth, it is worth investigating the possibility that resistance to infection as a manifestation of horizontal resistance can be measured even in the presence of vertical resistance (i.e., in the presence of hypersensitivity). It is possible that one could count the average number of infections per square centimetre of leaf surface, irrespective of whether the infections are hypersensitive flecks (indicating vertical resistance) or normal lesions. This would greatly expedite investigations. To test the possibility one could compare two or more rice lines or cultivars by inoculating them with a race of P. oryzae virulent on all of the lines (i.e., giving normal lesions on all); then repeating the experiment with a race avirulent on all the lines (i.e., giving only hypersensitive flecks on all the lines); then repeating it again with a race virulent on one line but not the others; and so on. If one then ranks the rice lines in order of decreasing resistance, starting with the line that gives the fewest infections (lesions or flecks) per square centimetre of leaf, the order of ranking should be independent of the race used. In other words, all the different races of P. oryzae should indicate the same rice line as being the most resistant.

I have written enough to show that a great amount of preliminary research is needed before one can begin to measure resistance to infection quantitatively.

Third: The selection of lines and cultivars in which the period from inoculation to sporulation is greater

A long period between inoculation and sporulation, i.e., a long

period needed for newly formed lesions to start releasing spores, is a manifestation of horizontal resistance that can be selected for. If plants of different rice lines or cultivars are inoculated and examined regularly, the period needed before sporulation can begin can easily be measured.

The isolate of P.oryzae used to compare the lines must be virulent on all the lines (i.e.,there must be no complications from vertical resistance). Artifacts must be avoided. (Avoid using detached leaves in a laboratory.) Plants must be of an appropriate age, and must be grown under natural conditions.

Fourth: The selection of lines and cultivars on which sporulation is less abundant

The lines to be selected are those that produce fewest spores per lesion.

Again, use virulent isolates of P. oryzae and avoid artifacts.

It is possible that these second, third and fourth projects will select much the same lines. That is, it is possible that lines which are the most difficult to infect (second project) are on the average also the lines in which the period needed for sporulation is longest (third project) and also those in which sporulation is less abundant (fourth project). If this is so, it will be a great help.

Fifth: The accumulation of resistance by breeding

Horizontal resistance is almost certainly polygenic in inheritance, and should be accumulated by a programme of breeding. Selected lines or cultivars could be paired, and appropriate segregates isolated in the F3 or later generations. The parent lines and the segregates would of course be selected both for agronomic characters and for horizontal resistance.

A long-term project suitable for an institution to undertake would be to prepare a composite. Selected parents would be paired in all possible combinations, the F1's bulked, and the bulked composite grown for several generations to remove most of the heterozygotes. Plants could then be selected on agronomic characters to start new lines, and the lines then tested for horizontal resistance.

An intrinsic difficulty with horizontal resistance

The benefit of horizontal resistance does not show fully at the start. Horizontal resistance is a slowing-down in the rate of infection. This slowing-down is less evident in single rows or small plots of the host plants, because inoculum moves in from outside. Only when the horizontally resistant plants cover whole fields or the whole countryside do the full effects of the slowing-down become apparent. Only if resistance to infection (the subject of the second suggested project) is very high, will the benefit of the resistance be immediately apparent.

One must take care therefore not to ignore amounts of resistance which could ultimately be effective but which are not very impressive while the new line is confined to small experimental plots.

Sixth: The combination of horizontal with vertical resistance

Vertical resistance is immediately apparent even in small plots. Indeed, in relation to the number of hectares on which a cultivar is cultivated, vertical resistance and horizontal resistance show opposite trends. Vertical resistance is best in small plots; by the time the vertically resistant cultivar is grown over a large area (e.g., by the time it becomes the dominant cultivar in a country) new virulent races of the pathogen are likely to have developed and so

'destroyed'the resistance. Horizontal resistance on the other hand keeps on gaining effectiveness in a cultivar when that cultivar is grown over a larger and larger area.

The ideal solution then is to combine the two sorts of resistance. The vertical resistance will keep the cultivar protected in its early years. By the time the cultivar is widely grown, horizontal resistance can take over.

The two forms of resistance can be combined by using a horizontally resistant line as the recurrent parent in a programme of backcrossing.

Continuity with the past

The sixth suggested project is really a suggestion for continuity with the past plus horizontal resistance. In the past, vertical resistance has been used without proper attention to the horizontal resistance or susceptibility of the cultivar in which it is used. The change suggested for the future is that genes for vertical resistance should be incorporated by backcrossing only into lines which have been selected for horizontal resistance. This would apply both to new genes for vertical resistance and to old genes that have been used and are judged to be still worth using in new cultivars.

The sixth suggested project would still require that the other suggested projects be carried out in order to supply the horizontally resistant lines needed as recurrent parents in backcrossing.

Organization of research

It would be proper for the Symposium to consider what research is needed and how to organize it.

The suggested projects indicate what research is needed: research on the quantitative relations involved in horizontal resistance. Apparatus must be devised and built. It may be unnecessary to repeat this research in different countries; it may be possible to concentrate it at an international centre. That is also a proper subject for the Symposium to consider.

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resistance and that we should be working on methods to facilitate the transfer of both types of resistance to rice varieties. The availability of the sexual stage of the causal fungus will enable us to study the genetics of pathogenicity. More information is needed on the genetics of resistance in the host.

It would be highly desirable to have a set of rice lines each with a single major gene for resistance to Pyricularia preferably in a common genetic background. A good example to follow is the case of resistance to powdery mildew in wheat (1) where variety of wheat (Chancellor) susceptible to all known races of the powdery fungus was selected and single major genes for resistance were transferred to this variety in a backcrossing program. Such a set of rice lines with single major genes for resistance would greatly facilitate the monitoring of genes for pathogenicity in Pyricularia in the various rice-growing areas of the world and would facilitate the identification of races of the fungus.

banana and pearl millet produced perithecia containing only a few ascospores. In addition, sterile perithecia (without ascospores) were produced by two other rice isolates.

Discussion

At the initiation of these studies Pyricularia oryzae Cav. was considered to be a specialized pathogenic form of P. grisea. It seemed reasonable to suppose that at one time this fungus produced a perfect stage in nature and that with the passage of time the genetic factors responsible for the production of the perfect stage have been disappearing from the fungus population. Since Pyricularia isolates from rice appear to be more highly specialized pathogenically than isolates from wild grasses, they are considered to be more highly evolved and therefore should have a lower probability of still retaining genetic factors for producing the perfect stage. Results thus far tend to confirm this idea. Only one isolate from rice, of 140 tested, was fully fertile whereas 10 of 62 isolates from other hosts have produced ascospores. Testing of these isolates is continuing and it may be that others will be found to be fertile.

While the emphasis at this symposium is on horizontal resistance to Pyricularia in rice, I think that we should not neglect vertical resistance. I believe that our eventual goal should be varieties with combined horizontal and vertical

crabgrass and also with 6 other rice isolates from this group. In one of these matings a rice isolate from the north coastal area of Peru produced fertile perithecia with a minus tester line derived from crabgrass isolates. Single ascospore isolates have been obtained from this cross and tests have been initiated to determine the pathogenicity of these ascospore isolates to rice and to crabgrass.

In other tests, Latterell's isolate 904 from banana produced a few perithecia with ascospores when mated with a plus isolate from crabgrass. Isolate 906, also from banana, produced a few fertile perithecia when mated with a minus isolate derived from crabgrass. A mating of isolate 904 with isolate 906 failed to produce perithecia. In matings with the nine isolates from pearl millet, one isolate produced a few fertile perithecia with few ascospores when mated with a minus isolate from crabgrass. Five of the 19 isolates from crabgrass from North Carolina were fertile; 3 of these were of the plus mating type and 2 belonged to the minus group. The 10 isolates from St. Augustine grass from North Carolina and the 9 isolates from this host from Peru failed to produce perithecia.

To date, 11 Pyricularia isolates have produced fertile perithecia. One of these is from rice, seven from crabgrass, two from banana, and one from pearl millet. The isolates from

when mated with certain other tester lines. Latterell's isolate 883 from rice from Arkansas and isolate 46 from rice from Peru produced perithecia but no ascospores when mated with some tester lines. These three isolates reacted only with lines of the minus mating type.

The 22 additional isolates obtained from Latterell were mated with each other in all possible combinations. Each isolate was also mated with ten tester lines of the plus mating type and with ten tester lines of the minus mating type. Isolate No. 776 from Digitaria from Florida produced fertile perithecia with 7 of the 10 minus tester lines. Isolate 904 from banana from Honduras produced two empty perithecia with one of the plus mating lines. The other matings produced no perithecia. Each of these 22 isolates was then mated with each of the 32 isolates from rice listed in the previous report (2). No perithecia were produced in these 704 matings.

Each of the 17 Pyricularia isolates obtained from rice from Peru in December 1970 was mated with 4 plus tester lines and 4 minus tester lines from crabgrass and also with 8 other rice isolates from this group. No perithecia were produced. Each of the 74 isolates obtained from rice from Peru in May 1971 was mated with 3 plus and 3 minus tester lines from

438 rice Taiwan (IE-3), 453 rice El Salvador (IB-33), 479 rice the Philippines (ID-13), 499 rice India (ID-8), 540 Oryza rufipogon Australia (IG-1), 549 rice India (IE-1), 559 rice Malaya (ID-16), 590 rice Japan (IH-1), 603 rice Arkansas, (IH-1), 649 rice India (ID-1), 721 rice Japan, 740 rice Sierra Leone (IB-35), 748 rice the Philippines (ID-14), 776 Digitaria Florida (II-1), 794 rice Dominican Republic (IG-1A), 825-D-6 rice Costa Rica X-ray (IB-1), 900 rice Louisiana (IE-5), 901 rice Louisiana (IG-1), 904 banana Honduras (II-1), 906 banana Honduras (II-1), 907 platano Honduras (II-1) and 908 Setaria Maryland (II-1). Nine cultures of P. grisea from pearl millet (Pennisetum glaucum (L.) R. Br.) from Georgia were obtained from Dr. Homer Wells. The following additional isolations were made by the author: 19 isolates from crabgrass from North Carolina, 10 isolates from St. Augustine grass from North Carolina and 9 isolates from this host from Peru, 17 isolates from rice from Peru in December 1970 and 74 isolates from rice from Peru in May 1971. Matings were made on Sach's agar as described previously (2).

Results

Further testing of the isolates listed in the previous report (2) showed that Latterell's isolate 888 from crabgrass from Arkansas which had produced very few perithecia when mated with certain tester lines produced an abundance of perithecia

PRODUCTION OF THE PERFECT STAGE OF *PYRICULARIA* FROM RICE AND OTHER HOSTS

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The perfect stage of *Pyricularia grisea* (Cke.) Sacc., *Ceratosphaeria grisea*, was produced in culture by mating two isolates of the fungus from crabgrass (*Digitaria sanguinalis* (L.) Scop.) from North Carolina (2). An isolate of the fungus from crabgrass from Arkansas also produced a few perithecia when mated with tester lines of fertile ascospore isolates from the original and subsequent crosses. Seven other isolates of *Pyricularia* from crabgrass from North Carolina, one isolate from St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze) from Peru and 32 isolates from rice (*Oryza sativa* L.) from various parts of the world failed to produce the perfect stage in matings with the tester isolates. This paper reports the results of further mating tests with these and with additional isolates of *Pyricularia* from rice and other hosts.

Materials and Methods

The following *Pyricularia* isolates with origin and race number (in parenthesis) were obtained from Dr. Frances Latterell:

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Resistencia horizontal al
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Octubre 8-12, 1971

Centro Internacional de Agricultura Tropical

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INTRODUCTION

Since the establishment of scientific rice breeding in Japan, great efforts have been made to develop the varieties possessing resistance to blast disease caused by Pyricularia oryzae Cav. As a result, some outstanding blast resistant varieties have been developed. These blast resistant varieties have greatly contributed to the stabilizing of rice production by controlling an epidemic of blast in Japan.

The blast resistant paddy rice varieties which have been developed hitherto in Japan were classified into four groups: 1) varieties developed from the crosses among Japanese domestic varieties, 2) varieties derived from the cross with the upland rice variety, 3) varieties possessing the resistance gene incorporated from Chinese ones of the japonica type, and 4) varieties possessing resistance gene or genes in the indica varieties. Of these, the varieties belonging to the third and fourth groups had been generally considered to be highly resistant to blast until they were unexpectedly affected by blast more severely than Japanese domestic varieties were.

Breakdown of high resistance from alien varieties occurred within three to five years after the release of the varieties

possessing resistance of this kind. The damage on the highly resistant varieties was recognized to be due to the selective propagation of newly developed pathotypes to the resistance gene or genes from alien varieties. Therefore, utilization of horizontal resistance to blast has been emphazized in the rice-breeding program in Japan.

Horizontal resistance has been called either field resistance or generalized resistance. In this report, the term "field resistance" will be employed in place of the term "horizontal resistance", because resistance showing horizontal reaction in a strict sense by Van der Plank (1963) has not been observed in any rice varieties by the Japanese investigators up to date. Rice breeders in Japan, therefore, classified blast resistance into two categories: "true resistance" and "field resistance". In this sense, true resistance is specific and qualitative resistance characterized by a hypersensitivity to the pathogen. On the other hand, field resistance is recognized to be remainders of resistance other than true resistance. For clarifying an essential nature of field resistance, main efforts of investigation in Japan have been paid on the basis of information on true resistance with the procedure of elimination method.

The present report will cover the recent progress of studies on field resistance to blast together with some important investigations on true resistance in rice breeding in Japan.

Existence of field resistance to blast

The varieties possessing the true resistance gene or genes exhibit a high resistance due to a hypersensitive reaction to the pathogen when the varieties are exposed to the fungus race without virulence to true resistance. In this case, field resistance of

the varieties can not be exhibited because true resistance plays epistatically over field resistance. Field resistance, therefore, is distinguished under such conditions where the fungus races virulent to all the true resistance gene or genes of given varieties are prevalent.

Information on the true resistance genes and on the virulence of fungus race were materially necessary to investigate field resistance to blast, because varietal difference on field resistance can be discriminated by the virulent fungus races only on the same bases of the true resistance genotype. In Japan, genotypes for true resistance to blast were estimated by the reaction pattern to the injection testing method using the seven standard fungus isolates selected by Ymasaki and Kiyosawa (1966). By the injection method and some additional means, 11 genes for true resistance to blast were found by Kiyosawa and his co-workers (1971). Those are Pi-a, Pi-i, Pi-ta, Pi-ta^2, Pi-z, Pi-z^t, Pi-k, Pi-k^s, Pi-k^p, Pi-k^h and Pi-m. By the reaction pattern to the seven standard isolates in the injection test, Japanese rice varieties, including domestic and newly bred varieties with alien blast resistance genes, are classified into 12 reaction types; Shin 2 type, Aichi-asahi type, Kanto 51 type, Ishikari-shiroke type, Yashiro-mochi type, Pi 4 type, Fuku-nishiki type, Toride 1 type, To-to type, Shinsetsu type, Shimokita type and Zenith type (Kiyosawa, 1967; Yokoo and Kiyosawa, 1970).

For dividing reaction types into more detail than the above system, some other fungus strains can be used as shown in Table 1. Thereby, 24 varietal groups can be discriminated according to the reaction pattern.

In the testing field of the blast nurseries, it has been observed that resistance to blast is different among the varieties possessing even the same genotype for true resistance. The varietal difference within the same genotype for true resistance is considered to be caused by the difference in field resistance of given varieties (Hirano et al., 1967; Hirano and Matsumoto, 1971; Asaga and Yoshimura, 1969).

Evaluation of degree of field resistance

If the varieties lack field resistance, they are severely affected by the virulent fungus races against the true resistance gene or genes. Field resistance of the varieties is estimated by the degree of damage in the field where the virulent fungus races are prevalent. In general, constitution of the fungus races varied with year, location and season (Goto et al., 1964; Yamada and Iwano, 1970). If, for example, in Fukuyama, Hiroshima Pref., the strains belonging to the N race group propagated in the early season of the rice-growing period, then the strains belonging to the C race group followed (Matsumoto and Okamoto, 1963; Okamoto and Matsumoto, 1964; Ezuka et al., 1969b). This phenomenon of race change was repeated every year. Major fungus strains of the N race group collected in Fukuyama-field probably belonged to the N-2 race, because they showed virulence to Pi-a gene of the Aichi-asahi type but did not attack the gene Pi-i of Ishikari-shiroke type. The strains of the C race group which followed the N race group were estimated to belong to the C-8 race, because they were virulent to the Pi-k and Pi-a genes of To-to type and avirulent to Pi-i gene (Ezuka et. al., 1969b).

In Soma, Fukushima Pref., however, the composition of fungus races was distinctly different from that in Fukuyama. The gene Pi-k did not show any resistant reaction from the early season of rice growth. The gene Pi-a exhibited moderate resistance, because the major fungus strains in Soma were virulent to the gene Pi-k but were avirulent to the gene Pi-a.

Resistance in the field, therefore, did not directly indicate field resistance itself because of the complex reaction against races. If field resistance of varieties is evaluated only by the observed value in the testing field, field resistance of the varieties possessing Pi-i gene may be ranked at a high level of resistance, and field resistance of the varieties possessing Pi-k gene may be classified as high grade when observed in the early season of the rice growth in Fukuyama. For the same reason, field resistance of the varieties with Pi-a gene may be graded at higher classes than those without Pi-a gene in Soma. Of course, there were a few pathogens virulent to Pi-i and Pi-a genes in Fukuyama and Soma, respectively. These facts indicate that a comparison of field resistance is worthwhile only when within the varieties of the same genotype for true resistance.

Some attempts were made to develop the testing method for evaluating field resistance of rice varieties under the different conditions: paddy field, upland nursery, and the greenhouse.

Evaluation of field resistance in the paddy field

Ezuka et al. (1969b) made an attempt to evaluate field resistance of rice varieties in the paddy field at two locations. Some

varieties, which were representative of Shin 2 type (+), Aichi-asahi type (Pi-a), Kanto 51 type (Pi-k), To-to type (Pi-a, Pi-k) and Ishikari-shirote type (Pi-i), were grown in the paddy fields of different localities, and their levels of field resistance were evaluated by the number of susceptible lesions per hill. Although the outbreak of blast in the varieties with Pi-k gene was delayed as compared with that in the varieties without Pi-k gene, marked differences among varieties within the same genotypic group were observed (Fig. 1). In this experiment, it was pointed out that the true resistance gene Pi-a had no influence on the degree of damage, probably due to prevalence of the fungus races virulent to Pi-a gene. For comparison of field resistance of given varieties, therefore, the varieties belonging to Shin 2 type and Aichi-asahi type, and those belonging to Kanto 51 type and To-to type, were lumped together as the same group, respectively.

The results indicated that Moko-ine, Norin 8, Norin 17, Norin 18 and Jukkoku showed a low level of field resistance, especially Moko-ine, which showed the lowest. Ginga, Norin 22, Homare-nishiki, Fujiminori and Shuho showed a fairly high level, and St 1 showed the highest of all. Among the varieties with Pi-k gene, Kusabue and Yuukara showed a low level of field resistance, but Chugoku 31 showed a much higher level than the others. Since the varieties of Ishikari-shiroke type showed only a few susceptible lesions because of minor races possessing virulence to the Pi-i gene, the varietal difference within this type was not recognized.

As a result, it appears that there was a different level of field resistance among the varieties possessing the same true resis-

tance, and that the severe damage of the so-called "highly-resistant variety" such as Kusabue and Yuukura, possessing the Pi-k gene from Chinese varieties, was caused by the lack of field resistance and by the rapid propagation of virulent pathogens.

Evaluation of field resistance in the blast nursery

Ezuka et al. (1969b) tried to evaluate the level of field resistance and to determine a fluctuation of field resistance throughout the year in the blast nursery beds. The tests were repeated six times from June to August. A progressive status of the disease severity in representative varieties is shown in Fig.2. Since the Pi-a gene appeared to have no effect in this experiment, the varieties were grouped into three: Shin 2 (+) and Aichi-asahi (Pi-a) group, Kanto 51 (Pi-k) and To-to (Pi-a, Pi-k) group and Ishikari-shiroke (Pi-i) group. The records were taken on the percentages of the diseased leaf area in total leaf area. Daily percentages of the diseased leaf area in given varieties were summed up by getting the approximate quantity of the integral calculus of the curved line in Fig. 3. The disease rating index devised by Sakurai and Toriyama (1967) is calculated from the ratio of susceptibility of a given variety to that of a standard variety which is the most susceptible variety chosen from the same true resistance genotype.

$$\text{Disease rating index} = \frac{\text{Summed up value of given variety}}{\text{Maximum summed up value in the group}} \times 100.$$

The disease rating indices of the representative varieties are shown in Fig. 4. The tendency of the varietal difference in the blast nurseries resembled closely those in the paddy field (Fig.5).

However, the difference among the varieties possessing the lower level of field resistance was observed more evidently in the paddy field than in the blast nurseries, but the difference among the varieties possessing the higher level of field resistance was recognized more apparently in the blast nurseries than in the paddy fields. The blast nurseries, therefore, have been used by many rice breeders for evaluating field resistance of their breeding materials.

Evaluation of field resistance by the seedling inoculation method

Niizeki (1967), Sakurai and Toriyama (1967), and Yunoki et al. (1970a) tried to evaluate field resistance of rice varieties by a spray method for seedlings, and Kiyosawa (1966a, b, 1970b) attempted an injection method. When field resistance is evaluated in the paddy field or in the blast nursery, the degree of damage is influenced by the weather conditions such as temperature and rainfall, and by the constitution of fungus races existing in the testing field. Conversely, the seedling inoculation method can use the virulent fungus strains to the testing rice varieties and can be conducted under artificially controlled conditions. The testing result, therefore, is expected to be evaluated at the same level in every test. At the same time, it may be observed whether or not the evaluation of field resistance is influenced by the fungus strains or environmental factors.

Niizeki (1967) conducted the seedling inoculation test in a greenhouse and the field test in a paddy field for three years repeatedly with the same varieties at the same place. He chose the testing paddy field where most of the fungus strains belonged

to the C-1 race group. The testing varieties were transplanted late under much nitrogen fertilizer for bringing out a natural epidemic of blast. The susceptible variety, Kusabue, in the testing field,was damaged heavily with stunting. For the seedling inoculation test, rice seedlings were grown in a nursery box in a greenhouse. The seedlings were inoculated by the spray method with suspension of fungus spores of C-1 race when the seedlings attained the fourth, fifth and sixth leaf-age.

