IV. GENETICS: ANALYSIS OF GENES FOR BLAST DISEASE RESISTANCE AND OTHER CHARACTERS

a. ANALYSIS OF GENES FOR BLAST DISEASE RESISTANCE CAUSED *BY PIRICURARIA ORYZAE*

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The blast disease caused by *Piricularia Oryzae* is the most important in most rice growing countries. It seriously damages rice when nitrogen fertilizer is heavily applied. Therefore, breeding for blast disease resistance is urgently needed and a number of promising resistant varieties have been selected in various countries. However, knowledges on the genetics of disease resistance are still limited.

In order to get more knowledges on the inheritance of blast disease resistance in rice, the writer has conducted genetic experiments on resistances to leaf as well as neck blast disease. Fourty-six rice varieties from Japan, U. S. A. and Taiwan were used as the cross parents.

1. Inheritance of resistance to blast disease in seedlings

Studies on seedling reaction to blast disease was conducted during the period March to July in 1958. Five resistant, four intermediate and seven susceptible varieties were used as cross parents. 13 F_2 crosses together with their parental strains were planted in a specially designed greenhouse, in where artificial inoculation of blast fungus onto the seedlings was made. The inoculum was composed of a number of isolates taken from infected leaves collected from various parts of Taiwan. Single lesion was cultured in artificial media and the inoculum was applied in the form of a conidial suspension. The rice seedlings were grown in fungus free soil in iron flats (30 cm×30 cm in size), placed in the green house. About 40–50 seedlings are planted in separated blocks in the same flat. Prior to the inoculation, 2% ammonium sulfate solution was topdressed to the flats twice, at the rate of 25 cc per flat for each application. When the seedlings reach at the height of 15 cm, the conidial suspension was sprayed onto the leaves of seedlings. The inoculation was repeated 3–4 times so as to minimize the chance of escaping from infection. The flats were then placed in an inoculation chamber where the moisture (80-90%) and temperature (25–28°C) were controlled. A week after inoculation, the flats were moved out of the inoculation chamber into either a wire cage or in a greenhouse, depending on the air tem-

perature outside. Three to four days later, the inoculated seedlings were examined and records with regard to the types of lesions were taken according to the following six grades. O, A and B types were considered as resistant, while, C, D and E type were considered as susceptible.

Grade	Degree of resistance	Lesion type	Description
O	HR	Immune	No visible sign of infection.
A	R	Brown spot	Small brownish-dark brown lesions or spots.
B	MR	Brown margin	Brownish lesions with a distinct border which would not increase in size.
C	MS	Grayish white center	Slowly expanding, lesions restricted in size.
D	S	Large lesions with dark green center	Large lesions surrounded by brownish bands, expanding.
E	HS	Large lesions with grayish center	Water soacked appearance, often without well defined border, variable in size, lesions are expanded drastically.

Note: HR: Highly resistant
R: Resistant
MR: Moderately resistant
MS: Moderately susceptible
S: Susceptible
HS: Highly susceptible

Variation in seedling reaction to blast disease of the parental strains and F_2s are shown in Table 1.

As the table shows, crosses between resistant and susceptible strains, the F_2 segregated into 3 resistant and 1 susceptible plants in the majority of the crosses, showing the resistance being controlled by a single dominant gene. The result agrees with what Sasaki (1922) as well as Oka (1957) reported.

In the cross between a susceptible variety J-302 (Nakamura, Japonica type) and a resistant variety T-234 (Wukao, Indica type), the susceptible plant being smaller in number than expected and the chi-square value did not show a good fit to the 3:1 ratio. Since seed fertility in the F_1 plant of this cross was 20%, gametic selection due to hybrid sterility may be one of the disturbing factors for such a deviation. It is also possible that excessive plants may also be due to "disease escaping."

The F_2s of the crosses, P-123×M-503, P-123×P-130, P-123×7114 and P-124×T-215 segregated into 9 resistant and 7 susceptible plants. The chi-square value showed good fits to the expected ratio. When we classify the degree of resistance into three classes, resistant (O+A), intermediate (B+C) and susceptible (D+E), a 9:3:4 ratio was observed in P-123×P-130 and P-123×7114. These facts indicate that at least two genes are concerned with regard to blast resistance. This assumption was confirmed by the second series of experiment conducted in the field condition.

