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Factors Affecting the Racial Distribution of *Pyricularia oryzae* II Comparison of Multiplication of Races C-1 and C-3 on Rice Varieties with Kanto 51-type Resistance

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八重樫博志* 小林尚志**：イネいもち病菌のレース分布支配要因
Ⅱ レースC-1, C-3の関東51号型品種における増殖比較

Abstract

Races C-1 and C-3 of *Pyricularia oryzae* were compared for their multiplying potential, *i.e.*, time needed for fungus infection, number of susceptible lesions formed, sporulation in lesions, lesion size, and racial competition on two Kanto 51-type rice varieties, Tatsumimochi and Fukei 69, which are compatible to both races. Race C-3 produced fewer spores and smaller lesions than race C-1 on Tatsumimochi, but on Fukei 69 there was no difference between the two races in lesion size or number of spores produced. Tatsumimochi appears to have a race-specific factor that inhibits lesion expansion of race C-3. When the two varieties were inoculated with a mixture of spores of the two races, race C-3 was reisolated less frequently than race C-1 on Tatsumimochi, but on Fukei 69 the two races were reisolated with about an equal frequency. Race C-3 requires slightly shorter time for infection than race C-1 on both varieties. Apparently even in varieties that have no major genes for resistance against the prevalent races, there may be some race-specific factors that influence the distribution of the races. Antagonism and competition among races may also play a role in racial distribution. (Received August 20, 1975)

Introduction

Information on factors affecting the racial distribution of the rice blast fungus is important in forecasting which races are likely to become prevalent in the future, in helping farmers decide which varieties to plant, and in helping breeders select sources of blast resistant genes. Many reports[6,7,8,11,15] indicate that racial distribution is determined mainly by the varieties grown in particular area or district. Most of these reports attribute the racial distribution to the selective multiplication of races that are able to attack the varieties. All races capable of attacking the varieties grown, however, are not always distributed uniformly there. Other reports [2,11,12,13,15] indicate that factors other than varieties may also affect the racial distribution. Yamanaka[13] postulated that the physiological and ecological characters of the pathogen and environmental factors may also affect racial distribution. There are few reports[3,4,9,14] that attemt to obtain experimental evidence on the effects of factors other than varieties on racial distribution. Preliminary experiments indicated that some of these factors may be affecting the distribution of races C-1 and C-3 on Kanto 51-type varieties. Five isolates of each of these two races were compared on two Kanto 51-type varieties for time required for infection, number of susceptible lesions, sporulation in lesions, lesion size, and racial competition to determine whether these factors may affect the distribution of these races.

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Materials and Methods

Five isolates each of races C-1 and C-3 and two Kanto 51-type rice varieties, Tatsumimochi and Fukei 69, were used as materials in the present experiments. These two varieties are susceptible to both races. Two methods of inoculation were used for most experiments. Plants in the 6-leaf stage were sprayed with a spore suspension, incubated in a moist chamber for 20 hours at 27-28 C, and then kept in a green house until lesions appeared. To measure sporulation and size of lesions, the punch method of inoculation was used on the 10th and 11th leaves of the main culm soon after expansion of the 11th leaf. The inoculated leaves were shaded to prevent rapid drying of the paste inoculum. Twelve days after inoculation the leaves were detached and incubated for 17-18 hours in the dark at 27-28 C to induce sporulation. Furuta's method[1)] was used to induce sporulation on oat meal agar. Conidia were counted with the aid of a hemocytometer using four replication per sample. Detached lesions or discs of oat meal agar 2 cm in diam were placed in small glass tubes with a measured amount of water containing a surfactant and subjected to supersonic vibrations[10)] to release conidia from the sporulating surface. To estimate lesion size, the image of the lesion was magnified by a profile projector, traced on paper, and measured by an automatic area meter.

Results

Sporulation in culture and number of susceptible lesions on the Chokai variety of rice

Since factors other than the specific relations between races C-1 and C-3 and the two Kanto 51-type varieties could influence racial distribution, the ability of the isolates to sporulate on oat meal agar and to produce lesions on a variety Chokai with no major genes for resistance to blast was determined. There was a wide variation among isolates (Table 1) in number of spores produced in culture.

Table 1. Sporulation on oat meal agar and number of susceptible lesions on variety Chokai by isolates of *Pyricularia oryzae*

Race	Isolate	Sporulation	No. of susceptible lesions
		(conidia/cm^2 $\times 10^4$)	(lesions/10cm leaf)[a)]
C-1	1 TH68-58	2[b)]	15[c)]
	2 TH68-128	18	13
	3 TH68-91	27	12
	4 TH68-178	53	15
	5 KEN60-19	74	12
C-3	11 TH66-100	30	23
	12 TH68-140	107	16
	13 TH67-31	118	15
	14 TH68-85	57	14
	15 TH67-22	238	15

a) Leaves were inoculated with conidia of the isolate by spray method.
b) Average number of three replicates.
c) Average number of lesions on two replicates of 10 plants.