The relationship between the grade of field resistance evaluated in the paddy field and by the seedling inoculation method is shown in Figs. 6 and 7. Field resistance evaluated at the sixth leaf-age showed a high correlation coefficient of 0.864,but that at the fourth leaf-age showed a low of 0.389, and that at the fifth leaf-age showed half way between them.

Niizeki (1967) evaluated the degree of field resistance of some varieties which were representative of the most susceptible to the most resistant ones traded by the rice breeder's experiences for a long time. At the sixth leaf-age the seedlings were inoculated with eight fungus strains belonging to different races. As shown in Fig. 8, the resistant varieties such as Homare-nishiki and Yamabiko showed resistance, and the susceptible varieties such as Jukkoku and Asahi showed a high susceptibility in this test. The grade evaluated by the seedling inoculation method coincided well with the grade estimated by the breeder's experiences, and the order of field resistance of the varieties was influenced little by the fungus strains, the amount of fertilizer application, temperature and seasons.

Niizeki (1967) concluded that field resistance was effective to a number of fungus strains, and only one fungus strain virulent

to all the testing materials was enough to evaluate the grade of field resistance when inoculation by spray was made of the spore suspension at the sixth leaf-age of varieties.

Sakurai and Toriyama (1967) and Yunoki et al. (1970a) also examined the inoculating conditions such as the amount of nitrogen fertilizer, leaf-age of seedlings and the concentration of spore suspension. Three fungus strains belonging to the N-1, C-1 and T-1 races were employed to inoculate the seedlings of varieties which were representative of the different levels of field resistance. Field resistance was evaluated by the number of susceptible lesions per seedling.

The relationships between field resistance and amount of the nitrogen fertilizer application is shown in Table 2. A large amount of nitrogen application (10 g of ammonium sulphate per 35 X 27 X 12 cm pot) markedly increased the number of susceptible lesions on the seedlings inoculated, and the differences of the number of susceptible lesions among the varieties increased with amount of nitrogen application.

As to the leaf-age, field resistance of the seedlings appeared to become higher with the increase of age (Table 3), and the varietal difference seemed to increase with the age (Fig. 9). For example, the ratio of resistant variety, Homare-nishiki, to susceptible variety, Jukkoku, was about one and half the number of susceptible lesions at the fifth leaf-age, but about three times at the seventh leaf-age.

The number of susceptible lesions per plant was also influenced by the concentration of spore suspension. When too dense or too

thin concentration of inoculum was used for seedling inoculation, it became difficult to determine the varietal difference of field resistance because the number of susceptible lesions was either too much or too little (Fig. 10). The optimum concentration of spore suspension was determined to be 100,000 to 250,000 spores per 1 ml of water.

The order of field resistance of varieties in each test determined by the seedling inoculation method coincided well with that in the paddy field and the blast nursery bed. Accordingly, Sakurai and Toriyama (1967) proposed the testing method for seedling inoculation as follows:

(1) inoculum is selected from the fungus strains possessing a pathogenicity to the varieties tested;
(2) a large amount of nitrogen fertilizer is applied;
(3) seedlings of the sixth to seventh leaf-age are inoculated;
(4) the concentration of spore suspension for inoculum is 100,000 to 250,000 spores per ml of water.

By this method, Yunoki et al. (1970a) evaluated field resistance of a number of varieties grown in Japan.

Kiyosawa (1966a) considered that field resistance would be evaluated by the injection method in a greenhouse when a weakly aggressive fungus strain was used for inoculation, and he examined his proposal by using the fungus strain Ken 54-04 which was chosen as an inspecifically and weakly aggressive strain. At the 5.3 leaf-age of seedlings, two fungus strains including Ken 54-04 and check strain Ken 54-20 were inoculated by the injection method with suspension of 200,000 spores per 1 ml, and the number of lesions

were counted according to their symptom types. As shown in Table 4, there were no varietal differences when the fungus strain Ken 54-20, possessing ordinary aggressiveness, was injected.

On the other hand, when the weakly aggressive strain Ken 54-04 was inoculated, the percentage of lesions showing the pg symptom type (gray center with purple margin, most susceptible type) in total lesions was found to be significantly different among the varieties tested. The degree of field resistance evaluated by this injection method showed considerably high correlation coefficients from 0.63 to 0.84 with the value observed in the paddy field and the blast nursery bed.

Kiyosawa (1966a) also examined a symptom type by the injection method in a total of 909 varieties, and tabled the results gotten by these two fungus strains to each of the true resistance genotypes of varieties. As shown in Table 5, about one-third of the varieties belonging to Shin 2 type (+) showed the S reaction type (pg symptom type excelled) when Ken 54-04 was injected, and the remainder of this varietal group showed more or less a resistant reaction, but the reaction to Ken 54-20 was almost all the S type. In Aichi-asahi type varieties possessing the Pi-a gene, almost all the varieties showed the S reaction type by Ken 54-20, but there were wide variations by Ken 54-04 (Table 6). The same tendency was observed in the varieties of Ishikari-shiroke type possessing Pi-i gene and of Shinsetsu type possessing Pi-i and Pi-a genes. The reaction of the foreign varieties is shown in Table 7. Though the deviation of frequency in table showed little

difference from that of the Japanese varieties, the weak aggressiveness of Ken 54-04 was also observed in the foreign varietal group.

Kiyosawa (1969) concluded that a weak aggressiveness of Ken 54-04 would be non-specific, and the resistance gene or genes to Ken 54-04 would also be non-specific. Therefore, he suggested that the resistance gene or genes of this kind would exhibit resistance more or less in a paddy field, because field resistance types were generally considered to be non-specific.

Evaluation of field resistance by the sheath inoculation method

The sheath inoculation method was proposed by Takahashi (1951) for evaluating resistance to blast. In this method, it was recommended by Takahashi (1967) that the highest degree of hyphal growth in the host cell was used as the criterion of susceptibility or resistance. The value of resistance evaluated by this method was more complex than that of the spray or injection methods, because the hyphal growth of pathogens in host cells was affected by both true resistance and field resistance of the varieties tested. The degree of field resistance evaluated by this method coincided with the disease rating index which was proposed by Sakurai and Toriyama (1967) within the same varietal group of the true resistance genotype in blast nurseries.

Field resistance and fungus strain

As the definition of field resistance of rice among the Japanese rice breeders is the remaining resistance except for true resistance, exhibition of field resistance was generally considered

to be non-specific to fungus strains. Early experiments by Niizeki (1967) and Sakurai and Toriyama (1967) showed the possibility of existence of non-specific resistance. In these experiments, the degree of field resistance was evaluated by the number or percentage of susceptible lesions, and the varieties showing susceptible lesions were classified into the same group against the pathotype.

When St 1 and Chugoku 31 were inoculated by the spray method with the virulent strains which showed pathogenicity by the injection and the spray method, only a few susceptible lesions were usually observed, and a few numbers of the susceptible lesions of these varieties were also observed in blast nursery beds. Therefore, it was considered that both St 1 and Chugoku 31 had the highest grade of field resistance. However, it was reported that St 1 was severely diseased in Fukushima Prefecture when these varieties were widely tested to ascertain their high field resistance by the blast nursery method all over Japan. Therefore, Yunoki et al. (1970b) tried to ascertain whether field resistance varied with the fungus strains or not.

In general, when the rice varieties possessing true resistance were inoculated with avirulent fungus strains by the spray method, no lesions usually formed on the seedlings, but except in a few cases susceptible lesions were rarely observed on the resistant varieties. In such cases, fungus strains isolated from these susceptible lesions on resistant varieties showed virulence to the varietal group from which fungus strains were isolated. Appearance of a new pathotype

of this kind was considered to be due to mutation of pathogenicity, and the different mutation ratios for different resistance genes were observed by Niizeki (1967).

Some fungus strains were isolated from the susceptible lesions of St 1 and Chugoku 31 in the blast nursery bed in Fukuyama, Hiroshima Pref., and were inoculated by the spray method to the seedlings of St 1 and Chugoku 31, respectively. As shown in Table 8 only a few susceptible lesions were found on the seedlings. Then, the fungus strains were reisolated from the susceptible lesions of inoculated seedlings, and reisolated fungus strains were again used to inoculate each variety. These reisolation tests were repeated three times, and the results were similar to the first isolation test. In this respect, it was considered that high resistance of St 1 and Chugoku 31 belonged to a different category from true or vertical resistance in rice.

Yunoki et al. (1970b) collected fungus strains from different locations and from different rice varieties, and tested their aggressiveness to St 1 and Chugoku 31. Of these collections, some fungus strains isolated from Kisa, Hiroshima Pref., showed strong aggressiveness to St 1 and Chugoku 31, and the number of susceptible lesions on both varieties was about equal to those on the usual varieties possessing the low level of field resistance (Table 9). Erosion of high resistance of these varieties was also observed when the fungus strains from Fukushima Pref. and some other strains were used to inoculate. Furthermore, it was observed that the resistance of these varieties was reduced to a low level when they were grown under unfavorable conditions in a greenhouse during the winter season.

Such breakdown of high resistance of St 1 and Chugoku 31 was apparently due to the specific reaction to the fungus strains, and this phenomenon is similar to the breakdown of vertical resistance.

High field resistance alike to St 1 and Chugoku 31 was found in Zenith and its derivatives by Ezuka et al. (1969b) and Yunoki et al. (1970b). Zenith and its derivatives were classified by the injection method into two varietal groups according to reaction pattern against the seven standard fungus strains; Zenith, Fukei 67 and Fukei 73 were classified into Zenith type possessing Pi-a and Pi-z genes; and 54 BC-68, Fuku-nishiki, Ou 243 and Ou 244 were classified into Fuku-nishiki type possessing Pi-z gene. Nevertheless, these varieties were clearly divided into two groups of different category from the above grouping when tested in the blast nursery bed in Fukuyama and inoculated by spray method with the virulent fungus strains such as FS 66-59, Chu 66-45 and TH 65-105. One is the high level of field resistance, to which Zenith, 54 BC-68 and Ou 244 belonged, and another is the low level of field resistance, to which Ou 243, Fukei 67, Fukei 73 and Fuku-nishiki belonged.

Though the varieties belonging to high level of field resistance showed a typical susceptible symptom, they developed only a few number of susceptible lesions. Breakdown of high resistance in Zenith and Ou 244 has not been observed in Japan, but the possibility of breakdown yet remains because susceptible reaction of Zenith was reported at some locations in the world during 1964-1965 in the International Uniform Blast Nurseries(FAO, 1966).

Some Japanese upland rice varieties, such as Kuroka and Fukuton, have also been found to have an extremely high field resistance like Zenith. By the injection method, Kuroka was found to have the true resistance gene Pi-a only, and Fukuton to have no true resistance gene (Ezuka et al., 1969a). Nevertheless, when tested in blast nursery beds and when inoculated by the spray method with virulent fungus strains to Pi-a gene, these varieties developed only a few lesions of moderately resistant symptom type, and were recognized to have a high level of field resistance (Ezuka et al.,1969b, Yunoki et al., 1970a). Recently, it was found that high field resistance in these upland rice varieties was specific to the fungus strains, because some fungus isolates could develop a number of susceptible lesions on these upland varieties (Sekiguchi, personal communication). This was quite unexpected evidence for the Japanese rice breeders. Up until this finding, they believed that the Japanese upland rice varieties should be a favorable gene source for high field resistance, because the Japanese upland rice had been planted for many years in Japan and had exhibited stable resistance to blast. However, breakdown of high level of field resistance in the upland rice varieties was only observed in a laboratory test, and it has not yet been proved under field conditions.

The other type of high field resistance may be due to a simultaneous effect of a true resistance gene to some fungus strains. Some varieties descended from the upland rice variety Sensho were found to have true resistance when inoculated by the spray method with the fungus strains belonging to the N-6 or C-6 races. These

varieties were observed to have a considerably high level of field resistance in the blast nursery beds and in the paddy fields where fungus strains virulent to these varieties were prevalent, as compared with the ordinary varieties susceptible to the N-6 and C-6 races (Nakanishi and Nishioka, 1967).

Conversely, it has been considered that an existence of the true resistance gene Pi-k brought decreasing field resistance against fungus strains virulent to Pi-k (Suzuki and Yoshimura, 1966; Iwano, Yamada and Yoshimura, 1969). However, it was found that the gene Pi-k was independent with the degree of field resistance (Asaga and Yoshimura, 1970). Decreasing field resistance in the varieties possessing Pi-k gene might be due to the Vertifolia effect pointed out by Van der Plank (1963).

Effect of fungus strains on field resistance of a middle or lower level was also investigated. According to the early investigations by Hirano et al. (1967) and Niizeki (1967), almost the same reaction to different fungus strains were observed on the respective varieties for different levels of field resistance. Hirano and Matsumoto (1971) repeated the seedling inoculation test with six fungus strains belonging to the C-1 race and with another two fungus strains. As shown in Table 10, highly significant correlations were obtained between fungus strains employed. As far as these results are concerned, middle to lower level of field resistance in rice had the same meaning of horizontal resistance as defined by Robinson (1969). However, the variation of field resistance of a middle to lower level was observed by Yunoki et al. (1970b) and Ito (personal communication).

Yunoki et al. (1970b) investigated the variability of field resistance in numerous varieties with fourteen fungus strains of six races which were collected from northern to southern Japan. The degree of field resistance was evaluated by the ratio of a susceptible lesion number on the given variety to that on the standard variety and was graded according to the following criteria: rr is less than 20, r 21 to 40, m 41 to 60, s 61 to 80 and ss is more than 81 percent. Some examples of results are shown in Table 11. Varieties such as Hatsu-nishiki showed wide range of variation from rr to ss; on the other hand some varieties such as Akibare showed a stable degree of resistance between rr to r; and some varieties such as Norin 29 and Aichi-asahi showed a constant susceptibility of ss. The varieties were grouped into a variable one or stable one for field resistance, and it was found that the varieties in the variable group by the seedling inoculation method were also variable when tested in the blast nurseries. To the contrary, the varieties showing a stability by the seedling inoculation method were also stable in the blast nurseries.

If field resistance of varieties possessing the same true resistance gene or genes is not influenced by the fungus strains, the order of field resistance in the varietal group with the same true resistance gene or genes is expected to be constant even in different environments such as locations and growing seasons. Interaction between variety, fungus strain and location was investigated by Ito in cooperation with five national agricultural experiment stations (personal communication, briefly reported

in Year Book Cent. Agr. Exp. Sta. 1969, 1970). Fifteen varieties, of which ten belonged to Kanto 51 type and five to Shin 2 type, were inoculated by spraying with six fungus strains under isolated nursery conditions. Of the six fungus strains, five belonged to the C-1 race and two to the N-2 race. The experiments were conducted at five locations with two replications each.

The degree of field resistance was graded from 0 (rr) to 11 (ss) at intervals of 0.5, and the results of analysis of variance on field resistance is shown in Table 12. Significant interaction was indicated between main factors: variety X fungus strain, variety X location and fungus strain X location. For example, Tatsumi-mochi, which was considered to be a moderately high level with the order of 3 to 4 within 15 varieties, showed marked variation of field resistance with fungus strain. When fungus strain Ken 53-11 was inoculated, Tatsumi-mochi was evaluated as a low level of resistance, and the order of resistance was 11 within 15.

Therefore, when the level of field resistance was evaluated due to the order by each fungus strain, the range varied with variety. In the same manner, the order evaluated in each location varied with variety. The variable range by fungus strains and by locations indicated a positive correlation (Fig. 11). As a result, it is concluded that field resistance of rice varieties varied with the fungus strains, so the term field resistance does not coincide strictly with the term horizontal resistance.

Inheritance of field resistance

A high level of field resistance is found in St 1 and Chugoku 31, descended from the Pakistan variety Modan by successive back-

crossing to Japanese paddy rice variety Norin 8. Inheritance of this high field resistance was investigated with eleven crosses by Toriyama, Yunoki and Shinoda (1968). High field resistance was dominant over susceptibility, and monogenic inheritance was observed through all the crosses investigated. Linkage relation between the high field resistance gene Pi-f and the true resistance gene Pi-k was also analyzed with the crosses involving Chugoku 31, which possessed both Pi-f and Pi-k genes; 14.5 percent of the recombination value between Pi-f and Pi-k was obtained, and the arrangement of genes on Chromosome 9 (1a linkage group; Group 8) was estimated (Fig. 12). (Toriyama et al., 1968.)

Inheritance of high field resistance of Ou 244, which was incorporated from Zenith, has not been investigated yet, but this trait segregated very clearly into either high field resistance or susceptibility in the offsprings of the crosses involving Ou 244. Major genic inheritance, therefore, was expected in this high field resistance of Ou 244, the same as that of St 1.

The genetic scheme of high resistance in Japanese upland rice was investigated with the crosses between upland rice Kuroka and the chromosome reciprocal translocation lines by Shinoda et al. (1970). High field resistance was dominant over susceptibility, and major genic inheritance was observed, but it was not determined whether the number of genes was two or three. By the analysis of linkage relationships between high field resistance and chromosome reciprocal point, it was found that one of the genes for high field resistance was located on Chromosome 4 (A linkage group; Group 3) and the other on Chromosome 11 (Pl linkage group; Group 2).

The other kind of moderately high field resistance was found in the same varieties descending from upland rice variety Sensho. Exhibition of this moderately high field resistance was estimated to be due to the simultaneous effect of a true resistance gene to the fungus races N-6 and C-6. This true resistance was recognized when the spray method was used for inoculation but did not exhibit even when injected with the same fungus strains. Though inheritance of this true resistance gene has not been investigated yet, it was estimated that true resistance was controlled by the major genic system from the view point of the varietal lineage.

Moderately high field resistance of Monare-nishiki, which was one of the descendants of Sensho, was investigated in its genetic system with F_3 line analysis under blast nursery conditions by Yunoki, Toriyama and Kiyosawa (1970). It was observed that moderately high field resistance was controlled by two pairs of complementary genes which were linked with 20 to 30 percent of recombination value. To know the relationship between field resistance and reaction to the injection with weakly aggressive fungus strain Ken 54-04, F_3 lines of the crosses involving Homare-nishiki and Ginga were employed for the injection test with Ken 54-04 by Kiyosawa (1970b). One major gene and two minor genes were estimated to control reaction to the Ken 54-04 injection, and the sensitivity to environmental conditions appeared to be dependent mainly upon the minor genes and partially upon the major gene. Comparison between the data by the injection method and by the blast nursery on the same F_3 lines indicated that exhibition of resistance to two testing methods was controlled by different genetic systems because of a non-significant correlation between them (Yunoki, Toriyama and Kiyosawa, 1970).

All the above mentioned examples of high field resistance were estimated to be specific to fungus strains or to be a simultaneous effect of true resistance gene. Major genic system of inheritance of field resistance, therefore, might be corresponding with the specific pattern of resistance to fungus strains.

Inheritance of a middle or lower level of field resistance and the relationship between field resistance and true resistance were analyzed with the F_3 and F_4 sister lines of three crosses by Asaga and Yoshimura (1969,1970,1971). Three crosses were made among Kusabue, Yamabiko and Norin 29, of which Kusabue had the Pi-k gene and extreme susceptibility, Yamabiko had the Pi-a gene and a middle level of field resistance, and Norin 29 had no true resistance genes and susceptibility. F_3 lines were grouped according to the true resistance genotypes: Pi-k and Pi-a group, Pi-k group, Pi-a group, + group and heterogenic group. The degree of field resistance was evaluated in the blast nursery where fungus strain Ken 60-19 belonging to the C-1 race was inoculated by spraying for eliminating the action of the true resistance genes. The mean and standard deviation of field resistance in each genotype are shown in Table 13.

Marked differences of field resistance were found among sister lines with the same true resistance genotypes, but the variable range within sister lines was less than the difference between the parental varieties, and the mean values were about the same of mid-parent. F_3 lines of the highest and the lowest degree of field resistance were selected from each true resistance

genotype group in each cross. Each F_4 family including 45 lines were tested for their resistance to stem rot in the paddy field. Differences within each F_4 family were observed on field resistance and marked differences between the two families selected from the same genotype were also observed. As shown in Fig. 13, a highly significant correlation was obtained between the F_3 lines and F_4 families. This positive correlation, 0.891***, and the nearly normal distribution of field resistance within the family, meant that the middle or lower level of field resistance was inherited and would be controlled by polygenic system.

In this experiment, field resistance evaluated in the blast nursery bed and paddy field showed a high correlation, and the resistance to stem rot also correlated well with the degree of field resistance.

Mathematical studies on field resistance

Mathematical study on epidemiology was established by Van der Plank (1963). He used the equations

$$\frac{dx}{dt} = \gamma X \qquad (1)$$

as a model of increase of infection, where χ is the proportion of disease and γ is infection rate, and

$$\frac{dx}{dt} = \gamma X(1-\chi) \qquad (2)$$

as a model when the amount of the host is limited, and the proportion of disease at t is

$$x = x_0 e^{rt} \qquad (3)$$

where x_0 is the initial proportion of disease, and when the logarithmic stage of an epidemic was considered

$$x_t = x_{t-i-p} e^{(i+p)r} \tag{4}$$

where p is the latent period and i is the infectious period.

Van der Plank (1963) pointed out that horizontal resistance reduces the infectious rate r, increases the latent period p and reduces infectious period i.

Independently of the work of Van der Plank, Kiyosawa (1965) proposed the following equations

$$\frac{dl}{dt} = \lambda l \tag{5}$$

$$\frac{dl}{dt} = \lambda l \left(1 - \frac{l}{L}\right) \tag{6}$$

$$l = l_0 e^{\lambda t} \tag{7}$$

$$l = \frac{L}{1 + k e^{-\lambda t}} \qquad \left(k = \frac{L - l_0}{l_0}\right) \tag{8}$$

where l_0 is the number of lesions at the initial time, l is the number of lesions at t, λ is the fitness of pathogens and k is the coefficient related with the number of lesions at the initial infection.

In these equations, l_0 depends upon true resistance of the variety but not upon the degree of field resistance. On the

contrary, λ depends upon the degree of field resistance but not upon true resistance from the point of view of variety. Namely, l_0 depends upon virulence but not upon aggressiveness or fitness, and λ depends upon agressiveness or fitness but not upon virulence from the point of view of fungus strain.

Kiyosawa (1969b) proposed that true resistance and field resistance were defined by the variables of equations. By this definition, field resistance is expressed by $1/\lambda$, and true resistance corresponds to l_0. He suggested that the varietal difference of field resistance should compare the value of $1/\lambda$ in each variety.