Table 1. Variations in seedling resistance to leaf blast disease in parental lines and F_2s in green house experiment

	Parents and crosses	Type of lesion						Resistant	Susceptible	Total	Segregation ratio	X^2	P
		O	A	B	C	D	E	(O+A+B)	(C+D+E)				
1960	P-123					115				115			
	P-123×M-503	15	8	12	18	10		35	28	62	9 : 7	0.013	0.8-0.9
	M-503	1	3	8						12			
	P-123×T-208	59	9	43	20	11		111	31	142	3 : 1	0.760	0.3-0.5
	T-208	54	46							100			
	P-123×P-167	72	28	19	14	13		119	27	146	3 : 1	3.297	0.05-0.1
	P-167	64	17	10						91			
	P-138					12	10			22			
	P-138×7101	18	12	26	9	12		56	21	77	3 : 1	0.212	0.5-0.7
	P-163	116	39	5	1	15	1			177			
	P-163×7245	23	86	23	13	28		132	41	173	3 : 1	0.156	0.5-0.7
	7245	99	16	1	1					117			
	P-163×9781	30	61	15	7	19		106	26	132	3 : 1	0.980	0.1-0.2
	9781	15	21	13	1					22			
	P-163×9783	42	67	7	6	39		116	45	161	3 : 1	0.748	0.3-0.5
	P-163×A-73	31	50	8	1	32		89	33	122	3 : 1	0.273	0.5-0.7
	J-302×T-234	102	32	23	16	6		157	22	179	3 : 1	15.421	0.01
	P-141×P-167	67	23	23	17	11		113	28	141	3 : 1	1.988	0.1-0.2
	P-141					8				8			
1961	P-123×P-130	39	59	12	12	49	1	110	62	172	9 : 7	4.148	0.02-0.05
		98		24		50					9 : 3 : 4	3.266	0.1-0.2
	P-130	22	37	5	4	30	2			100			
	P-123×7114	34	77	10	16	61	2	121	79	200	9 : 7	1.468	0.2-0.3
		111		26		63					9 : 3 : 4	6.927	0.02-0.05
	7114	55	39	1	4	1		95	5	100			
	P-124	45	20	3	8	24				100			
	P-124×T-215	34	22		5	31	2	56	38	94	9 : 7	0.421	0.5-0.7
	T-215	14	35	15	2					66			
	P-138×7247 (I)	11	17	7	12	3		35	14	49	3 : 1	0.333	0.5-0.7
	(II)	56	14	28	13	17	4	98	34	132	3 : 1	0.040	0.9-0.95
	7247 (I)	11	5	2	4					22			
	(II)	69	5	6	4	2				86			
	P-123 (II)			1	3	64	9			77			
	P-123×T-215 (I)	11	4	10	7	54	1	25	62	87	1 : 3	0.648	0.3-0.5
	(II)	20	3		3	58	6	23	67	90	1 : 3	0.015	0.8-0.9
	T-215 (I)	14	35	15	2					66			
	(II)	70	26	2	2								

(I) Data of 1960. (II) Data of 1961.

2. Inheritance of resistance to leaf blast disease in field experiment

During the second crop season of 1958, 14 F_2 crosses together with their parents were planted in an experimental field located at Tunshu, Taichung Prefecture, where the blast disease prevails in both the first and second crop seasons.

The leaf infection in adult plants was recorded by an index number indicating the percentage of lesion area, as shown in Table 2.

Table 2. Index-numbers showing the percentage of diseased leaf area

% of diseased leaf area	0	0.2	0.5	1	3	5	11	25	55	80	100	120
Index number	0	1	2	3	4	5	6	7	8	8.5	8.8	9

Since this classification was too complicated, the writer tried to group the index numbers into five classes by a statistical method so as to maximize the difference between line means relative to that within lines. However, the result showed that the grouping differed according to the season. Therefore, the resistant and susceptible classes in F_2 were determined by comparing with the variation range with those of parental lines. The frequency distributions as shown by the index-numbers given in Table 2 are shown in Table 3.

As Table 3 shows, the F_2 plants of most crosses between resistant strains were all resistant, but a cross, J-320×T-248, both parents being highly resistant, gave 36 highly resistant and 21 intermediate plants. This ratio is in a good fittness with a 9:7 ratio. This suggests that there may be resistant genes with complmentary effects. In crosses T-248×M-511 and T-220×T-248, most F_2 plants were resistant but a few were moderatly resistant. If this is due environmental modification or not is being tested.