Isolate 15, for example, produced more than 100 times as many conidia as isolate 1. In general, isolates of C-3 produced more spores than isolates of C-1. There was little difference in the number of susceptible lesions produced by the isolates on the Chokai variety (Table 1).

Time needed for fungus infection

Plants inoculated by spraying were inocubated in a moist chamber for 6,8,10, or 20 hours. Soon after incubation for the scheduled number of hours, the drops on the leaves were dried by an electric fan to stop further fungus penetration. The time required for infection was expressed as the percent infection (Table 2) obtained

Table 2. Percent infection of races C-1 and C-3 of *Pyricularia oryzae* to Kanto 51-type rice varieties after 6, 8, and 10 hr-incubation periods

Race	Isolate	Percent infection on two rice varieties[a]					
		Tatsumimochi			Fukei 69		
		6	8	10hr	6	8	10hr
C-1	1	0	6	23	0	9	40[b]
	2	0	14	51	0	20	63
	3	0	5	38	1	11	43
	4	0	9	33	0	20	70
	5	0	16	23	2	27	39
C-3	11	0	7	56	1	23	76
	12	8	30	39	19	39	67
	13	0	34	75	8	102	86
	14	2	23	81	19	44	55
	15	3	22	39	5	30	53

a) The percent infection for 6, 8, and 10hr-incubation periods were shown as the percentage of 20 hr-treatment in number of susceptible lesions.
b) Average number of two replicates. Ten plants were used for each treatment.

by dividing the number of susceptible lesions on plants incubated for 6,8, or 10 hours by the number of lesions on plants incubated for 20 hours for each isolate. The time needed for infection by race C-3 was slightly shorter than that by race C-1. The same tendency was observed on both rice varieties, Tatsumimochi and Fukei 69, indicating that there is no specific relations between race and variety in time needed for infection. Both races appeared to infect Fukei 69 more rapidly than Tatsumimochi. This is probably due to the high susceptibility of Fukei 69 to blast.

Number of susceptible lesions

The two Kanto 51-type varieties were inoculated by spraying under the same conditions. Both isolates produced more lesions on Fukei 69 than on Tatsumimochi with only small differences between races (Table 3).

Table 3. Number of susceptible lesions formed on Kanto 51-type rice varieties by spray inoculation with races C-1 and C-3 of *Pyricularia oryzae*

Race	Isolate	No. of susceptible lesion/10cm leaf Variety	
		Tatsumimochi	Fukei 69
C-1	1	8[a]	23
	2	8	14
	3	7	23
	4	9	27
	5	14	39
	(mean)	(9.2)	(25.2)
C-3	11	8	31
	12	4	19
	13	6	21
	14	10	36
	15	11	30
	(mean)	(7.8)	(27.4)

a) Average number of lesions on two replicates of 10 plants.

Sporulation and size of lesion

Sixteen pairs of isolates, one from each race, were separately inoculated on the same leaves of each of the two varieties to compare sporulation and lesion size. The results summarized in Fig.1 showed that isolates of races C-1 and C-3 produced about the same number of spores on Fukei 69. On Tatsumimochi, however, all the isolates of race C-3 produced fewer conidia than those of race C-1. This was

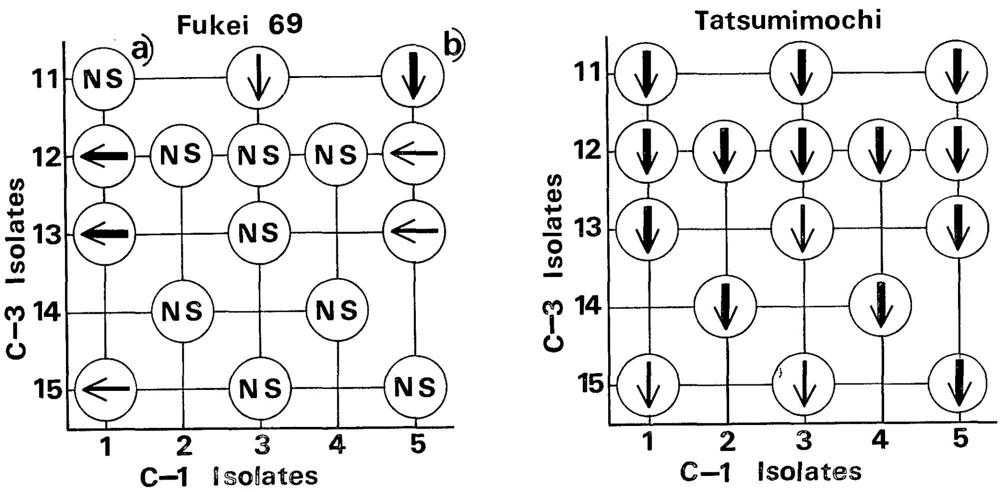

Fig. 1. Comarison of sporulation of races C-1 and C-3 of *Pyricularia oryzae* in lesions formed on Kanto 51-type rice varieties by punch inoculation

a) No significant difference in spore production between C-1 isolate and C-3 isolate.