The influence of an environmental condition and the increase of resistance with aging of the plant was theoretically given as follows:

$$\frac{dl}{dt} = \lambda l\left(1 - \frac{t}{T}\right) \tag{9}$$

$$l = k e^{\lambda (t - t^2/2T)} \tag{10}$$

$$l = \frac{L}{1 + k e^{-\lambda (t - t^2/2T)}} \tag{11}$$

where field resistance of plants linearly increases till a time (T) when increase of the number of lesions becomes 0; in other words, the disease increase terminates.

Using the equation (11), Chiba et al. (personal communication) investigated the effect of some factors on degree of field resis-

tance. The variable for field resistance, λ, was affected largely by yearly difference of climatic factors and the amount of additional fertilizer, and slightly by varietal difference among the factors investigated. A significant negative regression of λ against l_0 was found as a result of a density effect. The value of λ corrected by the regression coefficient showed that when the ranges of variation were λ by year, the variety and additional fertilizer were 0.32, 0.20 and 0.31, respectively. Average of λ's obtained under various conditions for four years was 0.36.

Conclusion

Field resistance to blast disease in rice is now being investigated not only by rice breeders but also plant pathologists in Japan. There are many problems to be investigated. As was reviewed in this paper, we have little information on field resistance in rice, especially on a varietal difference of latent and infectious periods.

Throughout this review, it was emphasized that studies of field resistance by Japanese investigators did not coincide in part with studies of horizontal resistance by Van der Plank (1963), and that the difference between true resistance and field resistance was due to the difference of a standpoint for recognition. In fact, descendants of upland rice variety Shensho, which was recognized to have true resistance by spraying with the C-6 or N-6 races, showed a high field resistance to numerous fungus strains in the paddy fields and blast nurseries.

In addition, such varieties as St 1, Ou 244 and Kuroka were recognized to have an extremely high field resistance according to a few susceptible lesions under spray inoculation, and were found to show specific reaction to some fungus strains on the number of susceptible lesions. Since in horizontal resistance there was no interaction between pathotype and pathodeme, the definition will be applicable in the limited field until the discovery of the new pathotype which shows the pathotype-pathodeme interaction. This is because the possibility of an existence of such new pathotypes can not be denied. The rice breeders have to give strong attention to developing promising varieties which possess high and stable field resistance to blast.

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Table 1: Reaction of varietal group to fungus strains

Inoculation method	Injection method										Spray method		Genotype estimated
Fungus strain	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168	Ina 168 -a-k+	Ina 168 -a-k-m+	Ina 168 -zt+	Ken 65-20		
Fungus race													
Reaction type	T-2	T-1	C-3	N-1	N-2	N-3	N-4				C-8	C-6	
Shin 2 type	S	S	S	S	S	MS	S					S	+
	S	S	S	S	S	MS	S					R	+, (?)
Aichi-asahi type	S	S	R	S	S	S	R					S	Pi-a
	S	S	R	S	S	S	R					R	Pi-a, (?)
Ishikari-shiroke type	M	S	M	S	MS	MR	M					S	Pi-i
	M	S	M	S	MS	MR	M					R	Pi-i, (?)
Shinsetsu type	M	S	R	S	MS	MR	R					S	Pi-a, Pi-i
	M	S	R	S	MS	MR	R					R	Pi-a, Pi-i, (?)
Kanto 51 type	MR	S	S	R^h	R^h	R^h	R^h	S	S		S	S	Pi-k
	MR	S	S	R^h	R^h	R^h	R^h				R	S	Pi-k, Pi-i
	MR	S	S	R^h	R^h	R^h	R^h	M	S		S	R	Pi-k, Pi-m
	MR	S	S	R^h	R^h	R^h	R^h				R	R	Pi-k, Pi-i, Pi-m
To-to type	MR	S	R	R^h	R^h	R^h	R^h	S	S		S	S	Pi-a, Pi-k
	MR	S	R	R^h	R^h	R^h	R^h				R	S	Pi-a, Pi-k, Pi-i
	MR	S	R	R^h	R^h	R^h	R^h	M	S		S	R	Pi-a, Pi-k, Pi-m
	MR	S	R	R^h	R^h	R^h	R^h				R	R	Pi-a, Pi-k, Pi-i, Pi-m
Tashiro-mochi type	S	S	M	MR	M	MR	S						Pi-ta
Shimokita type	S	S	R	MR	M	MR	R						Pi-a, Pi-ta
Pi 4 type	S	M	R^h	R	R	R	MR						$Pi\text{-}ta^2$
	S	M	R^h	R	R	R	R						Pi-a, $Pi\text{-}ta^2$
Fukunishiki type	M	M	M	MR	M	MR	M						Pi-z
Zenith type	M	M	R	MR	M	MR	R						Pi-a, Pi-z
Toride 1 type	R^h	R^h	R^h	R^h	R^h	R^h	R^h			S			$Pi\text{-}z^t$
	R^h	R^h	R^h	R^h	R^h	R^h	R^h			R			Pi-a, $Pi\text{-}z^t$

Table 2. Field resistance and amount of nitrogen fertilizer

Variety	Genotype for true resistance	Fungus strain (race)								
		Noku 373 (K-1)			Ken 60-19 (C-1)			Ken 53-33 (T-1)		
		low	middle	excess	low	middle	excess	low	middle	excess
St 1	Pi-f	2.8	5.8	9.2	3.9	5.3	8.4	4.4	6.8	10.7
Norin 8	+	19.6	25.6	35.4	16.2	21.8	30.7	17.5	25.6	40.5
Jukkoku	Pi-a	22.0	28.7	33.0	17.5	20.2	38.4	23.4	29.0	39.7
Fujiminori	Pi-a	7.9	10.6	24.2	8.7	17.6	18.9	6.9	14.8	21.2
Ishikari-shiroke	Pi-i	3.8	14.7	23.0	10.4	18.2	21.3	5.6	17.3	19.5
Kusabue	Pi-k	0	0	0	22.1	30.4	45.2	22.0	30.5	38.0
Sonshuraku	Pi-k	0	0	0	14.8	24.6	35.4	10.7	22.3	31.5
Mangetsu-mochi	Pi-k	0	0	0	15.0	23.8	26.1	13.5	17.8	22.8
Chugoku 31	Pi-f, Pi-k	0	0	0	2.3	3.6	6.7	1.8	4.9	8.6
Yuukara	Pi-a, Pi-k	0	0	0	20.8	32.6	40.8	18.9	21.3	41.4
Kongo	Pi-a, Pi-k, Pi-m	0	0	0	4.5	10.6	14.5	9.6	11.0	18.4

Note. Figures in the table mean the number of susceptible lesions.

Table 3. Leaf age and field resistance

Variety	Genotype for true resistance	Leaf age								
		5th			6th			7th		
		low	middle	excess	low	middle	excess	low	middle	excess
St 1	Pi-f	9.8	14.3	14.8	6.3	5.5	10.7	3.9	3.5	7.4
Norin 8	+	36.6	47.5	55.1	19.6	24.8	35.4	18.9	29.6	33.4
Jukkoku	Pi-a	48.4	60.9	63.4	18.7	33.3	46.8	16.9	34.7	45.0
Shuho	Pi-a	32.4	33.6	49.8	15.4	20.5	28.3	13.7	18.1	23.7
Homare-nishiki	Pi-a	29.6	40.3	42.4	10.6	17.4	24.5	11.4	20.4	15.0
Kusabue	Pi-k	34.7	58.7	70.9	24.8	29.8	43.6	24.6	27.9	41.0
Sonshuraku	Pi-k	30.5	50.4	58.3	28.3	21.4	29.1	14.7	13.4	27.5
Mangetsu-mochi	Pi-k	28.7	45.6	54.2	13.6	27.3	31.6	15.1	15.4	20.8
Chugoku 31	Pi-f, Pi-k	10.8	20.8	19.7	4.4	3.8	7.4	1.8	1.5	3.1
Kongo	Pi-a, Pi-k, Pi-m	19.5	29.4	35.8	8.7	14.2	20.6	9.7	16.7	14.6

Note. Figures in the table mean the number of susceptible lesions.

Table 4. Reaction to fungus strains by injection method

Variety	Percentage of susceptible lesions showing pg symptom						Evaluation by exceeding symptom type	
	Ken 54-04 (weakly aggressive strain)			Ken 54-20 (ordinary aggressive strain)				
	Mean value	Order	Significance	Mean value	Order	Significance	Ken 54-04	Ken 54-20
Aichi-asahi	67.3	1	**	88.1	1	not	S	S
Moko-ine	44.7	2		83.2	4		MS	S
Kameno-o	31.1	3	c	72.1	7		MS	S
Shin 2	26.1	4	c	87.8	2		MS	S
Ta-sensho	13.8	5	b	87.2	3		M	S
Futaba	7.6	6	a b	70.6	8		M	S
Norin 8	5.4	7	a	81.0	6		M	S
Shinju	2.4	8	a	81.7	5		M	S
Norin 22	2.2	9	a	69.8	9		M	S
Ginga	0.6	10	a	62.6	10		MR	S

Note. Item of significance is measured by Duncan's multiple range test (1% level)

MR; moderately resistance. M: medium. MS: moderate susceptibility.

S: susceptibility

Table 5. Resistance of 'Shin 2' type evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20

Fungus strain Ken 54-04		R^h	R	MR	M	MS	S	Total
Fungus strain Ken 54-20	R^h							0
	R							0
	MR							0
	M							0
	MS				5	2	1	8
	S	1	4	18	74	24	52	173
Total		1	4	18	79	26	53	181

Note. R^h : High resistance, R: Resistance, MR : Moderate resistance, M : Medium, MS : Moderate susceptibility, S : Susceptibility

Table 6. Resistance of 'Aichi-asahi' type evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20

Fungus strain Ken 54-04		R^h	R	MR	M	MS	S	Total
Fungus strain Ken 54-20	R^h							0
	R							0
	MR							0
	M							0
	MS				3	1		4
	S	1		11	47	25	60	144
	Total	1		11	50	26	60	148

Note. See Table 5.

Table 7. Resistance of foreign varieties evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20

Fungus strain Ken 54-04		R^h	R	MR	M	MS	S	Total
Fungus strain Ken 54-20	R^h	15	2					17
	R	2	19	2				23
	MR	4	6	18	3		1	32
	M		6	25	67	2	1	101
	MS				23	7		30
	S			2	8	6	11	27
	Total	21	33	47	101	15	13	230

Note. See Table 5

Table 8. Resistance of "St 1" and "Chugoku 31" to fungus strains isolated repeatedly from "St 1" and "Chugoku 31"

Fungus strain	Variety from which isolated	race	Number of susceptible lesions											
			St 1			Chugoku 31			Norin 22			Moko-ine		
			1*	2*	3*	1	2	3	1	2	3	1	2	3
Chu 66-10	St 1	C-8	2.2	5.2	5.4	0	0	0	12.0	13.8	35.8	42.0	39.7	223.7
Chu 66-11	St 1	C-8	0.9	0	1.0	0.5	0.6	0.8	12.9	2.1	8.1	55.0	12.2	70.6
Chu 66-12	St 1	C-8	1.9	1.3	2.2	0.3	0.9	0.8	15.5	2.8	7.9	82.0	14.4	82.4
Chu 66-13	St 1	?	2.9	0.2	2.7	1.8	0	3.3	16.3	0.3	33.5	50.6	1.4	201.9
Chu 66-14	St 1	N-1	8.8	1.3	10.4	0	0	0	15.9	2.3	49.2	71.4	3.6	357.8
Chu 66-15	St 1	N-1	0.2	3.5	3.7	0.3	8.9	0.3	23.0	6.5	28.5	134.9	48.8	97.9
Chu 66-22	Chugoku 31	C-8	2.4	8.6	1.0	2.6	10.3	1.7	23.9	7.2	15.5	134.7	32.4	108.1
Chu 66-23	Chugoku 31	?	0.8	1.2	1.6	1.6	0.2	0.4	18.8	2.2	21.2	89.7	8.8	179.5
Chu 66-24	Chugoku 31	?	0.6	9.6	0.1	0.2	4.9	0.3	50.9	9.7	4.1	224.0	37.0	56.2
Chu 66-25	Chugoku 31	?	1.4	1.1	0.2	0.2	0.5	0.1	21.4	0.4	24.0	81.4	6.2	213.3
Chu 66-26	Chugoku 31	?	0.1	3.9	0.4	0.1	4.5	0.3	3.1	1.2	4.9	12.6	5.6	29.0
Chu 66-27	Chugoku 31	?	1.3	2.3	0.1	0.2	1.2	0.1	19.7	2.0	9.9	112.6	17.6	103.3
Ken 60-19	Kanto 52	C-1	1.9	4.3	1.6	1.0	2.4	1.2	3.3	7.2	6.2	86.3	22.7	115.0

* means times of isolating

Table 9. Field resistance and the fungus strains collected from different locations

Fungus strain	Location collected	Variety isolated	Race	Number of susceptible lesions					
				St 1	Chugoku 31	Norin 22	Aichi-asahi	Homare-nishiki	Kanto 51
Chu 66- 1	Akana	Koshi-hikari	C-8	0.6	0.	4.3	10.6	0.	6.0
Chu 66- 3	do	do	C-1	0.2	1.6	4.8	12.2	2.0	14.8
Chu 66-32	Fukuyama	Nakei 212	T-2	1.0	0	11.4	42.6	12.7	0
Chu 66-35	do	do	N-6	3.2	0	15.6	28.2	0	0
Chu 66-38	do	do	C-1	0.8	0.4	10.5	22.3	2.5	38.4
Chu 66-46	do	Fuku-nishiki	N-1	0	0	12.0	33.5	1.2	0
Chu 66-51	Kisa	Norin 18	N-2	1.0	0	21.4	34.2	4.4	0
Chu 66-58	do	Senshu-raku	N-2	45.0	0	18.2	33.8	24.0	0
Chu 66-59	do	do	C-3	21.6	0	13.5	0	0	41.0
Chu 66-64	do	Kanto 51	C-1	7.5	31.0	22.0	57.0	22.4	107.0
Chu 66-65	do	do	C-1	64.4	16.3	40.3	44.0	9.0	35.9

Table 10. Correlation coefficient between field resistance evaluated by different fungus strains

Fungus strain	A C-1	E C-1	F C-1	H C-8
E C-1	0.939***			
F C-1	0.971***	0.954***		
H C-8	0.953***	0.972***	0.973***	
I N-1	0.935***	0.970***	0.898***	0.985***

*** 0.1 percent level

Table 11. Range of variation of field resistance of varieties possessing middle to low level of resistance

Fungus strain	Race	Variety / Genotype	Hatsu-nishiki	Kansai 6	Shin-ei	Kogane-nishiki	Yama-biko	Reimei	Akibare	Fujisaka 5
			+	+	+	+	Pi-a	Pi-a	Pi-a	Pi-i
Ken 53-33	T-1		ss	rr	r	r	m	rr	rr	r
Hoku 63-27	T-1		rr	rr	rr	r	r	rr	rr	ss
Ken 60-19	C-1		s	rr	s	s	ss	r	r	r
Ina 72	C-3		m	rr	rr	r	-	-	-	-
Hoku 1	N-1		r	r	r	r	r	r	r	r
Hoku 373	N-1		r	rr	r	rr	r	r	r	rr
P - 12	N-1		ss	rr	m	rr	m	r	r	m
Ken 54-20	N-2		rr	rr	m	ss	rr	rr	r	-
P - 26	N-2		s	r	s	s	m	r	r	-
H 67-1	N-2		ss	r	r	m	m	r	r	-
H 67-4	N-2		ss	r	s	m	s	r	r	-
FS 67-14	N-2		ss	m	r	m	s	s	rr	-
FS 66-59-9	N-2		ss	rr	s	rr	m	rr	rr	-
Ina 168	N-4		r	rr	rr	rr	-	-	-	-

rr: high resistance. r: resistance. m: medium. s: susceptibility

ss: high susceptibility

Table 12. Analysis of variance of field resistance by varieties, locations and fungus strains

Source of Variance	Degree of freedom	Mean square	F
Varieties	14	63.6	43.27**
Locations	4	302.6	205.82**
Fungus strains	4	120.2	81.74**
Varieties X Locations	56	4.6	3.11**
Varieties X Fungus strains	56	2.3	1.56*
Locations X Fungus strains	16	44.1	30.03**
Error	224	1.5	

** and * : Significance at 1 and 5 percent level, respectively.

Table 13. Mean and standard deviation of field resistance in F_3 lines

Cross	Genotype	Number of lines	Mean	Max.	Min.	Standard deviation
Yamabiko	Pi-k, Pi-a	23	22.3	34	15	5.3
	Pi-k	17	22.1	30	13	4.7
X	Pi-a	16	21.1	32	12	5.5
Kusabue	-	18	20.9	33	12	4.9
	Heter	264	21.3	37	10	5.2
Norin 29	Pi-k	85	27.7	38	16	4.8
X	-	76	26.0	38	15	5.0
Kusabue	Hetero	159	26.9	37	12	5.2
Yamabiko	Pi-a	60	14.9	27	10	2.8
X	-	52	16.4	23	10	3.5
Norin 29	Hetero	153	16.2	26	10	3.3
Kusabue	Pi-k, Pi-a	1	33.6	39	20	3.6
Norin 29	-	1	18.5	27	9	3.9
Yamabiko	Pi-a	1	11.7	18	7	2.1

Note: Figures in the table mean the degree of field resistance; 0 to 100: susceptibility to resistance.

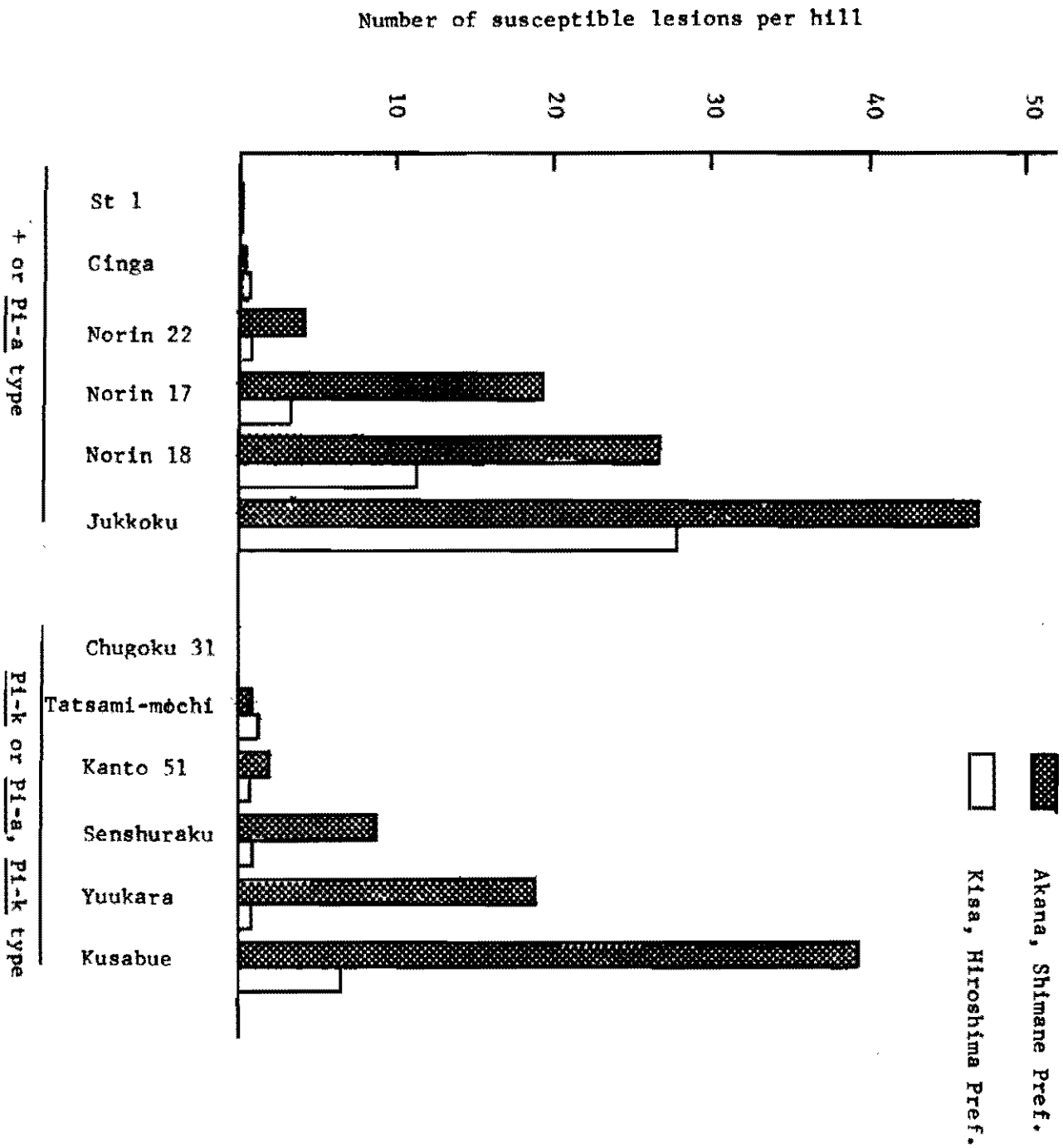


Fig. 1. Varietal difference of field resistance evaluated by the paddy field test.