As shown in the same table, the F_1 and F_2 plants of crosses between susceptible strains, such as P-123×D-1, P-123×C-1, P-123×7156 and P-321×P-123, were all susceptible. However, a few resistant plants were found in the cross P-123×H-25. In the F_2 of P-123×7156, both parents were susceptible, 57 highly resistant and 38 susceptible plants were found. They fitted quite well to a 9:7 ratio. It then seems that at least two dominant genes with complementary effect are responsible for the resistance to leaf blast disease.

In crosses between susceptible and resistant strains such as P-123×T248, P-123×7237 and P-123×U-401, the F_1s were resistant, and the F_2s showed a 3 resistant to 1 susceptible ratio. The F_2 of P-123 and 7126 gave a countinous range of variation in resistances. These differences in segregation ratio indicate that different genes are responsible for the resistance of parental strains. We may assume that a resistant gene R_1 (Okada's symbol 1956) gives higher resistance than R_2 and the presence of both of them brings about a high resistance to leaf blsease. Based on this assumption, the genotypes of the parental strains used may be putatively assumed.

During the first crop season of 1959, F_2 crosses wear again planted at the Tunshu Experimental field together with their parental lines in order to get more

Table 3. Variations of F_2 and parental plants in leaf blast resistance in field experiment

Parents and crosses	Disease index number												Total	Segr. ratio	X^2
	0	1	2	3	4	5	6	7	8	8.5	8.8	9			
P-169		13	3	1									17		
P-169×T-220	77	26		1	1								105	1 : 0	
T-220	67	95	14	13	6								195		
J-320		20											20		
J-320×T-248	4	32	9	5	4	2	1						57		
	(36)				(21)									9 : 7	1.067
T-248	34	4											38		
T-248×M-511	370	90	42	11	2	10	2						527	1 : 0	
M-511	142	48	2	1									193		
T-220×T-248	71	24	10	3		3	2	1					114	1 : 0	
P-123						5	3	15	19				42		
P-123×H-25		2	1	1	2	3	20	108	154	16	6	5	318		
H-25								11	21	11	10	19	72		
P-123×D-1							3	103	204	58	2	6	376	0 : 1	
D-1								6	16	14	2		38		
P-123×C-1							1	36	188	67	23	32	347	0 : 1	
C-1						19	20	25	30	21	5		120		
P-123×7164						1		19	37	45	55	95	252	0 : 1	
7164									7	19	17	3	46		
J-321									4	10	17	79	110		
J-321×P-123							6	10	17	52	25		110	0 : 1	
P-123×7165	1	13	19	24	17	8	5	7	1				95		
		(57)				(38)								9 : 7	0.542
7165					15	22	14	22	25	3			101		
P-123×T-248		25	12	12	4	9	9	4	2	1			78		
			(53)				(25)							3 : 1	2.068
T-248	116	67	20	3									206		
P-123×7126	26	80	33	19	18	5	5	5	4				195	(?)	
7126	8												8		
P-123×7237	6	50	24	23	16	19	9	4	6				157		
			(119)				(38)							3 : 1	0.411
7237	21	54	5	3	1								94		
J-321									4	10	17	79	110		
J-321×U-401		57	42	67	43	35	34	24	15	6	4	3	330		
			(244)				(89)							3 : 1	0.198
U-401	1	89	32	18	9	2							151		

Table 4. Segregation for neck blast resistance

Crosses	% of diseased ear										Total	Segregation ratio	X^2
	10	20	30	40	50	60	70	80	90	100			
P-140	6	3	3	7	4						23		
P-140×T-233	35	12	10	9	8	4	4	4	2	6	94		
	(74)					(20)						3 : 1	0.695
T-233	5	1	8	6	3						26		
P-167						8	18	15	21	18	80		
P-167×La-8-4	14	11	14	15	9	4	5	7	8	9	96		
	(67)					(29)						3 : 1	1.389
La-8-4	12	7	2	3	3						27		
P-167×P-168	35	10	4	7	2	2	2	5		8	75		
	(58)					(17)						3 : 1	0.218
J-302						1	3		3	15	22		
J-302×T-234	18	4	10	13	20	4	9	7	2	3	90		
	(55)					(25)						3 : 1	0.370
P-123							4	3	3	3	13		
P-123×J-303	10	11	21	14	9	9	13	3	2	3	95		
	(56)					(39)						9 : 7	0.280
J-303					2	2	2	2	2	4	14		
P-124					7	2	0	7	7	8	40		
P-124×T-208	62	27	18	18	21	7	7	10	5	4	179		
	(107)					(72)						9 : 7	0.904
T-208	36	37									73		
P-123×P-130	4	11	14	15	10	7	2	11	2	27	102		
	(61)						(13)		(28)			9 : 3 : 4	2.432
P-130		1	6	4	6	4	7	2			30		
P-124×T-208	62	27	13	18	21	7	7	10	50	4	179		
	(89)		(36)		(35)			(19)				9 : 3 : 3 : 1	7.047
T-208	36	2									38		
P-124×T-215	12	2	2	4	7	8	11	7	8	15	69		
	(27)					(42)						27 : 37	0.265
T-215	7	6	7	4	6						30		
P-138							1		5	24	30		
P-138×T-215	18	2	4	3	7	8	4	6	2	38	92		
	(42)						(50)					27 : 37	0.454
T-215	7	6	7	4	6						30		

