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Genetic Study on the Resistance of Blast Disease in the Rice Variety Tongil (Ⅱ)

Shin Han Kwon · Jeung Haing Oh

Genetics and Breeding Laboratory
Korea Atomic Energy Research Institute, Seoul, Korea

水稻 統一品種의 稻熱病抵抗性에 關한 遺傳學的 硏究(Ⅱ)

權臣漢 · 吳正行

韓國原子力硏究所 遺傳育種學硏究室

ABSTRACT

The present study was conducted to determine the number of genes controlling resistance of variety Tongil to three prevalent blast strains and any relationships among the genes in the inheritance of resistance of the variety to blast. Tongil was crossed with five local varieties and the progenies were tested with blast isolates E-7 and 100-14 by injection inoculation method.

Results indicated that a single dominant gene conditioned resistance of variety Tongil and resistance to the isolate E-7 was controlled by a gene different from that controlling resistance to the isolate 100-14. In previous study it was identified that the gene controlling resistance to the isolate E-7 was different from the gene for resistance to isolate T-1. Therefore based on the foregoing results it may be inferred that the variety Tongil has at least three or more resistant genes to blast.

Introduction

It is evident that rice blast disease has remained as limiting factor of rice production. Many investigators ave developed disease resistant varieties to control ɔis disease in order to attain self-sufficiency in rice roduction. Fortunately the rice variety Tongil, which ʼas recently developed by introduction of blast ɘsistant genes from indica type variety, has been ɘsistant to prevalent blast races in this country. Inasmuch as Tongil is a blast resistant variety, it is significant to determine its gene constitution and inheritance of the blast resistance for further systematic breeding of other varieties.

Since it was reported by Sasaki[14] that resistance or susceptibility to blast behaved as a Mendelian character controlled by one pair of gene, the gene for resistance being dominant over its allelomorph, the researches on this aspect have made a repid progress. Sometimes it was reported [13] that susceptibility was controlled by a major gene, dominant apparently while most investigations[3-6,10-12,15,16] have shcwed resistance to be dominant over susceptibility. Recently author and his colleagues[9] also reported that resistance of the variety Tongil to common blast isolates T-1 and E-7 was controlled by two different dominant genes.

The present study, a continuation of the previous work, was conducted to determine any genetic relationship between the former isolates and a mutant induced by X-ray irradiation in the inheritance of resistance of the variety Tongil.

Materials and Methods

Five susceptible varieties, Milsung, Palgueng, Shin-2, Jungkuk-41 and Kwandong-89, were crossed with variery Tongil to determine the number of genes controlling its resistance. These materials were grown in plastic boxes in the growth chamber and tested with two blast isolates, E-7 and 100-14 with different pathogenicity. The first inoculation was done at about 3.5-4 leaf stage of growth by injection method[7], in which spore suspension was injected into vacant tubular space of emerging leaf sheath with a hypodermic syringe. A second inoculation one week later using the above method was also conducted on the same seedlings. Blast fungus for the tests was cultured on PDA at 28°C for 10-14 days and the mycelial mats were transferred to OMA media for sporulation. Four days after sporulation under fluorescent light, spore suspension was prepared for inoculation at a concentration of 20,000 spores per ml. A week following the injection, the number and type of lesion on the leaves were measured. The lesions were grouped into four types: brown spotes(b-type), small lesion less than 2 mm long in diameter with brown margin(bg-type), large lesion more than 2 mm long in diameter with brown margin(bG-type) and large lesion with purple or uncolored margin(pG-type). The plants were classified into three groups, plants similar in resistance to the resistant parent(r), plants resistant to a less extent than the resistant parent(m), and plants similar to the susceptible parent(s). In a F_2 analysis, the results of this scoring were directly used for examination of segregation ratio by Chi-square test.

Results and Discussion

In this study blast isolates T-1, E-7 were isolated from leaves obtained from farmers field and a mutant isolate 100-14 was originated from a prevalent blast race N-1 by X-ray irradiation[8]. These isolates were identified with Japanese differential variety set as T-1, C-3 and C-5, respectively. The five local varieties were susceptible to all the isolates except for the variety Milsung which was moderately resistant against mutant isolate 100-14(Table 1).

Table 1. Blast reactions of parent varieties inoculated by injection method, with different isolate of *Pyricularia oryzae* Cav.