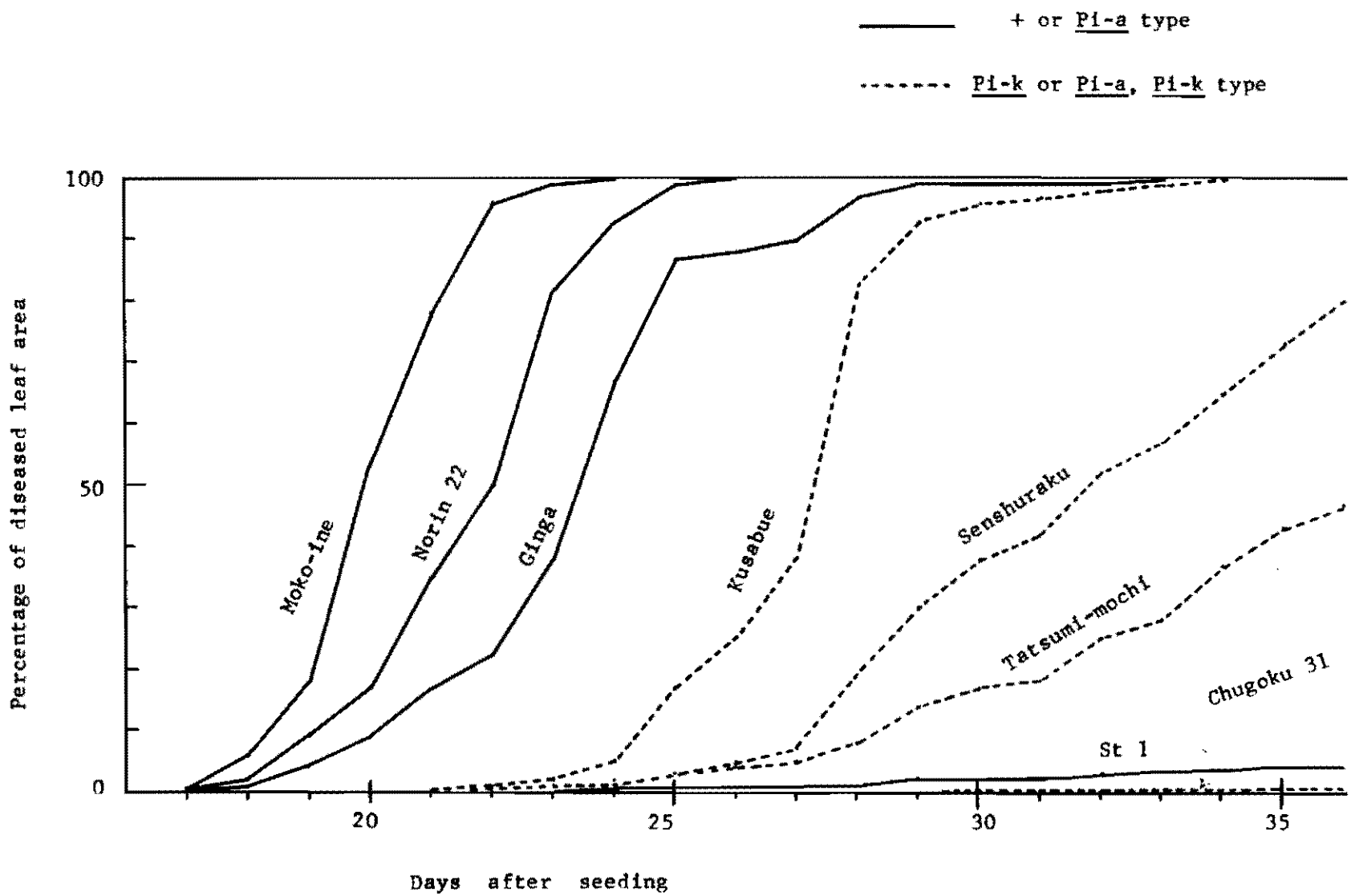


Fig. 2. Progressive status of the disease severity in the blast nursery test.

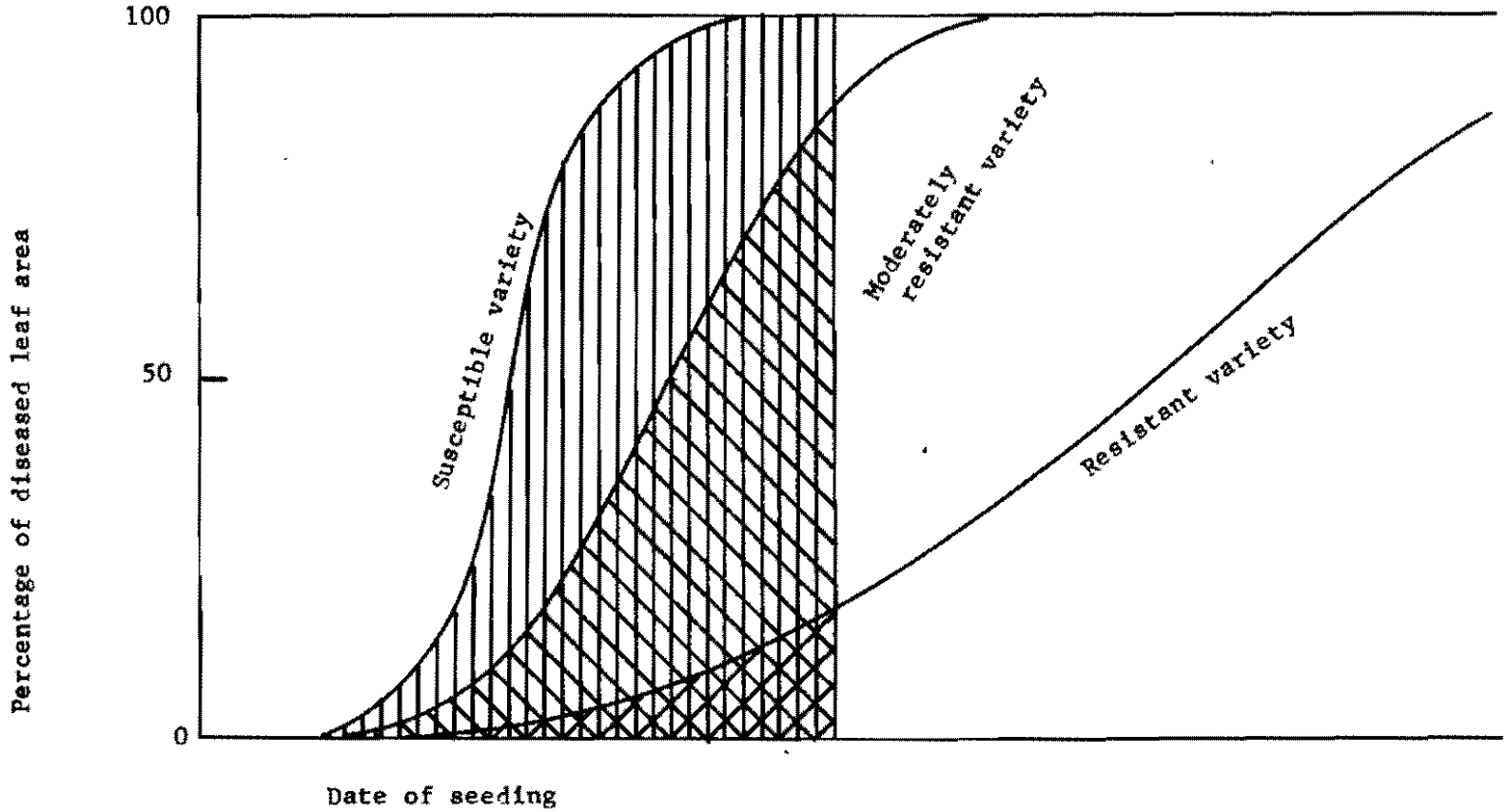


Fig. 3. Model figure of daily progress of percentage of diseased leaf area on seedlings in the blast nursery bed.

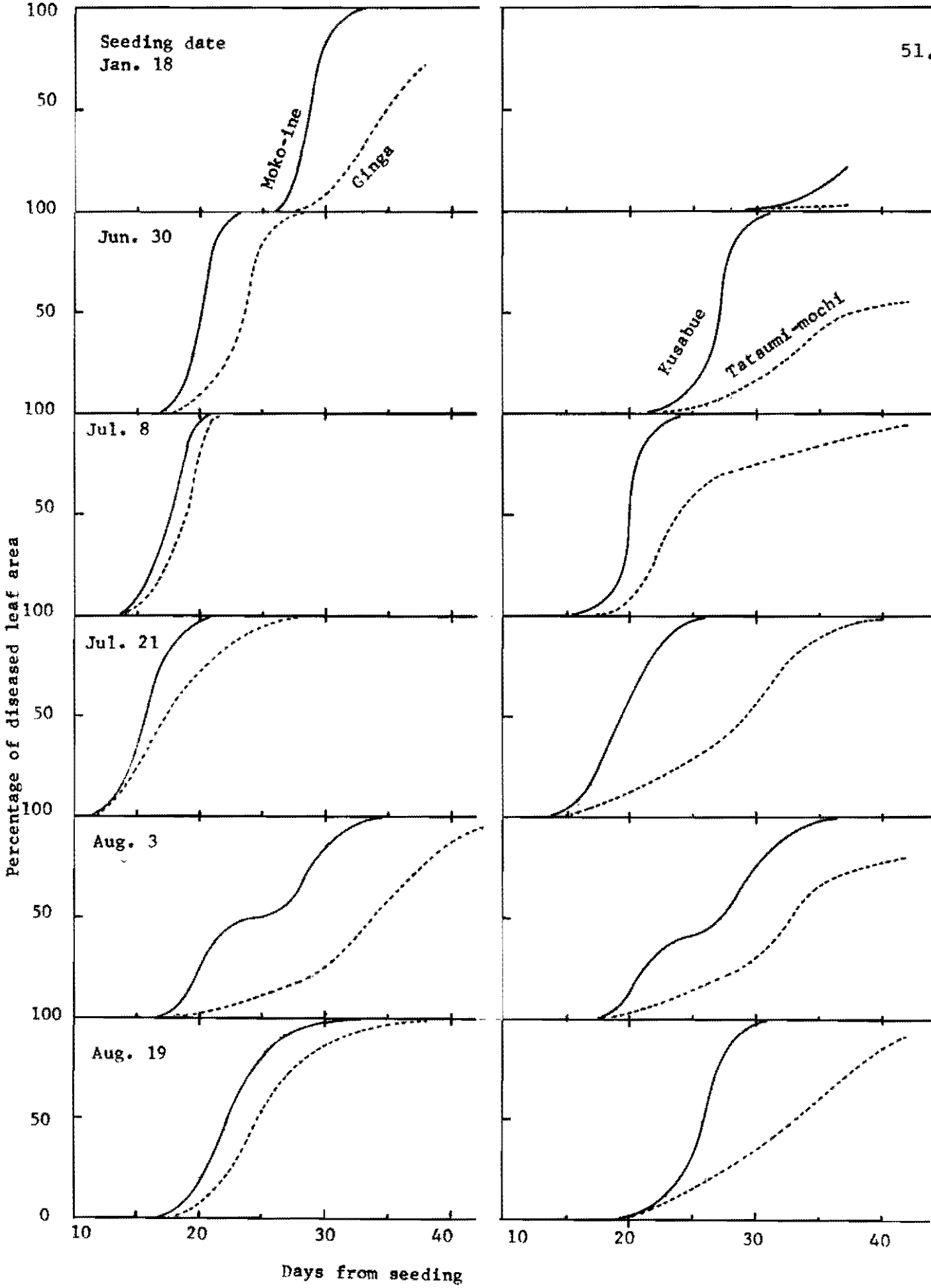


Fig. 4. Progress of disease severity of representative varieties in the blast nursery bed at different seeding time

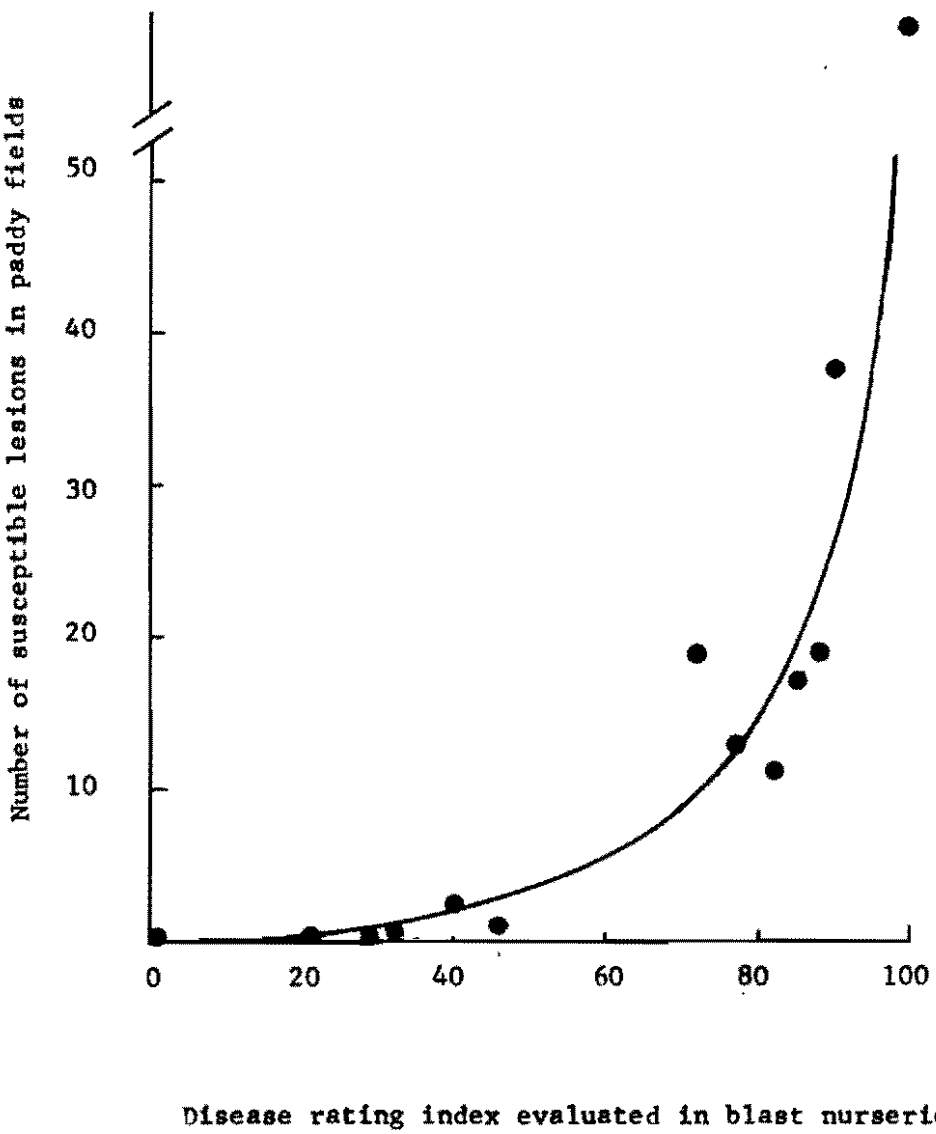


Fig. 5. Relationship between field resistance evaluated by paddy field test and that by nursery test.

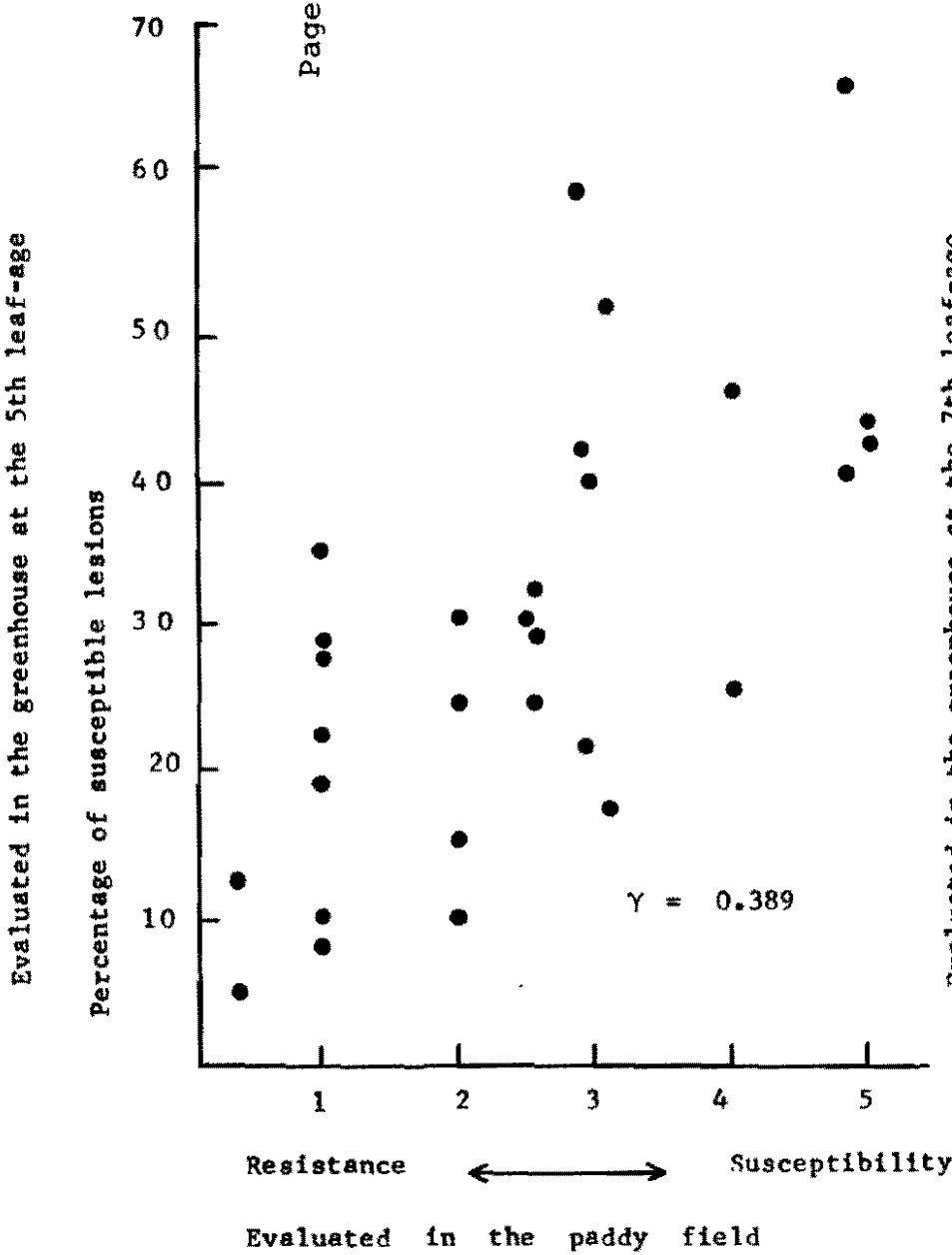


Fig. 6. Correlation between the degree of field resistance evaluated at the 5th leaf-age and that evaluated by paddy field test.

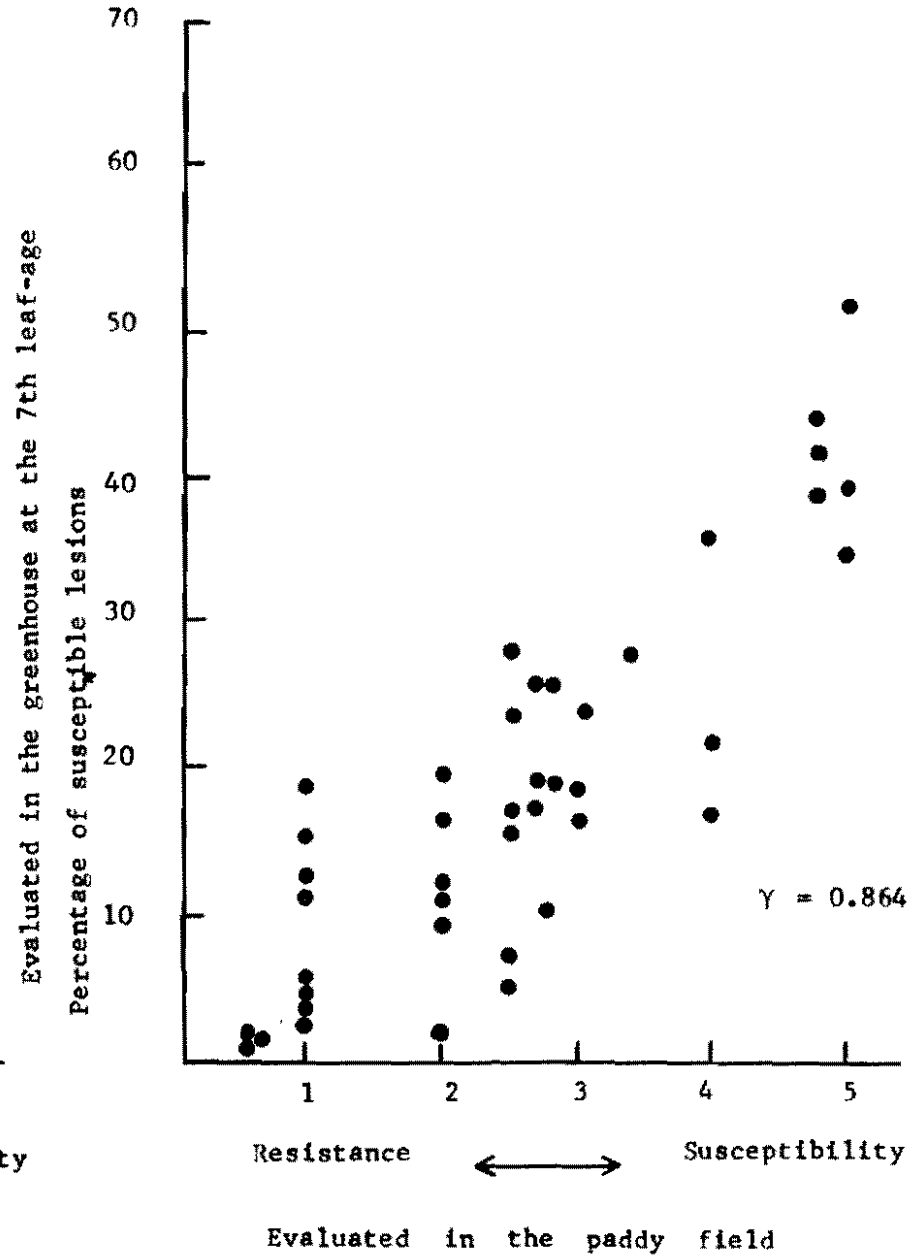
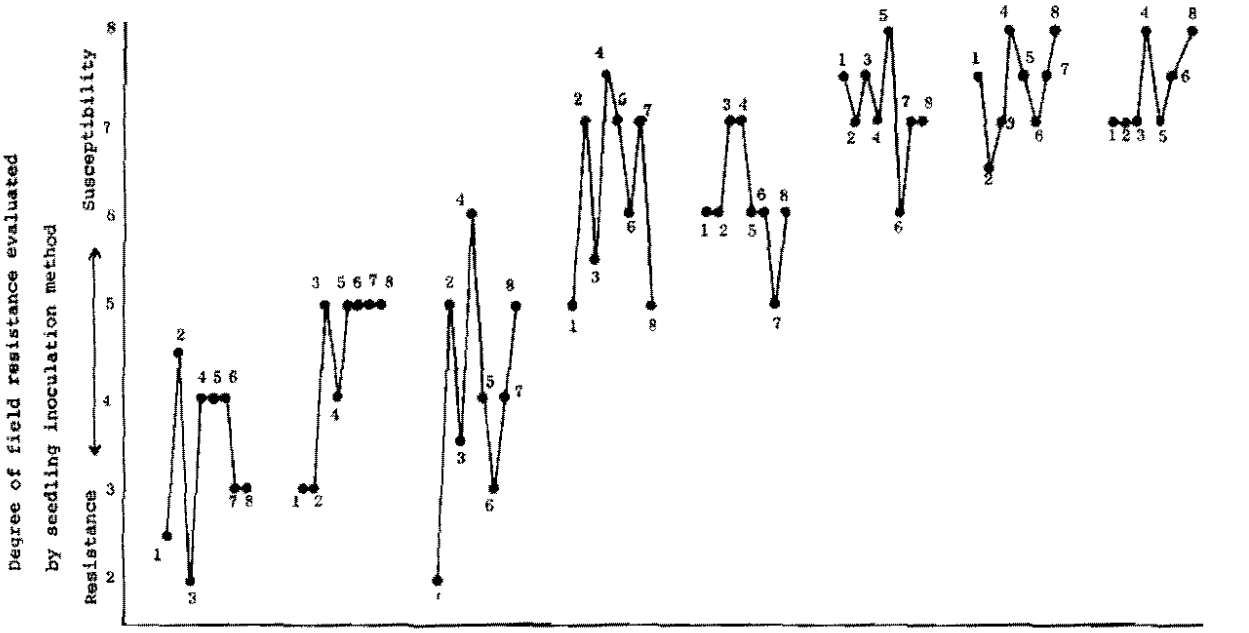