results on the inheritance of leaf blast resistance. However, the prevalence of the disease was not serious due to unfavorable environmental conditions in that year. Only the neck blast was quite serious at maturity and the inheritance of neck blast resistance could be observed.

The degree of damage by neck blast disease was expressed by the percentage

of diseased panicles. The range of variation in parental lines was compared with those in F_2 to determine the border of resistant and susceptible classes. The data thus obtained are given in Table 4. As the table shows, 3:1, 9:7, 3:4 and 27:37 ratios were assumed. There might be at least three genes controlling the resistance to neck blast disease.

As will be shown later the resistance to neck blast is in some crosses correlated with the resistance to leaf blast but in other crosses not correlated siginficantly. This suggests that genes which modify neck resistance are present. The writer has already assumed two pairs of genes R_1 and R_2 for leaf resistance. They may be effective also for neck resistance. Assuming that three genes are involved in neck resistance, we may assume that the third gene is responsible for neck resistance. Then the observed segregation ratios of neck resistance can be explained well in most crosses.

DISCUSSION

Hsu: How many genes are involve in the resistance to leaf blast in your study?

Hsieh: This experiment was conducted under a controlled condition in this Institute, and under natural conditions at Tunshn, Taichung Prefecture. It was found that the F_1 and most F_2 plants of crosses between resistant varieties were resistant. Those between two susceptible varieties were mostly susceptible. Crosses between resistant and susceptible varieties showed a 3:1 and 9:7 ratios, showing that at least 1–2 genes are responsible for resistance.

Oka: According to your data, some parental varieties show a wide range of variation in resistance. This seems to be due to the impurity of materials. Such materials are not suitable for genetic study.

Lin: I think the index number is too complicated and must be simplified.

Hsieh: I noticed this problem before, and I am going to group the 9 classes into 4–5 classes by a statistical method so as to maximize the difference between line means relative to that within lines.

Oka: Linkage relations between genes for resistance to blast disease and other characters have not been studied so intensively by previous workers, you had better put more emphasis on this problem.

Hsieh: Yes, one of the difficulties in this experiment is that we can't record various characters at the same time in the same field due to the death of infected plants. In order to overcome this difficulty, 2–3 tillers of plant grown in Toughu were taken back to Taipei, and were planted with completly the same arrangement as that in the original field, and kept them in a good growing condition; then various characters could be recorded and related to the degree of blast resistance. From the preliminary study it seems that one of the resistant genes is linked with liguleless gene and phenol reaction genes. More will be done in order to confirm this assumption.

Oka: In your coming experiment it is desired to increase crosses between resistant

×resistant and susceptible×susceptible to see whether or not there is complementary action of genes.

Hsieh: Yes, we will do this in the next year.

Ku: In your experiment under the field condition, you can't control the purity of pathogenes. Therefore, the segregation ratio may be disturbed by different races of the pathegene. Have you ever considered this problem?

Hsieh: This is a quite important problem. As Mr. Hung's report shows we have different races in Taiwan, but we don't know yet whether they appear at the same time at the same locality or not. We can only carry out experiments on the assumption that the different races will not occur in the same locality at the same time. Hashioka (1950) formerly assumed that we do not need to be afraid so much about the disturbing effect of races on segregation ratios. Stricty speaking we must use a single race in genetic studies but we can't do this in a large scale, because it is difficult to get a large amount of inoculum suply. Anyway, I think we must overcome this difficulty.