Variety	Isolate	T-1	E-7	100-14
	Race identified	T-1	C-3	C-5
Tongil		R*	R	R
Milsung		S	S	MR
Palgueng		S	S	S
Shin-2		S	S	S
Chungkuk-41		S	S	S
Kwandong-89		S	S	S

*R : resistant reaction
MR : mederately resistant
S : susceptible reaction

In order to determine the number of genes controlling the resistance of the variety Tongil, the parents, F_1 and F_2 populations of each crosses were inoculated with the isolate E-7 of blast fungus(Table 2). All of the F_1 plants from crosses between variety Tongil and susceptible varieties had a high level of resistance almost identical to that of the resistant variety Tongil. No susceptible plant was detected suggesting that resistant gene was completely dominant over its allele susceptibility. The F_2 populations of the cross between Shin-2 and Tongil showed a segregation ratio of 3 : 1 when inoculated with E-7 isolate, indicating a participation of one dominant gene for resistance in the variety Tongil. The F_2 population of Kwandong-89 x Tongil also gave a good fit of 3 : 1 ratio of resistant and susceptible seedlings

This indicated that resistance of the variety Tongil to the isolate was conditioned by a single dominant gene. Monogenic segregation in both F_2 populations of Palgueng x Tongil and Jungkuk-41 x Tongil also showed that a single dominant gene conditioned resistance to the isolate. Even in the cross of Milsung x Tongil, an almost perfect 3 : 1 ratio of resistant and susceptible seedlings occured in the F_2 population when intermediate types were included in resistant types for Chi-square test due to frequency distribution of infection type. Actually one of the most difficult problems encountered in this study was classification of the resistant and susceptible grades from the variable reactions found in hybrid populations, there

Table 2. Distribution of infection types on parents, F_1 and F_2 populations from Tong-il x local varieties inoculated with isolate E-7 of *Pyricularia oryzae* Cav.

Exp. no	Cross	Population	Number of plant				Assumed ratio	P for goodness of fit
			r	m	S	total		
I	Tongil x Shin-2	Tongil	22	0	0	22		
		Shin-2	0	1	23	24		
		F_1	9	0	0	9		
		F_2 Observed	30	2*	11	43	3 : I	0.9<
		Expected	32.25		10.75			
II	Tongil x Kwandong-89	Tongil	22	0	0	22		
		Kwandong-89	0	0	22	22		
		F_1	10	0	0	10		
		F_2 Observed	49	3	19	71	3 : 1	0.5~0.75
		Expected	53.25		17.75			
III	Tongil x Palgueng	Tongil	22	0	0	22		
		Palgueng	0	1	24	25		
		F_1	10	0	0	10		
		F_2 Observed	49	1	18	68	3 : 1	0.75~0.9
		Expected	51		17			
IV	Tongil x Chungkuk-41	Tongil	22	0	0	22		
		Chungkuk-41	0	2	27	27		
		F_1	9	0	0	9		
		F_2 Observed	50	8	19	77	3 : 1	0.9<
		Expected	57.75		19.25			
V	Tongil x Milsung	Tongil	22	0	0	22		
		Milsung	0	1	29	30		
		F_1	7	0	0	7		
		F_2 Observed	59	5	21	85	3 : 1	0.9<
		Expected	63.75		21.25			

*"m" was included in "r" for Chi-square test due to distribution frequency of infection type.

often being a full range of reactions from resistance to susceptibility. The growth of the seedlings was synchronized to minimize this problem encountered in the study. In another test, the parents and the progenies of each crosses were inoculated with a mutant isolate 100-14 of the blast fungus (Table 3). All progenies (F_2) gave a good fit of 3 : 1 segregation ratio of resistant and susceptible seedlings with the exception of the F_2 populations of the cross between varieties Milsung and Tongil. This may be explained by the moderately resistant reaction of variety Milsung to the isolate used, even though the degree of resistance was less than that of the variety Tongil. The foregoing results indicated that the resistance of the variety Tongil to isolate 100-14 was conditioned by a single dominant gene.

In the experiment to determine the relationships between the resistant genes concerned, F_2 seedlings were first inoculated with isolate E-7, followed one week later by inoculation with another isolate 100-14. Only the plants showing resistant reaction to isolate E-7 were inoculated, because the degree of infection by the second inoculation could not be accurately determined on the plants infected heavily by first

Table 3. Distribution of infection types on parents and F_2 population from Tongil x local varieties inoculated with isolate 100—14 of *Pyricularia oryzae* Cav,

Exp. no.	Cross	Population	Number of plant			Assumed ratio	P for goodness of fit
			R	S	total		
I	Tongil x Palgueng	Tongil	29	0	29		
		Palgueng	1	33	34		
		F_2 Observed	36	10	46	3 : 1	0.5—0.75
		Expected	34.5	11.5			
II	Tongil x Chungkuk-41	Tongil	29	0	29		
		Chungkuk-41	0	32	32		
		F_2 Observed	50	13	63	3 : 1	0.25—0.5
		Expected	47.25	15.75			
III	Tongil x Kwandong-89	Tongil	29	0	29		
		Kwandong-89	2	32	34		
		F_2 Observed	64	12	76	3 : 1	0.05—0.1
		Expected	57	19			
IV	Tongil x Milsung	Tongil	29	0	29		
		Milsung	30	4	34		
		F_2 Observed	57	5	62	3 : 1	0.005>
		Expected	46.5	14.5			