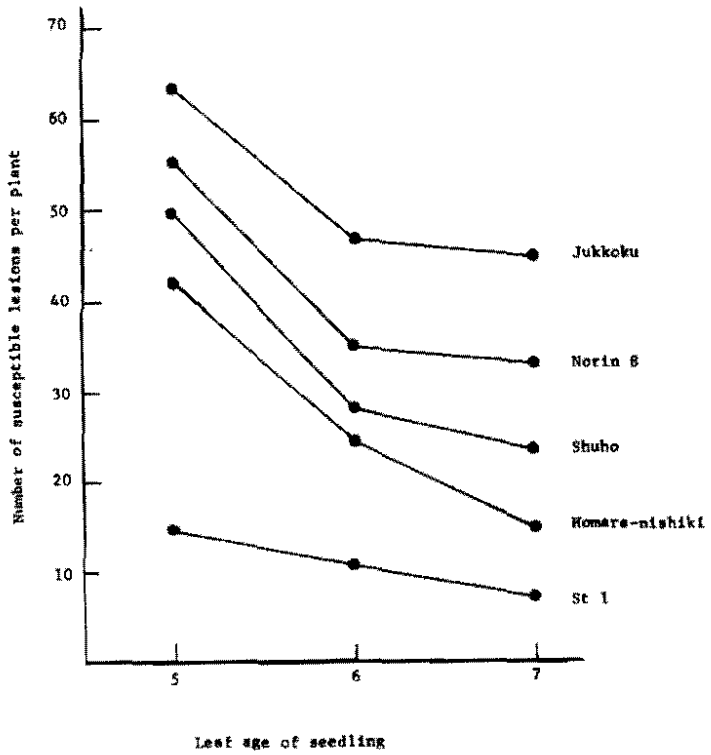
Fig. 7. Correlation between the degree of field resistance evaluated at the 7th leaf-age and that evaluated by paddy field test.



Variety	Homare-nishiki	Yamabiko	Norin 22	Nagiho	Norin 18	Kinmaze	Jukkoku	Asahi
Degree of field resistance (Breeder's experience)	3	3	5	5	7	7	9	9

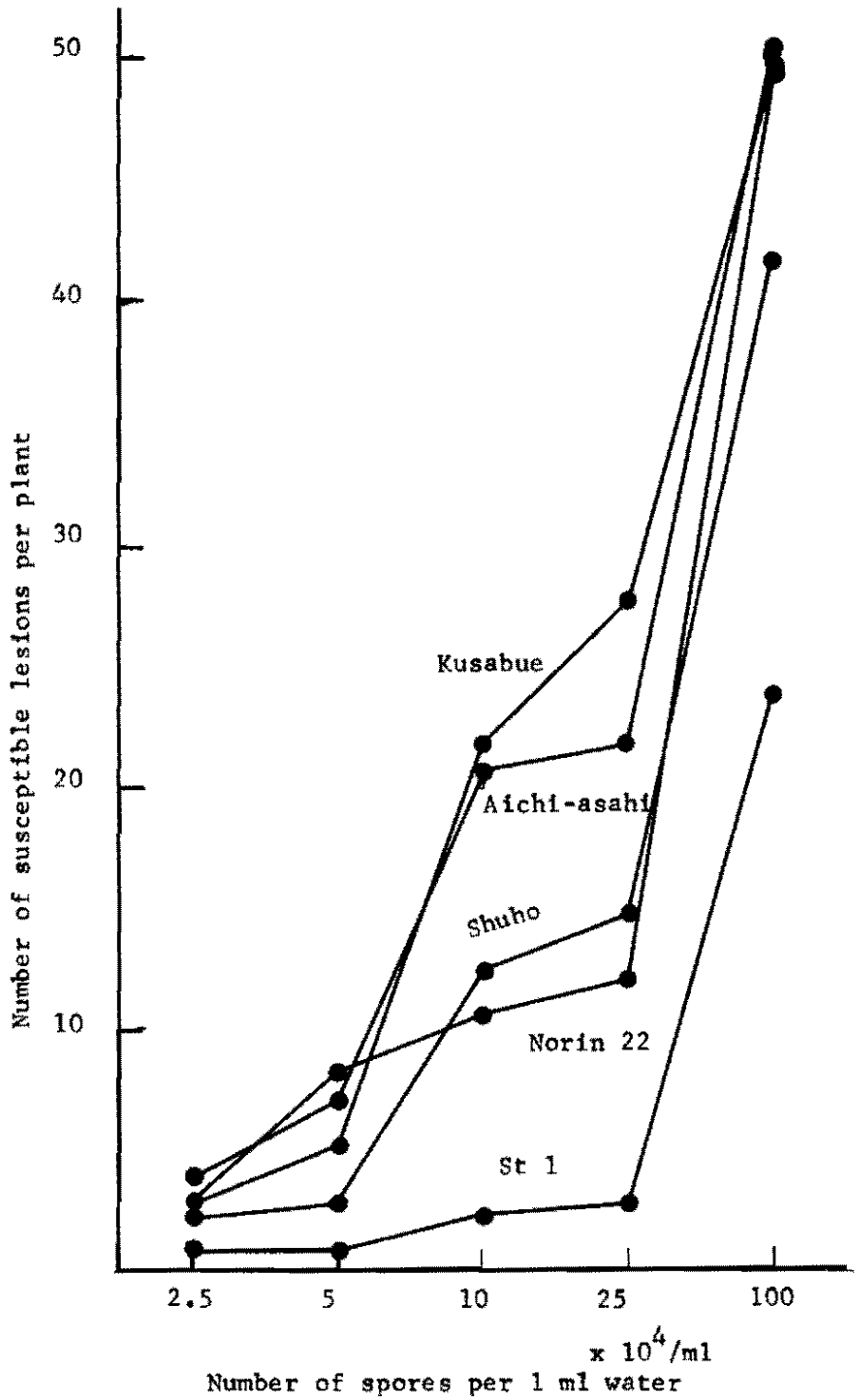
Note: Numbers in figure are Dr. Ikeno's strain [illegible]

Fig. 8. Field resistance of some varieties evaluated by spraying with 8 fungus strains.



Note: Ken 60-19 was inoculated

Fig. 9. Relation between the number of susceptible



Note: Ken 53-33 was inoculated

Fig. 10. Relation between the number of susceptible lesions and the concentration of inoculum.

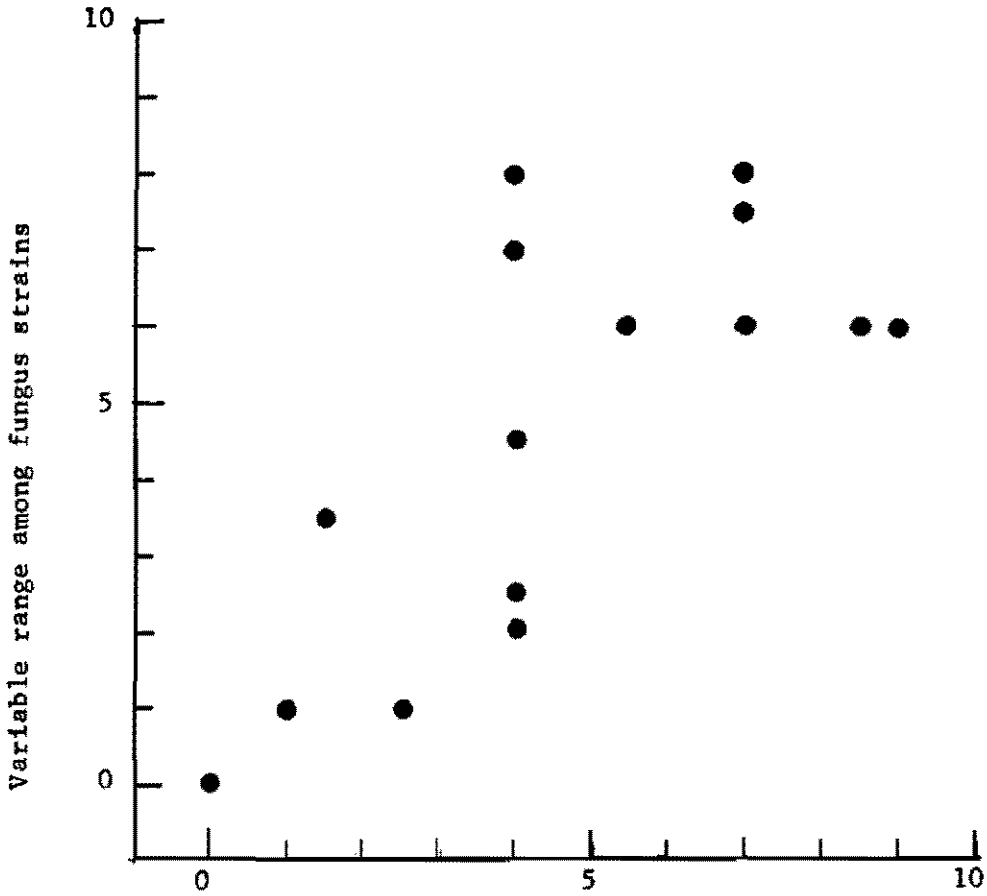


Fig. 11. Correlation between variable ranges of varietal resistance determined by fungus strains and those by locations.

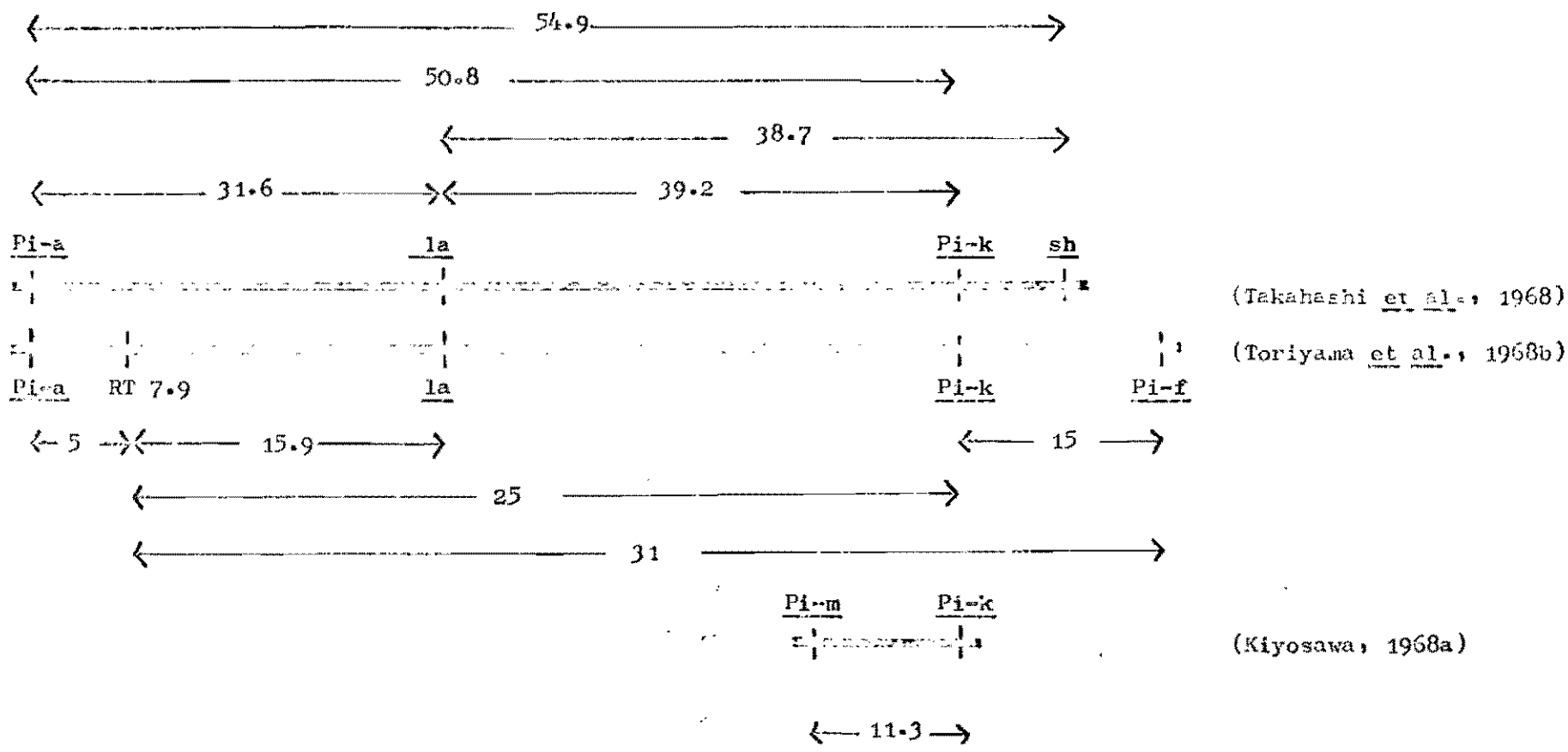


Fig. 12: Linkage relationship among genes for blast resistance and some other traits belonging to the la linkage group

Pi-a : blast resistance la : lazy growth habit Pi-k : blast resistance sh : shattering of grains

Pi-f : field resistance to blast RT 7.9 : reciprocal translocation point between Chromosome 7 and 9

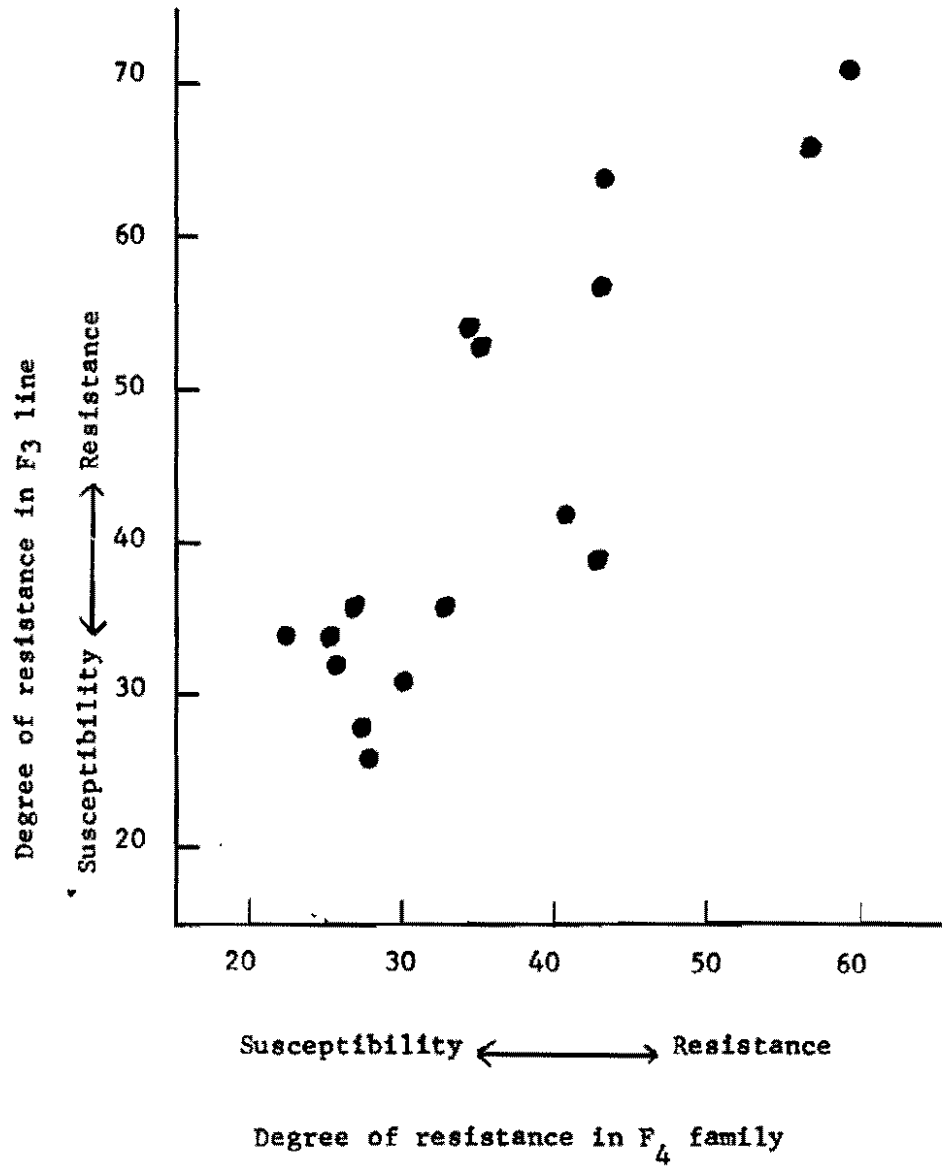


Fig. 13. Parent-offspring correlation of field resistance between F_3 lines and F_4 families.

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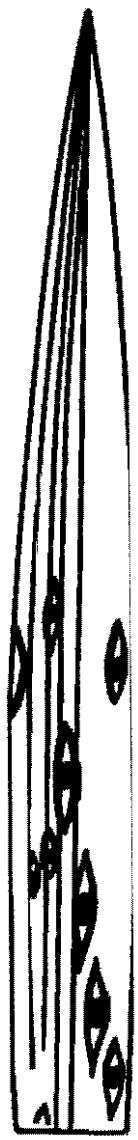

RECENT ADVANCE IN THE STUDIES ON HORIZONTAL RESISTANCE FOR THE BLAST DISEASE OF RICE IN JAPAN

Takuji Kozaka

SEMINARIO
sobre
Resistencia horizontal al
Añublo del Arroz

Octubre 8-12, 1971

RECENT ADVANCE IN THE STUDIES ON HORIZONTAL RESISTANCE FOR THE BLAST DISEASE OF RICE IN JAPAN

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Since a systemic rice breeding program with the cooperation of eight National and forty-seven Prefectural Agricultural Experiment Stations was organized in 1927 in Japan, much effort has been directed to the breeding of blast resistant varieties, using Japanese paddy rice in the first stage, and by the use of resistant varieties derived from Japanese upland rice, then by the use of resistant varieties derived from Chinese and indica varieties.

In these decades, special efforts were made to introduce race-specific resistance genes of foreign varieties into Japanese paddy rice, because most of the foreign varieties were found to be immune to most races of the causal fungus in Japan. Many new varieties were developed in this way. The breeding processes of representative new varieties were reviewed in detail by R. Ito (1963), K. Nagai (1966) and T. Hirano (1967).

These new varieties showed excellent resistance for some years after being discharged as commercial varieties, but lost their high resistance due to the occurrence of a new race or races within three years in the case of the shortest period, and about ten years in most cases. The dynamic changes of predominant races make a breeding program of resistant varieties very complicated, and the importance of the use of horizontal resistance has been re-recognized. Further researches have started in Japan from the point of distinguishing

between vertical or race specific and horizontal resistance of the rice varieties.

In this paper horizontal resistance is understood as a quantitative resistance which can be measured by either the number or the size of lesions of susceptible reaction type, but not as a qualitative one detected by the reaction type of lesions or by the hypersensitive reaction of host cells. Exact definition for horizontal resistance is another problem.

I. Varietal difference in horizontal resistance of rice

1. Testing methods

The most widely used ones are described below:

a) Grouping test varieties on the basis of blast resistance genotype or of reaction type to the races.

For the test of horizontal resistance, it is of primary importance to use compatible races that are highly virulent to all varieties to be tested. For this purpose, the test varieties are grouped generally on the basis of race-specific resistance genotype or of reaction type to the races found in Japan, then two or three test isolates are selected for each variety group.

Almost all varieties in Japan have been tested for their reaction to the races in Japan, and grouped into more than eleven groups of reaction type. Genetic studies have also been conducted for the representative varieties of each reaction type, and eight or more major resistance genes are identified (Kiyosawa, 1967) (Ezuka et al, 1969). Most Japanese varieties are included in three or four reaction types (Table 1). (Yamada et al, 1969.)

Table 1. Number of Japanese origin varieties of major reaction types to the races found in Japan.

Reaction type	Resist. gene	Reaction to the races C-3	C-6	N-1	N-2	N-3	N-4	N-5	Number of varieties included	
Shin 2 type	no	S	S	S	S	S	S	S	910	(67%)
Aichiasahi type	Pi-a	R	S	S	S	S	R	R	332	(25)
Ishikari-shiroke type	Pi-i	R	S	S	R	R	R	S	47	(3)
Others									57	
					Total				1349	

Standard test isolates for each variety group have also been selected, but as described later there remain some unsolved problems in selecting test isolates.

b) Seedling tests by spray inoculation under greenhouse conditions.

The seedlings of the varieties to be tested are grown in small seedling boxes under upland conditions as in the case of race identification. They are inoculated by spraying with spore suspension at 7 to 8 leaf stage, and incubated at 26°C for 18 to 20 hours, then kept in an air conditioned greenhouse at 26°C for about two weeks. Evaluation is made on the first two leaves from the top at the time of inoculation on the basis of lesion area (Fig. 1).

c) Nursery test by inoculation.

Preparation of nursery is the same as in the case of the"International Uniform Blast Nursery Program"by FAO. Border rows consisting

of the most susceptible varieties are inoculated with infected leaves by scattering; these are prepared in advance in another nursery bed by spray inoculation with test isolates, and cut into small pieces 3-5 cm long at the time of inoculation.

Evaluation is made on the basis of percent of diseased leaf area when the disease covers almost all leaves of the most susceptible varieties.

Special caution is taken to arrange in order to develop the disease from 7 to 9 leaf stage; too early or too late inoculation should be avoided.

d) Field test.

Test varieties are transplanted by ordinary cultivating methods in rows, three to five rows of two to three meters long per variety. The most susceptible variety is mixed with every two or three test varieties as border rows. Inoculation is often made 10 to 20 days after transplanting by scattering on border rows with diseased leaves, which are prepared in advance in the same way as in the nursery test. Some times they are under natural infection.

Evaluation for leaf blast is usually made at the young panicle formation stage to booting stage on the basis of percent of diseased leaf area or of percent of diseased leaves. Evaluation for neck blast is made about 25 days after heading on the basis of percent of diseased panicle.

2. Representative results of the tests for horizontal resistance

Intensive tests for horizontal resistance of rice were conducted

in Japan in several National Agricultural Experiment Stations in 1966 - 1968, in which almost all varieties obtained in Japan (including foreign varieties or inbred varieties with foreign varieties) were tested by nursery or field tests.

The results showed that there is a clear difference in horizontal resistance among the varieties of the same blast resistance genotype or reaction type to the known races in Japan. The resistant varieties have either a fewer number of lesions or smaller size of lesions of susceptible reaction type, or both, in comparison with the susceptible varieties.

The representative results are shown in Table 2.

The results obtained also indicate that some varieties are very consistent in their horizontal resistance, but some other varieties - more than ten percent of the total - fluctuate considerably among localities and years, in spite of the condition of the same race constitution. (See Table 2.)

II. The major factors influential to the evaluation of horizontal resistance of varieties

A joint work on horizontal resistance of 59 selected varieties of five representative blast resistance genotypes was carried out in 1967 - 1968 in ten localities throughout Japan in order to detect the major factors responsible for the fluctuation of the varieties in resistance. The results are summarized as follows:

1. Influences of isolates used

It was found generally that the isolates, compatible to all the test varieties and belonging to the same race, differ from each other in their virulence to the varieties (Table 3).

Table 2. Number of varieties of different degree of horizontal resistance included in representative variety groups of reaction types to the races in Japan (Ezuka et al, 1969).

Reaction type	Resist. genotype	Horizontal resistance	Number and name of varieties included
Shin 2 type	+	rr	13-- Chiyohikari, Ohu 247 Tokai 26, Sanin 63,Harima, Shito,St.1, Suzuhara-mochi Norinrikuto 24, Rikuto-Kan-to 83,Sensho,Fukuton,Riku-to Norin mochi 26
		r	30
		m	36
		s	34
		ss	22
Aichiasahi type	Pi-a	rr	10-- 65A-8,Heirokumochi, Rikuto Norin 1,Shinhakaburi, Hiderishirazu,Kuroka,Kiri-shima,Kahei,Hirayama,Akamai
		r	27
		m	21
		s	30
		ss	10
Ishikari-shiroke type	Pi-i	rr	7-- Yoneshiro,Hokkai220, Joiku 232, Soraiku 9, Sora-kei 22, Toyamawase, Kumochi
		r	8
		m	3
		s	1
		ss	2
Kanto 51	Pi-k	rr	1--- Reishiko
		r	3
		m	8
		s	9
		ss	12

Table 3. The number of lesions of susceptible type on a variety Manryo by spray inoculation with different isolates of the races at 20 x 10^4 / ml spore concentration (Suzuki and Yamada 1969).

Races identified	Isolates	Number of susceptible lesions per 60 plants
C-1	1	1472
	2	810
	3	1917
	5	540
	6	1080
	7	900
	8	990
	9	2160
	10	2120
N-1	1	540
	2	240
	3	630
	4	780
	5	1170
	6	960
	8	2160
	9	1170
N-2	1	2730
	2	1250
	3	1619
	5	1258
	6	2130
	7	1730
	8	1120
	9	490

A highly virulent isolate produces a large number or larger size of susceptible reaction type lesions on the plants in comparison to the isolates of the less virulent. It is, moreover, recognized that the degree of resistance of the varieties is somewhat different to each of the isolates.

Fig. 2 shows one of the tests with different isolates of the same Japanese race C-1, in which the isolate Ken 66-19 was the most virulent and Ken-117 the least virulent, and a variety such as Ugonishiki was variable in a wider range in its resistance to the isolates. Fig. 3 also shows a similar result indicating that Norin 8 and Norin 25 were very variable in resistance to each isolates (See Figs. 2 and 3).

An other extreme example is observed in a line St.1. This variety shows high horizontal resistance to many isolates of all known races in Japan, producing a very small number of lesions of susceptible reaction. Some isolates collected from Fukushima, Ibaragi and Hiroshima prefecture, however, showed high virulence to the variety, producing as many lesions as in the most susceptible varieties as shown in Fig. 4 (Sakurai, 1969). A major gene Pi-f is assumed to control this type of resistance in St.1 (Toriyama et al, unpublished).

A variety St. 1 was derived from the fifth backcross involving Norin 8 as a recurrent parent and Modan as a donor for the purpose of developing stripe virus-resistant varieties. Norin 8 is a Japanese paddy variety susceptible to stripe, while Modan is a typical indica variety of Pakistan origin, showing highly resistant to stripe. St.1 is highly resistant to stripe as well as Modan.

A very similar result was also obtained in a variety Minehikari. Minehikari showed high horizontal resistance to all isolates of Japanese race C-8 until 1968, but became susceptible to the isolates of the same race collected from Aichi prefecture in 1969 (Aichi Agr. Exp. Sts.1969). Genetic studies have not yet been made. Minehikari

is one of the leading varieties in central Japan, derived from multiple crossing between a Chinese variety Hokushi-taimai and a Japanese upland and lowland variety by breeders Drs. Iwatsuki and Ujihara. It is immune to Japanese N race group, most predominant races in Japan, and also highly horizontal resistant for more than ten years to Japanese races C-8 and C-1, the second dominant races.

As mentioned at the beginning, I understand horizontal resistance as a quantitative one which can be measured by either the number or the size of lesion of susceptible reaction type, but not as a qualitative resistance detected by the reaction type of lesion or by the hypersensitive reaction of host cells. In this tentative definition, the resistance observed in St. 1 and Minehikari is, beyond doubt, considered to be horizontal resistance, because they produce a small number of lesions to some isolates and a larger number of lesions to some other isolates, but the resistance of these varieties is found to be an isolate specific, probably controlled by a single major gene. Isolate specific resistance does not differ essentially from race specific resistance, which is known generally to be qualitative.

As many varieties have been recognized as highly horizontal resistant with the tests using a limited number of isolates, there is a small possibility that some of them may be highly susceptible to some unknown isolates, with a higher possibility in more resistant varieties.

These facts suggest much difficulty in evaluating horizontal resistance of the varieties with a limited number of isolates.

2. Aging of the plants

It is well known that the plants are more susceptible at the younger stage, 4-5 leaf stage, and become more resistant with aging (Table 4).

Table 4. Change of varietal resistance of rice with aging. Figures indicate the degree of disease of 0 (healthy) to 10 (dead). (Central Agr.Exp.Sta. 1967.)

Varieties	Resist. genes	Leaf stages at the time of inoculation				
		4	6	8	10	12
Kongo	Pi-k,a	5.0	5.5	2.3	2.2	0.2
Esunan 30	Pi-k,a	7.0	7.0	4.3	3.5	1.0
Senshuraku	Pi-k,a	-	-	4.8	1.5	0.7
Kanto 59	Pi-k,a	10.0	8.5	5.5	4.5	1.8
Kusabue	Pi-k,a	10.0	9.0	6.3	5.5	0.8
Shinano-hikari	Pi-i	9.0	8.0	3.0	0.7	1.5
Takane-nishiki	Pi-i,a	8.5	8.0	4.8	3.7	0.5
Homare-nishiki	Pi-a	7.0	7.0	1.8	0.7	0.3
Aichiasahi	Pi-a	9.5	8.5	6.3	5.5	2.0
Kogane-nishiki	-	-	-	4.5	2.1	1.0
Norin 22	Pi-a	8.5	7.5	4.8	2.0	0.8
Norin 29	Pi-a	9.5	8.0	5.2	4.3	0.8

The used isolate: Ken 60-19

The rate of increase of resistance with aging differs among varieties, although it was influenced by other factors such as fertilizer. Some varieties which are of the most susceptible at younger leaf stage become more resistant at the older stage in comparison with some other susceptible varieties.

In many cases, a definite varietal difference in horizontal resistance is exhibited well after 8 to 10 leaf stages.

The results indicated in Fig. 5 show that the susceptible varieties did not become resistant by the time of 9-10 leaf stage, while resistant varieties become more resistant in earlier stages.

In the field under natural conditions, the disease occurs generally soon after transplanting, at 8 to 9 leaf stage, in southern Japan, and after 10 leaf stage in northern Japan. These facts support the importance for the practical purposes of the evaluation of horizontal resistance of older plants after 8-9 leaf stage.

3. Effects of fertilizer and shading of sunshine

In Japan a good supply of nitrogen fertilizer and a long spell of cloudy days are major factors that favor the disease. Therefore, the difference in the response of the varieties to those two factors are important. The results showed that the order of the varieties in resistance was not so greatly disturbed by these factors. One of the results is shown in Fig. 6.

4. Influence of temperature

a) Air temperature at the time of inoculation.

As little research was made, a definite conclusion has not yet been obtained. Results indicated in Fig. 7 showed that the optimum

temperature for infection is somewhat different among varieties, but generally the susceptible varieties are infected severely in any test temperature.

b) Air temperature after inoculation.

A definite conclusion has not yet been obtained. One of the results is shown in Table 6. Fewer varietal differences in response to the temperature were found.

Table 6. Effects of air temperature after inoculation on varietal resistance. The figures indicate number of lesions per leaf (Central Agr. Exp. Sta. 1968).

Varieties	Resistant genes	Isolates used								
		Th-65-252			Ken 64-117			Ken 53-11		
		20°	25°	30°C	20°	25°	30°C	20°	25°	30°C
Sanin 68	Pi-k	13.8	10.5	8.5	6.6	2.7	2.2	1.6	1.6	0.5
Senshuraku	Pi-k	5.1	5.5	3.3	3.6	2.9	1.5	2.0	1.3	0.1
Kanto 59	Pi-k	14.7	11.0	11.1	4.0	2.5	3.0	2.3	1.3	0.3
Kusabue	Pi-k	10.3	10.5	9.6	7.7	8.1	5.0	3.2	1.4	0.7
Ohu 248	Pi-k	5.3	4.4	2.9	1.7	0.9	0.2	0.4	0.1	0.4
Senbonasahi	Pi-a	4.9	5.7	1.4	1.8	1.3	2.0	1.2	0.4	0.4
Kinmaze	Pi-a	10.1	6.7	2.1	3.1	1.2	0.3	0.7	0.2	0.0
Yamabiko	Pi-a	5.6	5.1	2.8	2.1	0.4	1.0	0.6	0.2	0.1
Chiyohikari	+	4.8	4.5	2.6	3.3	2.4	4.5	0.4	0.3	0.0
Kogane-nishiki	Pi-a	3.3	2.3	0.9	0.8	2.4	0.1	0.1	0.1	0.0
Norin 29	Pi-a	13.4	15.8	8.8	7.6	6.8	2.9	2.0	1.8	0.4
St.1	Pi-a	6.3	2.8	1.0	3.2	1.0	0.1	0.6	0.0	0.2
Tozan 38	Pi-a	8.6	10.2	2.3	3.8	2.4	1.7	0.6	1.2	0.2

Inoculated at 7 leaf stage, evaluation was made 10 days after inoculation.

5. Influences of spore concentration, and of mixed inoculation with more than two isolates.

With the increase of spore concentration, the number of lesions produced increases, but the rate of increase sometimes differs slightly among varieties (Fig. 7) even in the case with the same isolates.

Figure 8 shows a representative example of mixed inoculation. As shown in the figure, if one of the two isolates to be mixed is incompatible with the test varieties, and the other is compatible, the number of lesions or size of lesions produced on the compatible varieties decrease proportionally to the mixing ratio of incompatible isolates, due to the interaction between isolates, which is recognized to be the effect of production of phytoalexin (Ohata and Kozaka, 1967).

Mixed inoculation should be made with the isolates which are both virulent to the varieties to be tested.

III. Inheritance of horizontal resistance

A few research varieties, Norin 22, Homarenishiki and Ginga, have been made. These varieties are all very old ones bred in Japan and recognized to be highly horizontal resistant experimentally for a long time. Recent experiments also support this.

Kiyosawa (1970) reported that segregation of the F_3 progenies of the hybrids of Homarenishiki x Aichiasahi and Aichiasahi x Ginga against isolate Ken 54-04 under various environmental conditions could consistently be explained by one major gene and two minor genes. He also reported that Homarenishiki and Ginga have probably

at least one common gene, and that a major gene of Homarenishiki and Ginga behaved independently to the race-specific resistance genes Pi-a, Pi-k and Pi-i, which are carried in Aichiasahi,Kanto 51 and Ishikarishiroke, respectively.

Kiyosawa, Matsumoto and Lee (1967) suggested that the horizontal resistance of Norin 22 against isolate Ken 54-04 was controlled by one major and two or more minor genes by the analysis of segregation of the F_3 progenies of the hybrids of Norin 22 x Aichiasahi.

These results, however, were obtained by a particular isolate Ken 54-04 using an injecting method into sheathes. This isolate shows very weak virulence to the varieties tested.

Some questions still remain, however, about what method is most adequate to study the inheritance of horizontal resistance.

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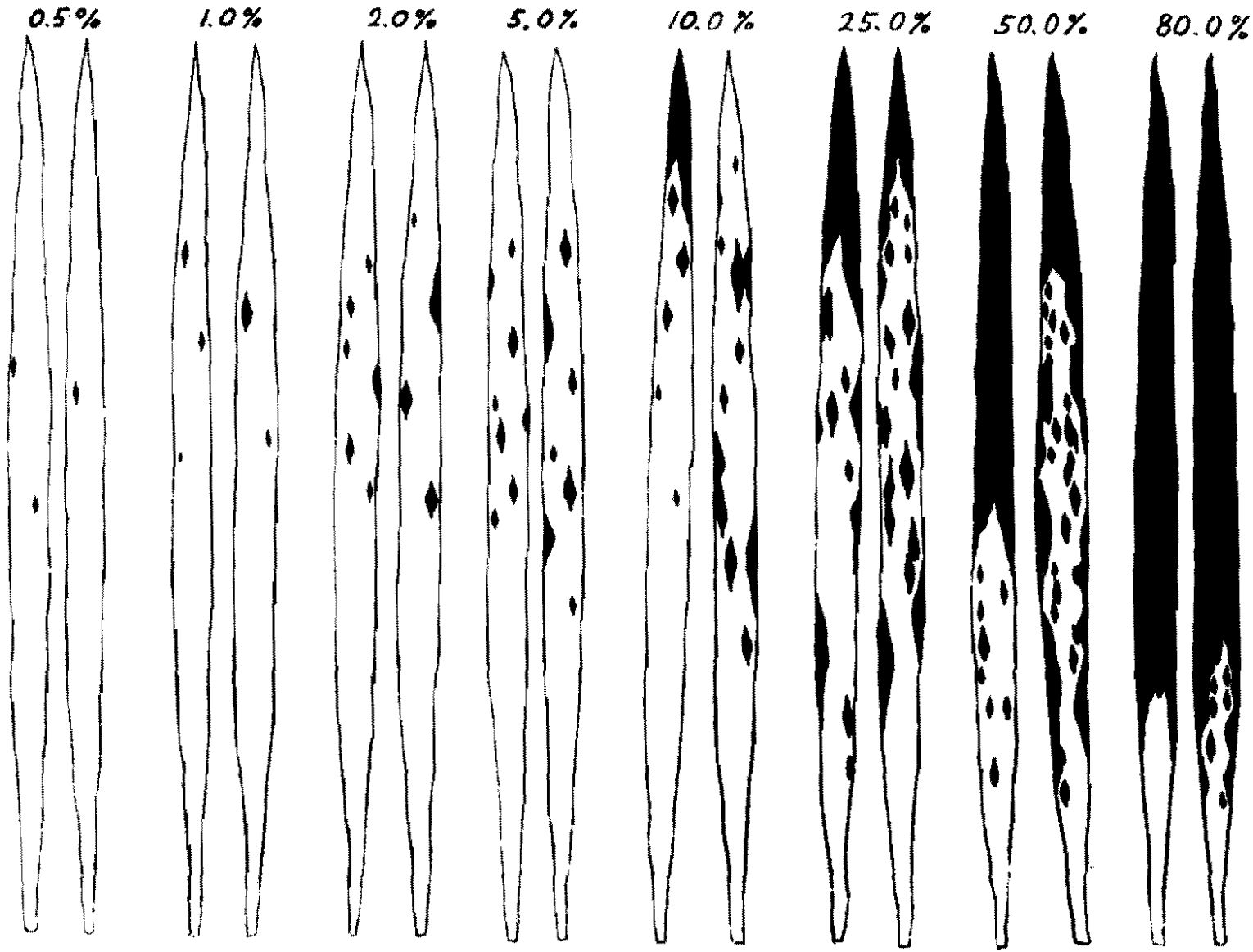
Fig. 1 A standard scale for disease rating on the basis of per cent of diseased leaf area



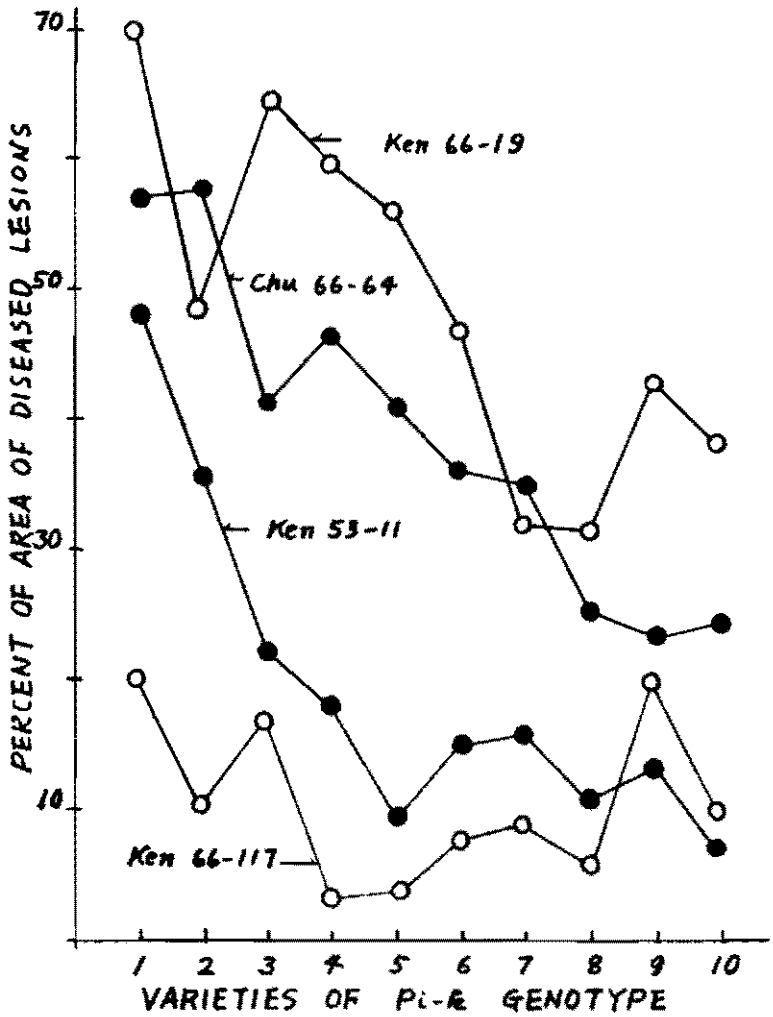


Fig. 2 Difference in varietal responses to the isolates. The listed varieties: 1 Kanto 51, 2 Sanin 68, 3 Kusabue, 4 Fu-69, 5 Tatsumi-mochi, 6 Kanto 59, 7 Ohu 248 8 Mangetsumochi, 9 Ugonishiki, 10 Senshuraku

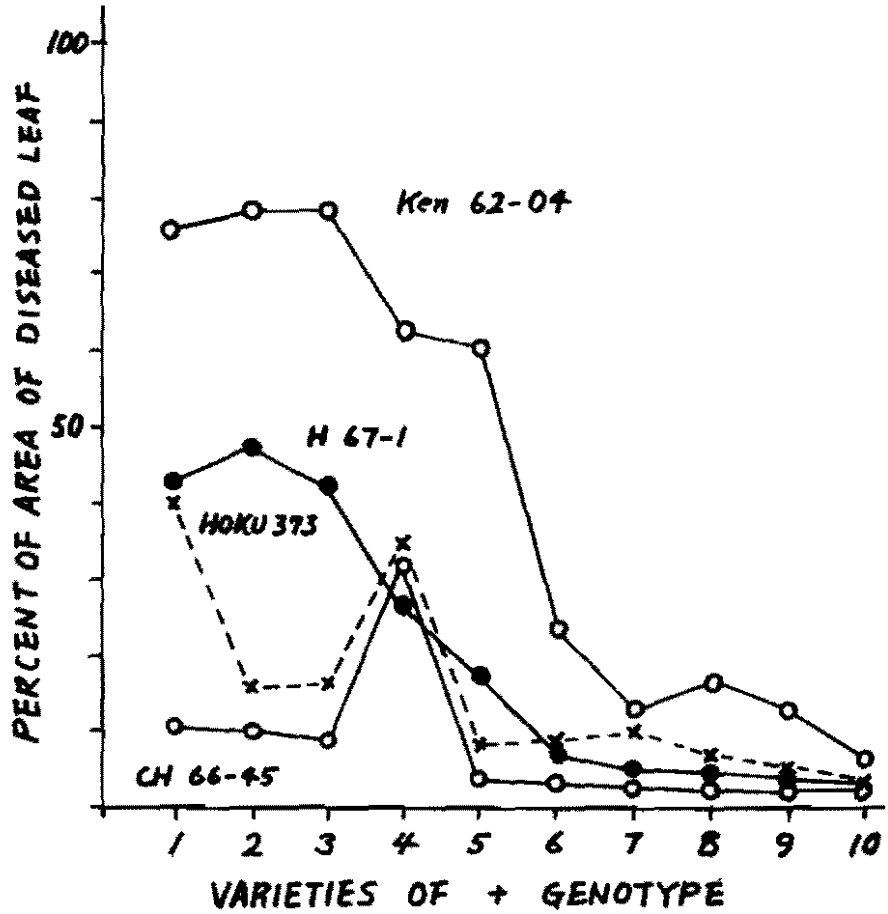


Fig. 3 Difference in varietal responses to the isolates. The listed varieties: 1 Norin 29, 2 Manryo, 3 Koshihikari, 4 Norin 8, 5 Norin 25, 6 Norin 22, 7 Nihonbare, 8 Chiyohikari, 9 Koganenishiki, 10 Ginga