inoculation(Table 4). The F_2 population of Shin-2 x Tongil inoculated with the two isolates segregated into 3 : 1 for isolate E-7. The plants resistant to isolate E-7 segregated into a good fit of 3 : 1 ratio when inoculated with isoalate 100—14. This was expected when resistance to the isolate E-7 was controlled by one gene different from the gene controlling resistance to isolate 100-14. The F_2 populations of the other four crosses was segregated also into 3 : 1 when inoculated with isolate 100-14 in the second inoculation. This indicated that resistance to the two isolates was controlled by one and a different gene respectively, consistant with the former result. However a segregation ratio of Milsung x Tongil in the second inoculation showed only a poor fit of 3 : 1 ratio due to the moderatly resistant reaction of the mother variety Milsung to isolate 100-14.

Recently several workers studied the inheritance of blast resistance with known races. Atkins and Johnston[1] found two resistant genes Pi-1 and Pi-6. Yamasaki and Kiyosawa[17] named at least 11 resistant genes identified among several varieties inculuding Indica type varieties, using seven isolates identified in Japan and concluded that a resistant variety might have one to three or more resistant genes. Hsieh et al.[2] also reported four resistant genes, calling genes, Pi-4, Pi-13, Pi-22 and Pi-25 corresponding to the respective blast races used for the test. According

Table 4. Segregation in F_2 populations of cross between Tongil and local varieties inoculated with two isolates of *Pyricularia oryzae* Cav.

Exp. no.	Cross	Fungus isolate	Number of plant r	m	s	total	Assumed ratio	P for goodness of fit
I	Tongil(R,R*) x	E-7	30	2	11	43	3 : 1	0.9<
	Shin-2(S,S)	100-14**	19	3	10	32	3 : 1	0.25—0.5
II	Tongil(R,R) x	E-7	49	3	19	71	3 : 1	0.5—0.75
	Kwandong-89(S,S)	100-14	28	4	20	52	3 : 1	0.01—0.025
III	Tongil(R,R) x	E-7	49	1	18	68	3 : 1	0.75—0.9
	Palgueng(S,S)	100-14	31	3	16	50	3 : 1	0.5—0.75
IV	Tongil(R,R) x	E-7	52	6	19	77	3 : 1	0.9<
	Chungkuk-41(S,S)	100-14	38	3	17	58	3 : 1	0.25—0.5
V	Tongil(R,R) x	E-7	59	5	21	85	3 : 1	0.9<
	Milsung(S,R)	100-14	59	3	2	64	3 : 1	0.005>

*Letters in parenthesis are reactions of the variety to isolates E-7 and 100-14, respectively.

**Second inoculation was made by isolate 100-14 on the plants which showed resistant reaction to the first inoculation with E-7 isolate.

to present study, the variety Tong-il was assumed to carry three different resistant genes and it was expected to reveal more resistant genes in relation to pathogenicity of used isolates by further systematic studies.

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摘　　要

統一品種의 稻熱病抵抗性 遺傳子構成과 抵抗性의 遺傳에 關聯하는 이들 遺傳子의 相互關係를 究明하기 위하여 統一品種과 5個의 罹病性 在來品種, 밀성, 팔굉, 新2號, 中國 41號 및 關東 89號 等을 交雜하여 얻어진 F_1 및 F_2 集團에 稻熱病菌株 E-7과 100-14를 각각 注射 接種하여 그들의 分離比를 Chi-square test하였다.

1. 遺傳子分析에 使用된 稻熱病菌株 E-7, T-1 및 100-14는 日本判別品種에 依하여 각각 C-3, T-1 및 C-5로 同定되었다.
2. F_1植物體는 接種菌株에 對하여 抵抗性 母品種과 동일한 水準의 抵抗性을 나타냈으며 이것은 稻熱病 抵抗性이 罹病性에 對하여 完全優性임을 示唆한 것으로 보였다.
3. F_2 集團에서는 接種菌株 E-7과 100-14에 對하여 抵抗性과 罹病性의 分離比가 각각 3 : 1로 나타나 이들 菌株에 對한 統一의 抵抗性은 單性遺傳을 하는 것으로 보였다.
4. 接種菌株 E-7에 對하여 抵抗性인 F_2 集團에 100-14 菌株를 2차로 接種한 結果 抵抗性과 罹病性이 3 : 1로 分離되어 이 두 菌株에 對한 統一의 抵抗性은 서로 다른 別個의 遺傳子에 依하여 發現되는 것으로 보였다.
5. 本 實驗의 結果를 綜合하면 接種菌株 T-1, E-7 및 100-14에 對한 抵抗性은 서로 다른 遺傳子에 依하여 支配되므로 統一品種은 적어도 3個 이상의 稻熱病 抵抗性 遺傳子를 갖는 것으로 추정되었다.