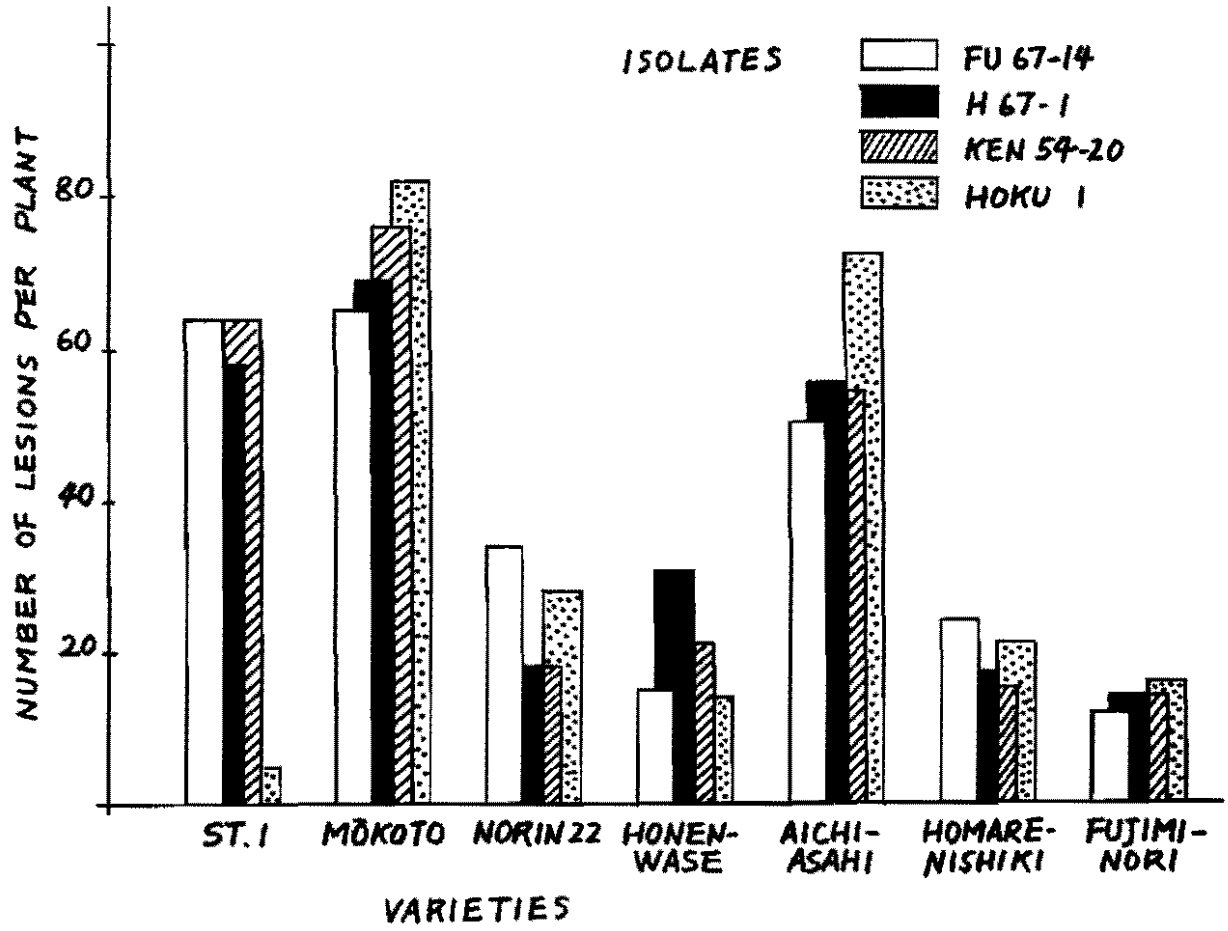


Fig. 4 Varietal resistance to the isolates of the same race, note: response of a variety St. 1 (Sakurai, 1969)

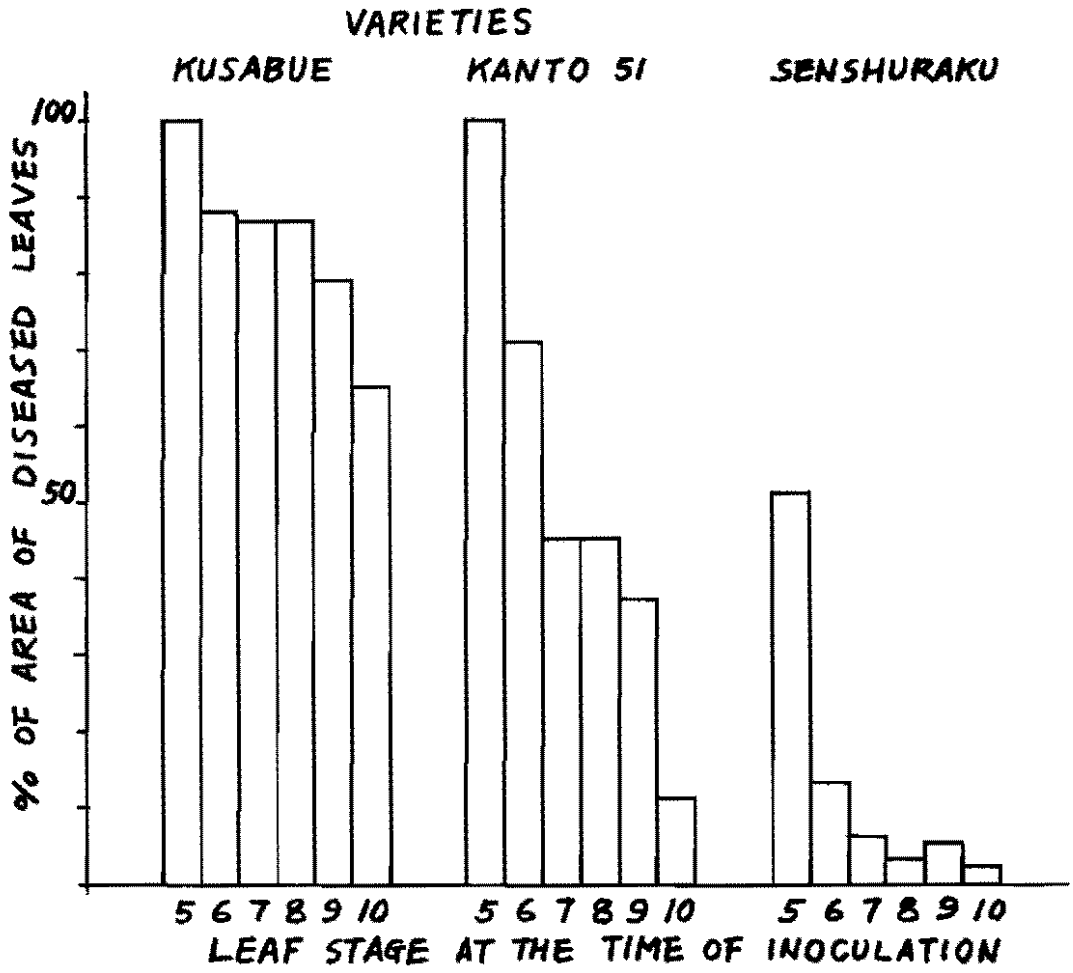


Fig. 5 Varietal difference in increasing of resistance with aging. (Central Agr. Exp. Sta. 1968)

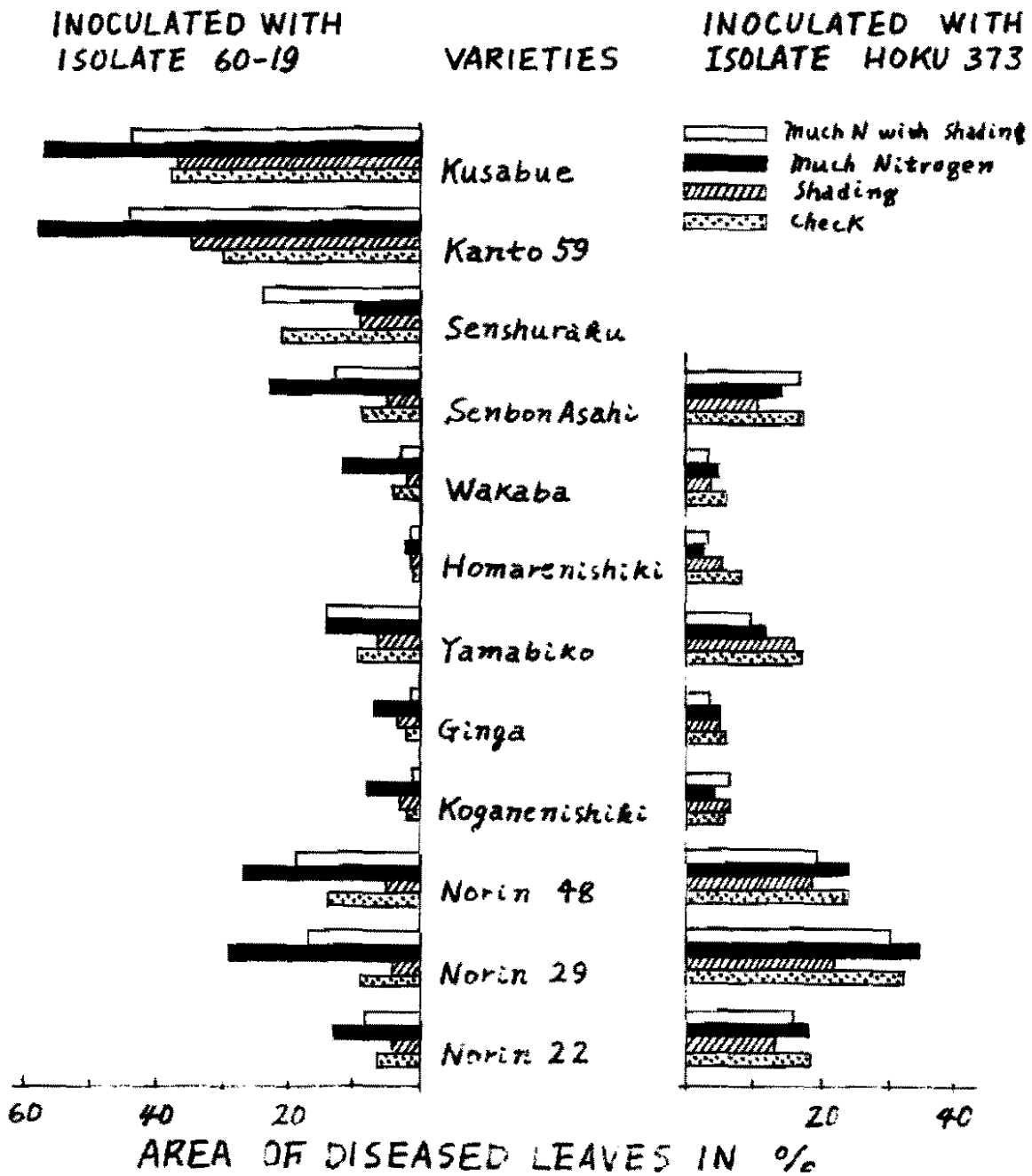


Fig. 6 Effects of nitrogen fertilizer and shading of sunshine on the varietal resistance (Central Agr. Exp. Sta. 1967)

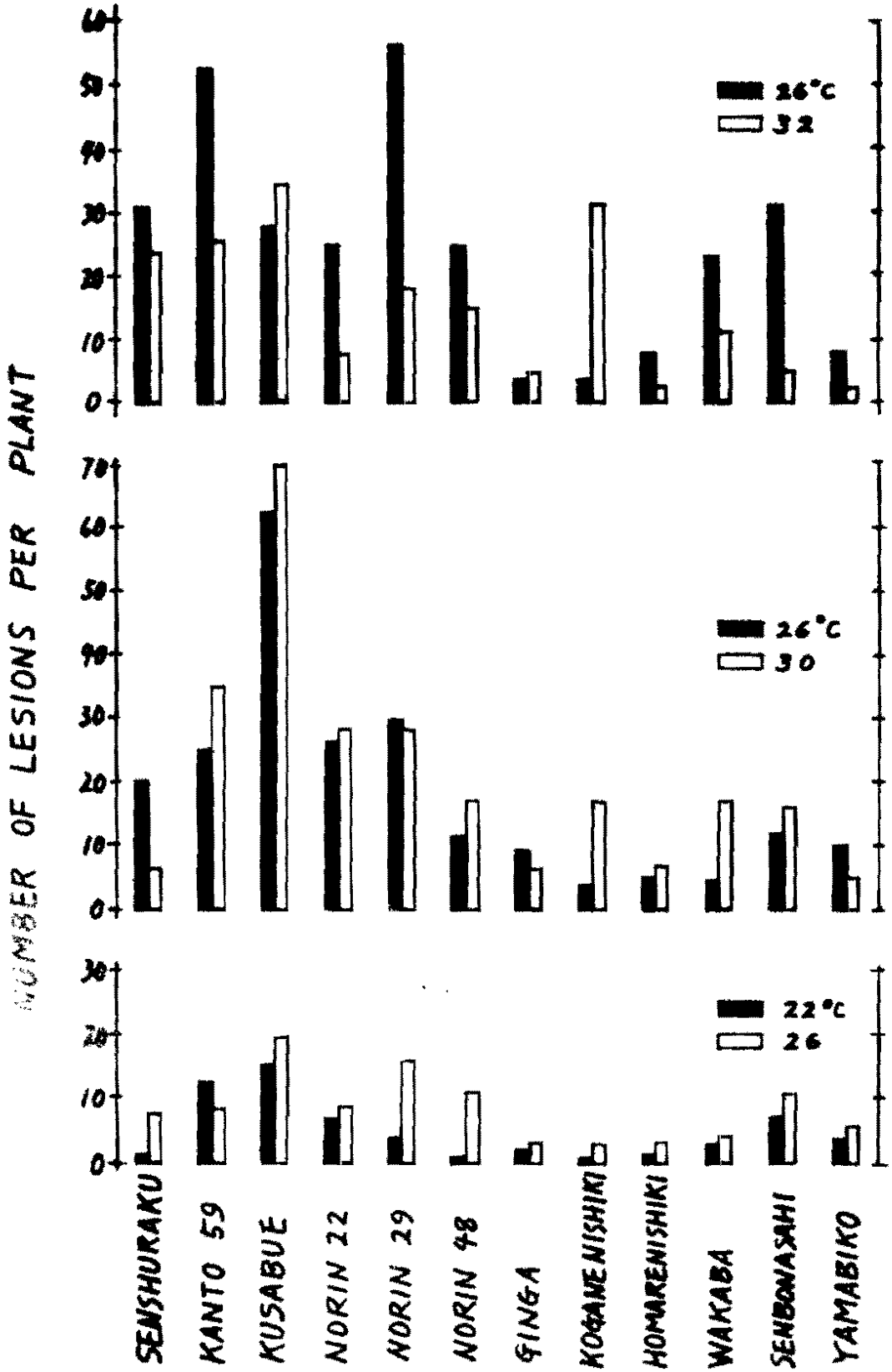


Fig. 7 Effects of temperature at the time of inoculation on varietal resistance, incubated for 25 hrs at selected temperatures (Agr. Exp. Sta. 1967)

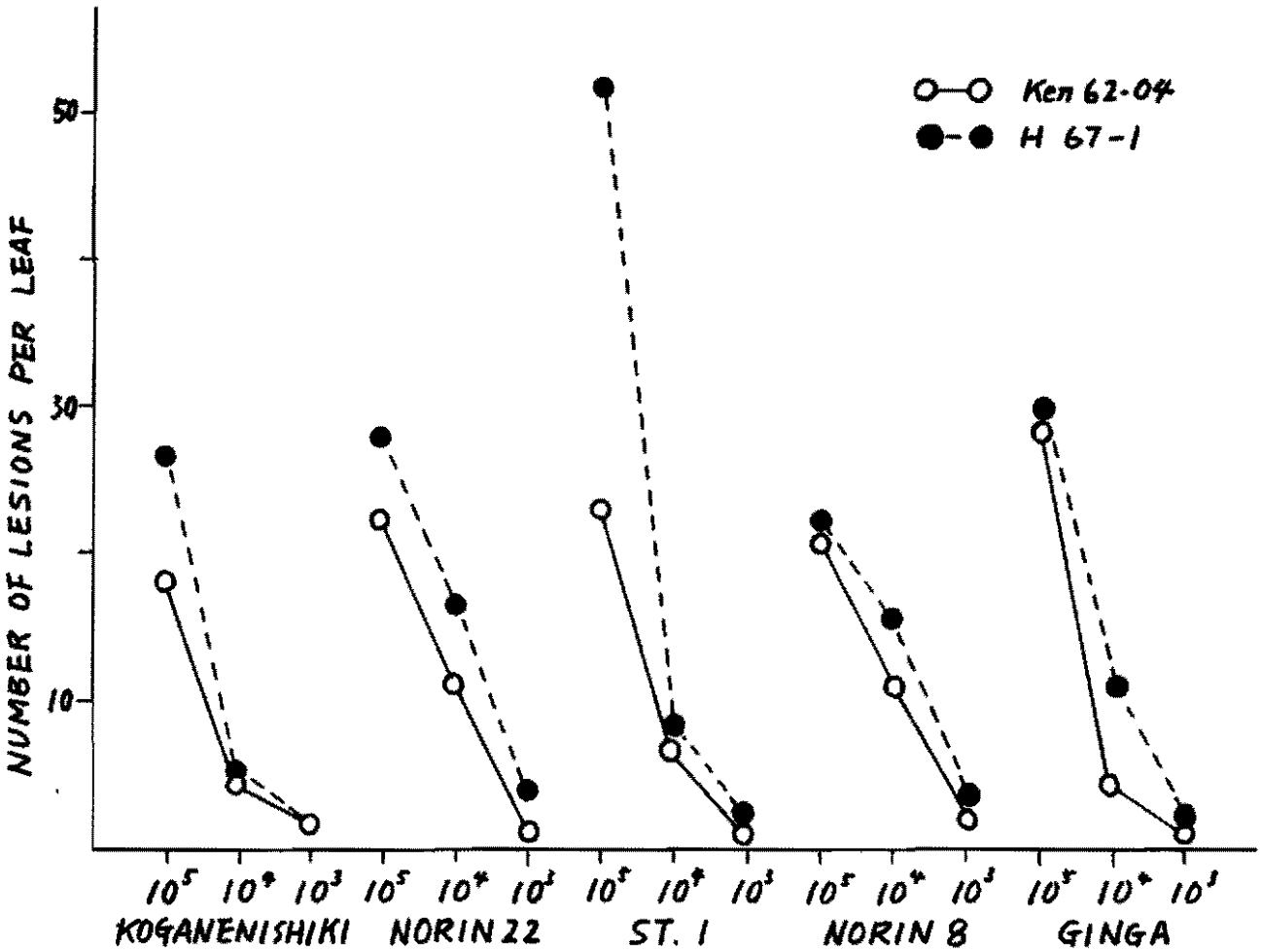


Fig. 8 Effects of spore concentration for inoculation on varietal resistance (Central Agr. Exp. Sta. 1967)

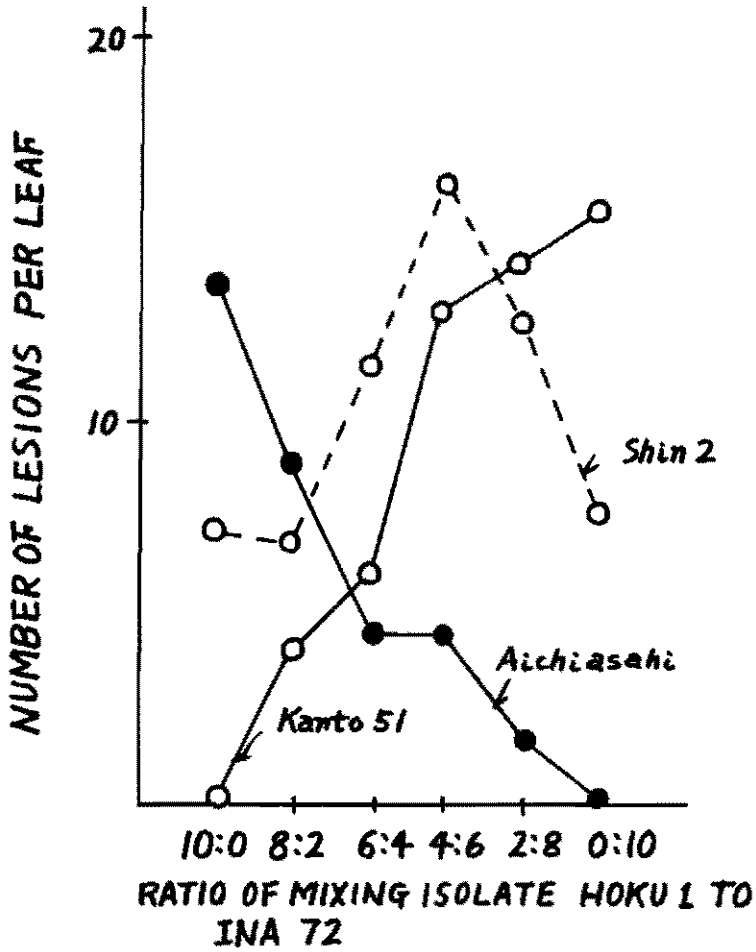


Fig.9 Effect of mixed inoculation with two isolates on varietal resistance. In figure, a variety Shin 2, is susceptible to one of two isolates and immune to the other (Nat. Inst. Agr. Sci. 1968)

GEOGRAPHICAL DISTRIBUTIONS AND PREDOMINANT RACES OF PYRICULARIA ORYZAE

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Several countries other than Japan, U.S.A. and Taiwan have carried out studies on the pathogenic races of the rice blast fungus since the symposium on the rice blast disease held at the International Rice Research Institute (IRRI) in 1963.

It will be useful to the study of horizontal resistance to the disease of rice to take a view of geographical distribution of pathogenic and predominant races in certain countries from the data which have been reported.

I. Geographical distribution of the pathogenic races

According to the paper, "Pathogenic races of Pyricularia oryzae Cav. in Asian and some other countries" by S. Matsumoto et al., nearly two hundred specimens of diseased plants in thirteen countries were tested on their pathogenic races at the National Institute of Agricultural Sciences of Japan from 1962 to 1966. At the same time, workers at the Japan-United States Cooperative Blast Project conducted inoculation tests of the isolates which had been exchanged between both countries. (Figure 1 and Table 1).

From the results of these tests, four geographical areas are distinguishable on the basis of race distribution: 1) Japanese, 2) Philippine, 3) Indian and 4) American. The races found in each of these areas have some pathogenic characteristics while showing differences from each other. The common characteristics in the pathogenicity of the races in each area are summarized below:

1) Races found in the Japanese area

Major races encountered in Korea and Taiwan belong to those characterized in Japan, namely, Japanese race N-1, N-2, N-3, N-4 and N-5. They are pathogenic to all varieties of Japonica type, i.e., Homarenishiki, Ginga, Norin 20, Norin 22 and Caloro, but are nonpathogenic to the majority of Indica type varieties, i.e., Tetep, Tadukan, Usen, NP-125, Raminad Str. 3 and Wag wag. Many varieties of Japonica type are cultivated widely in this area.

2) Races found in the Philippine area

Most major races found in the Philippines, Vietnam, Cambodia and Thailand and to some extent in Taiwan and Indonesia are identical to or closely resemble some of the US races 5, 6 and 11. As shown in Table 1, they show similar reaction patterns onthe Japanese differential varieties, with susceptible reaction on only two or three varieties, namely, Usen, Ishikarishiroke and Aichiasahi. They are characterized by: a) nonpathogenicity to all varieties of Japonica type except Aichiasahi and Caloro, thus indicating a striking contrast to the reaction observed with races in the Japanese area, and b) nonpathogenicity to the so-called Chinese type varieties, i.e., Chokoto, Yakeiko, Kanto 51, C.I. 5309 and Dular, also indicating a striking contrast to the reaction of races in the Indian area. Among the varieties of Indica type, these races are all pathogenic to Usen, and some of them are pathogenic to Raminad Str. 3 and Wag wag.

3) Races found in the Indian area

Major races found in India, Ceylon and West Pakistan, and some races in Indonesia, are identical or show close similarity to the US race 8 or 9. As shown in Table 1, all the major races in this area are nonpathogenic to most of the varieties of Japonica type, and in this respect are similar to the races in the Philippine area. However, they are pathogenic to most varieties of the Chinese type such as Chokoto, Yakeiko, Kanto 51, C.I. 5309 and Dular, thus indicating a striking contrast to the reaction of the races in the Philippine area. They also differ in that they are pathogenic to NP-125, which is resistant to most isolates from other areas.

4) Races found in the American area

According to the results reported by United States workers, the most prevalent races were US race 3 and 6 followed by US race 16.

G. E. Galvez reported that US race 6, which corresponded to ID-13, was the most prevalent one in Colombia, which was followed by the races II-1, IB-7, IA-1 and IB-38 in the proposed international race number by IRRI. Of these, the race II-1 showed pathogenic reactions to Aichiasahi and Bluebonnet 50, which were used as supplemental varieties in contrast with the isolates quoted in previous

papers which were suspected as cultural variants or other species of Pyricularia. On the other hand, the races with a distinct pathogenicity to Zenith were collected from most of this area, namely U.S.A., Mexico, El Salvador, Nicaragua, Costa Rica, Colombia, Venezuela and Brazil, and the wider distribution of these kinds of races could be shown to be characteristic of this area. Zenith and Gulfrose are cultivated in this area.

Among areas not included in the above, isolates from Hong Kong did not show different patterns to Philippine isolates but did show distinct pathogenicity to Dular, which is nonpathogenic to almost all the Philippine races. It is suspected that the isolates from Hong Kong indicate characteristics of isolates of mainland China, especially southern China, because their pathogenicity differs from those of neighboring countries such as Taiwan, the Philippines and Vietnam.

Isolates from Guinea in West Africa were similar to Indian races and showed a wider spectrum of reaction on the differential varieties. However, it would be better to omit them from the grouping, because tested isolates were few, and no further information is available on the races of this area.

Isolates from Hungary, Egypt and Australia showed only narrower spectrums of reaction on the differential varieties, and tested isolates were too few to group them into areas.

II. Predominant races in several countries

Research on predominant or common races of the fungus suggests the global distribution of the races. Predominant races in a certain country can be presumed from the existing data, though the data do not always aim at systematic sampling to know the actual distribution of the races.

The frequency of races differentiated in Japan in 1961 (Table 2) did not result from systematic sampling during the year-long study throughout the country. Japanese race N-2 was the most prevalent race, followed by race N-1. Because the full-scale breakdown of resistance of so-called Chinese type varieties started two or three years later, C-1 and C-2 were collected in only limited areas in 1961.

Results of last year's isolates (Table 2) were insufficient because data of four prefectural experimental stations were simply accumulated without any statistical consideration. Considerable numbers of N-2 and N-1 were collected throughout other countries, though there was some increase or decrease in limited areas. C-8 increased strikingly in number and over a large area from one isolate in 1961 to 220 in 1970, while none of C-2 was collected; C-1 still had considerable numbers but did not show as wide distributions as C-8. The increase of C group races can be explained by the increase in cultivation of the varieties which were originated from so-called Chinese type varieties, though there is inadequate explanation for the striking increase of C-8, which overwhelms other races. It is interesting that predominant races in T, C, and N groups, which are T-2, C-8, and N-2, respectively, show the same reaction on the N-group differentials in Japanese differential varieties compared with those of other races.

M. Yamada, who studied systematic sampling of the pathogenic races of the fungus, pointed out the pathogenic strength of C-8 as the reason for dominating other races. Though inexact, "pathogenic strength" means aggressiveness or horizontal pathogenicity, which is quantitative as well as horizontal resistance.

As the composition of pathogenic races in Japan is simpler than those in other countries such as the Philippines, a relationship between the predominant races and the races which were derived from them appears clearer in Japan than the other countries. From the viewpoint of the pathogenic gene, serial changing of the major races in Japan might be explained as follows:

N-2, which corresponds to international race IH-1, turns into N-1 by obtaining pathogenic gene from Ishikarishiroke, which is named Av-i^+ by Kiyosawa, and those two races, N-2 and N-1, turn into C-8 and C-1 respectively by obtaining pathogenic gene, Av-k^+, from Kanto 51.

Table 3 shows the unpublished results of my work in Ceylon from 1967 to 1969. In addition to the set of international differential varieties, seven important varieties in Ceylon at the time were tested as supplemental varieties, which divided the international races into subraces.

From the result, the races corresponding to international races IE-1, ID-13

and IC-17, occupied major parts of Ceylonese races. Among them there seems to be serial passages of changing from one subrace to another by the addition of pathogenicity to a certain variety. For example, IE-1 group appears to be varied from subrace No. 26 to No. 22 one after another by the addition of pathogenicity to Aichiasahi, Podiwee a-8, H-4, and IR-8-68, successively. IE-1 and IC-17 seem predominant races because of their frequencies. However, ID-13 is still doubtful, because it did not include the subrace pathogenic to H-4, which at that time was the most widely distributed variety.

Table 4 shows the results of the race differentiation in India by S. Y. Padmanabhan et al. IC-17 was the most predominant race and IE-1 also showed a larger frequency in Ceylon. On the other hand, ID-1 and IA-number races which were infrequent in Ceylon were commonly distributed in India. Of these, most of the IA-number races from India showed pathogenicity to Dular and Kanto 51, while Philippine IA-number races are mostly nonpathogenic to both varieties. Generally speaking, India and Ceylon can be included in the same race composition group (pages 1-5), and the predominant races of both countries also can be the same or close to each other.

Table 5 was taken from the Annual Report of the International Rice Research Institute 1967, from which races possessing less than four isolates were omitted. IA-group races, especially IA-109, which adds the pathogenicity of Raminad Str. 3 to ID-13, occupied greater parts of the isolates tested. In the same report, the results of differentiation of Philippine races by Philippine differentials, together with the number of isolates, were also reported. It was impossible from the data of the Annual Report to directly relate international races to Philippine races, because corresponding tables were not available. Among the Philippine races, P 8, P 15, P 12, and P 30, respectively, showed larger frequencies and they were presumed to be derived from IA-109 or adjacent races from their reaction to Philippine differentials. From those results, IA-109 instead of ID-13 could be a predominant race. Unfortunately, the annual reports of following years at IRRI did not give cumulative numbers of isolates of reported races but only the number of isolates of new races in 1968 and the reaction patterns of newly discovered Philippine races. Therefore, the changing trend in frequencies of races could not be obtained although increases of races pathogenic to so-called Chinese varieties could be known.

Discussion

With the advance of the differentiation study of the races, many new races which were started with a limited numbers of specimens have been discovered in many countries. But in general view of geographical distribution of the races and their predominant race might not be influenced that much. If this is so, the following hypothesis arises: The actual process of obtaining pathogenicity from a new variety or a new resistant gene is still unknown. But it is presumed that a new pathogenic race to a new variety or a new resistant gene occurs from the predominant race at a certain place in that time. For instance, in Japan, the occurrences of C-3, C-8, T-2 and also races pathogenic to Fukunishiki, which has a resistant gene from Zenith, could be good examples of this case; and in Ceylon the races pathogenic to H-4 and IR-8-68 also could be presumed to be derived from predominant races at the time. Considering this hypothesis, it would be interesting to know the predominant races of a certain place by using differential varieties which include several indigenous varieties, and to check the race of the varieties on which it is desirable to have a special resistant gene grown in blast nurseries in all important rice-growing areas throughout the world.

The race of blast fungus corresponding to "race 0" of Phytophthora infestants has not yet been discovered, i.e., there may be no rice variety to be susceptible to all the isolates of Pyricularia oryzae in terms of vertical resistance. Caloro, Usen, P. Perumal and some other varieties showed susceptibility to the all isolates obtained from a certain country, but they still might not be susceptible to the all of those from other countries. In this regard, a special affinity of the predominant or common races in a certain place to the variety which is indigenous or widely cultivated in the same place could be one of the reasons. In this, evaluation of the resistance of the variety to the blast fungus should be stressed, especially where the variety which aims to be widely applied at every place has the possibility of being cultivated.

Table 1. Geographic distribution of the representative races of rice blast fungus

Variety	Reaction of races																		
	a[2]	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
Zenith																		+	+	+
Rexoro	+	+				+	+	+				+	+	+	+	+	+	+	+	+
Lacrosse	+	+	+			+	+	+	+	+	+		+	+	+	+			+	+
Caloro	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+
Sha-tiao-tsao P	+	+		+		+	+	+	+	+			+	+	+	+	+		+	+
Sha-tiao-tsao S	+	+		+		+	+	+	+	+		+	+	+	+	+	+	+	+	+
C.I. 5309	+												+	+	+	+			+	+
Dular	+												+	+	+	+	+	+	+	+
NP-125																+	+		+	+
Raminad Str. 3							+			+										
Wag-wag							+			+							+			
Taichung 65	+		+	+	+	+	+							+	+	+	+		+	+
Tetep																			±	
Tadukan																			±	
Usen						+	+	+	+	+	+	+	+	+				+	+	+
Chokoto	+												+	+	+	+			+	
Yakeiko	+												+	+	+	+	+		+	?
Kanto 51	+												+	+	+	+	+		+	?
Ishikari-shiroke	+	+		+		+	+	+					+	+	+	+	+		+	+
Homare-nishiki	+	+	+															±	+	
Ginga	+	+	+	+	+													+	+	±
Norin 22	+	+	+	+	+													+	+	±
Aichi-asahi	+	+	+			+	+	+	+	+	+	+	+	+	+	+		+	+	+
Norin 20	+	+	+	+	+													+	+	+
Japan	x[1]	x	x	x	x															
Korea		x	x	x																
Taiwan		x		x	x	x	x													
Philippines						x	x					x								
Vietnam							x	x	x	x	x									
Thailand						x			x	x	x									
Cambodia						x								x						
Indonesia						x		x				x	x	x		x				
India													x		x	x	x			
West Pakistan																	x			
Ceylon							x								x		x			
U.S.A.																		x	x	
Brazil																				x

1) existence of race

2)

Reaction	Name of races	Reaction	Name of races	Reaction	Name of races
a	Japan C-1	h	ID- 13 (JU-2)	o	IE- 1 (JU-2)
b	Japan N-1	i	ID- 13 (JU-3)	p	IC- 17 (JU-2)
c	Japan N-2	j	IA-109 (JU-2)	q	IC- 17 (JU-1)
d	IG- 1 (JU-3)	k	ID- 15 (JU-1)	r	IB- 54
e	IH- 1 (JU-1)	l	ID- 14 (JU-1)	s	IB- 33
f	ID- 13 (JU-1)	m	ID- 1 (JU-1)	t	IB- ?
g	IA-109 (JU-1)	n	ID- 1 (JU-3)		

International race numbers are based on the proposed number by IRRI.

Table 2. Japanese races of rice blast fungus

	Group T			Group C									Group N					
	1	2	3	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6
Variety																		
Te-tep	±																	
Tadukan	±	±																
Usen	+	+	+															
Chokoto	+			+	±		+		+		+							
Yakeiko	+			+	±	+		+		+	+	+						
Kanto 51	+			+	+	+	+	+	+	+	+	+						
Ishikarishiroke	+		+	+	+		+	+	+			+	+				+	+
Homarenishiki	+	+	+	+	+		+	+		+	+		+	+				
Ginga	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+	
Norin 22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aichiasahi	+	+	+	+	+		+	+	+	+	+		+	+	+			+
Norin 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
No. of isolates* in 1961	1	1	3	52	30	0	1	1	9	10	1	2	51	113	6	14	3	6
No. of isolates** in 1970	0	33	0	129	0	17	0	0	0	0	220	0	137	220	20	2	3	0
International Race Group	IC 1	ID 15	ID 13	IE 1 or IF 1	IE 1	IF 3	IE 1	IE 1	IE 1	IF 3	IF 3	IF 1 ?	IG 1	IH 1	IH 1	IH 1	IG 1	?

International race numbers are based on the proposed number by IRRI.
(* Goto, K. et al. 1964, ** Aichi, Hokkaido, Nagano, and Ooita Agr. Exp. Sta. 1971)

Table 3. Pathogenic races of rice blast fungus in Ceylon (1967-1969)

ubrace	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	0
ariety																												
1. Raminad str. 3	+	+																										
2. Zenith			±													±												
3. NP-125	+	+	+	+	+	+	+	+	+	+	+	+	+															
4. Usen			+	+	+									+	+	+	+	+	+	+	+							
5. Dular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	+	+	+		
6. Kanto 51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	+	+	+		
7. Sha-tiao-tsao S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	
8. Caloro	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+		+	+	+	+	+	+	
9. M-302			+	+		+																						
10. IR-8-68			±				+	+						+								+						
11. H-4	+		+	+		+	+		+					+								+	+					
12. Ptb 16			+	+	+											+	+		+									
13. Podiwee a-8	+		+	+	+	+	+	+	+	+				+	+	+	+		+			+	+	+				
14. Aichiasahi	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+		+	
15. P.Perumal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
International race:	A 81	A 81	C 1	C 1	C 1	C 17	C 17	C 17	C 17	C 17	C 17	C 17	C 18	D 1	D 1	D 13?	D 13	D 13	D 14	D 15	D 16	E 1	E 1	E 1	E 1	E 1	G 1	
Number of isolates:	3	1	1	3	2	2	10	2	26	14	11	35	7	1	5	2	23	6	7	2	2	10	16	18	4	13	4	21

International race numbers are based on the proposed number by IRRI.

Table 4. Reaction of international rice differentials to pathogenic races of *Pyricularia oryzae* in India

International race group	International race	Reaction per differential variety: Raminad Str. 3	Zenith	NP 125	Usen	Dular	Kanto 51	C.I. 8970 (S)	Caloro	No. isolate
IA	1* (97)**	+			+	+	+	+	+	3
	4 (113)	+				+	+	+	+	5
	5 (101)	+			+	+		+	+	1
	6 (65)	+		+	+	+	+	+	+	3
	7 (114)	+				+	+	+		2
	8 (116)	+				+	+			1
	9 (122)	+					+	+		1
	10 (121)	+					+	+	+	1
	11 (81)	+		+		+	+	+	+	13
IC	1 (1)			+	+	+	+	+	+	12
	3 (17)			+		+	+	+	+	31
	4 (19)			+		+	+		+	1
	6 (18)			+		+	+	+		3
	7 (20)			+		+	+			1
	8 (24)			+		+				1
ID	1 (1)				+	+	+	+	+	19
	3 (5)				+	+		+	+	1
	10 (15)				+				+	1
	12 (2)				+	+	+	+		1
IE	1 (1)					+	+	+	+	13
	2 (3)					+	+		+	1
	3 (6)					+		+		1
	4 (4)					+	+			3
	5 (2)					+	+	+		1
	6 (5)					+		+	+	1
	7 (2)					+	+	+		1
	8 (8)					+				1
IF	1 (1)						+	+	+	5
	3 (2)						+	+		2
	4 (4)						+			1
IJ(II)**	1 (1)									1

blank: resistant
\+ : susceptible
* : International race number in original paper
** : International race number proposed by IRRI.
(S.Y. Padmanabhan *et al.* 1970)

Table 5. Races of Pyricularia oryzae in the Philippines in international numbers (1967)

	International race No.												
	IA-45	IA-46	IA-65	IA-109	IA-110	IA-111	IA-112	IA-126	IB-45	IC-1a	ID-13	ID-14	ID-16
Variety													
Raminad str. 3	+	+	+	+	+	+	+	+					
Zenith	+	+							+				
NP 125			+							+			
Usen	+	+	+	+	+	+	+		+	+	+	+	+
Dular			+							+			
Kanto 51			+							+			
Sha-tiao-tsao S	+	+	+	+	+			+	+	+	+	+	
Caloro	+		+	+		+			+	+	+		
No. of isolates	28	28	25	230	81	8	29	5	6	14	21	7	6

(from IRRI Annual Report 1967)

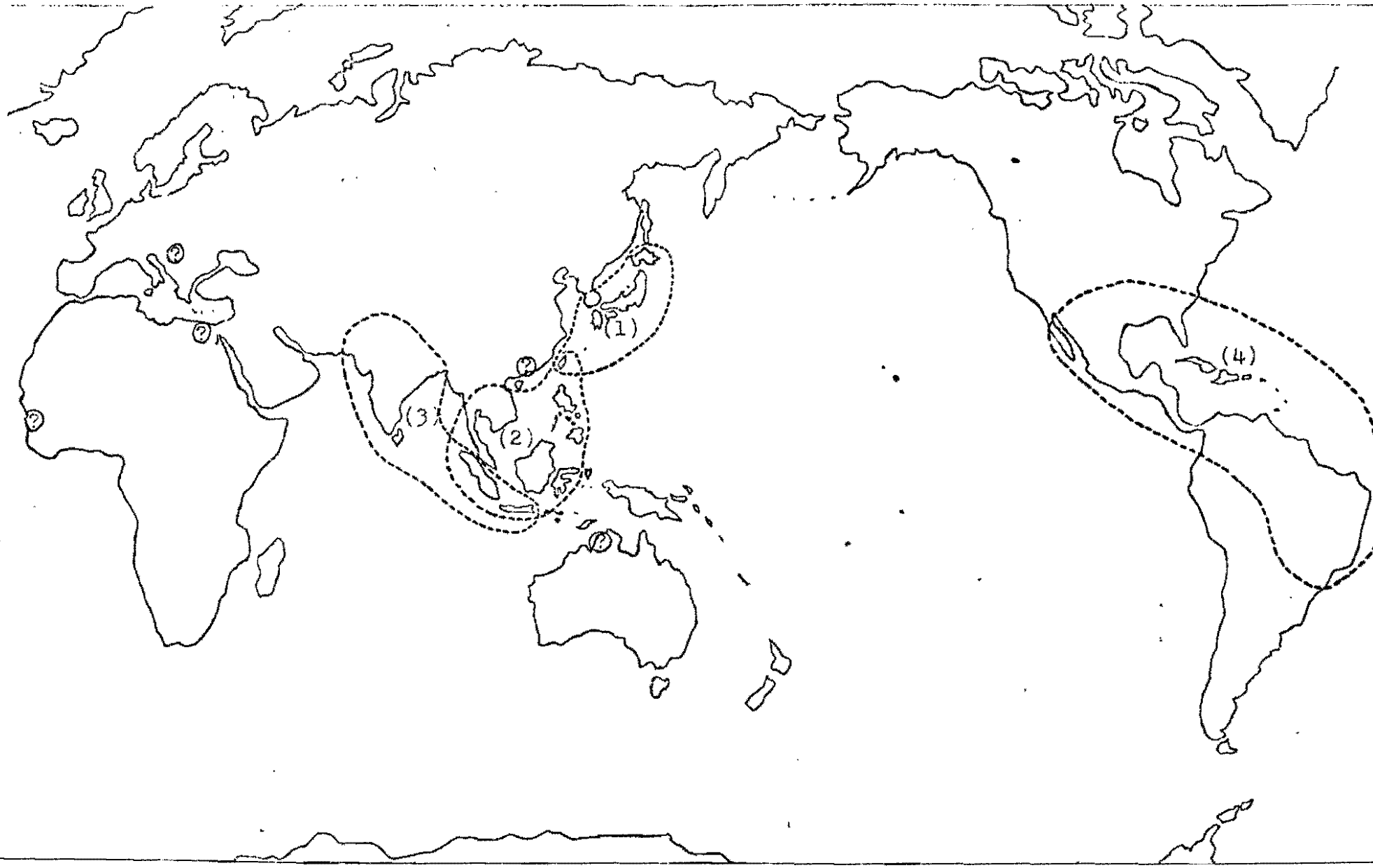

Fig. 1. Geographical grouping of pathogenic races of Pyricularia oryzae

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