b) Arrow pointing down indicates C-1 isolate ploduced more spores, arrow pointing left indicates C-3 isolate produced more spores; thin arrow indicates significance at 5% level and thick arrow indicates significanceat at 1% level.

true even in the pairing of isolate 15 of C-3, the most fertile on oat meal agar, with isolate 1 of C-1, the least fertile on oat meal agar. As shown in Fig. 2, the two races produced lesions of equal size on Fukei 69, but C-3 produced smaller lesions than C-1 on Tatsumimochi. The difference in sporulation between races C-1 and C-3 on this variety seems to be largely due to the difference in size of lesions. The variety Tatsumimochi appears to have a factor which specifically reduces the lesion size and sporulation of race C-3.

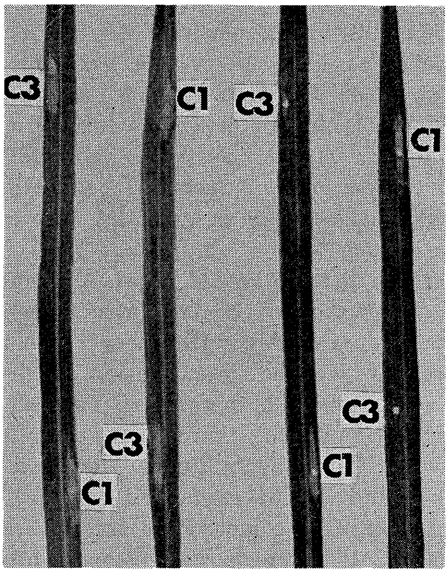

Fig. 2. Comparison of lesion size formed by races C-1 and C-3 of *Pyricularia oryzae* on Kanto 51-type rice varieties by punch inoculation

Isolation frequency of races C-1 and C-3

An equal amonunt of spore suspension of the two races was mixed evenly in each combination of isolates and sprayed on the varieties. About ten susceptible lesions were collected at random from each variety for race determination (Table 4). The frequency of reisolation of races C-1 and C-3 varied according to the host variety and the combination of isolates. The two races were reisolated at an about equal frequency from Fukei 69, whereas on Tatsumimochi the isolation frequency of race C-3 was fairy low compared with that of race C-1.

Table 4. Reisolation frequency of races C-1 and C-3 from Kanto 51-type rice varieties inoculated with a mixed suspension of the two races of *Pyricularia oryzae* by spraying

Variety	Race	Combination of isolates mixed										
		1+11	3+11	5+11	2+12	1+13	3+13	5+13	2+14	1+15	3+15	5+15
Tatsumimochi	C-3 / C-1	4 / ⑥ a)	3 / ⑤	1 / ⑧	2 / ⑧	3 / ⑤	1 / ⑨	2 / ⑧	1 / ⑨	5 / 5	⑥ / 4	2 / ⑧
Fukei 69	C-3 / C-1	5 / 5	⑦ / 3	2 / ⑧	4 / ⑥	⑧ / 2	⑧ / 2	4 / ⑥	4 / ⑥	⑩ / 0	2 / ⑧	⑧ / 2

a) The higher number is circled.

Since both Tatsumimochi and Fukei 69 were inoculated by the same mixture inocula, the difference in frequecy of reisolation of races C-1 and C-3 from these two varieties is most likely due to racial selection by the host variety or to racial competition at the infection site.

Discussion

It may be generally considered that the distribution of races of rice blast fungus is the result of occurrence of new races and differences in multiplication of these races. In this study differences in multiplication of races C-1 and C-3 were studied on the varieties Tatsumimochi and Fukei 69. Fewer conidia were produced by race C3- than by race C-1 on Tatsumimochi, but not on Fukei 69 or on oat meal agar. The smaller size of lesions of race C-3 on Tatsumimochi seems to be principally responsible for the lower number of spores produced. A lower incidence of neck-rot induced by C-3 on this variety has also been observed (K. Shindo, personal communication). Tatsumimochi appears to have a race-specific factor that limit expansion of lesions of race C-3. This variety is known to have a higher degree of field resistance than Fukei 69. The lower number of lesions formed on Tatsumimochi than on Fukei 69 by both races in this study may be an expression of this field resistance. However, the smaller lesion size of race C-3 cannot be accounted for by field resistance because the size of the lesions of race C-1 were as large on Tatsumimochi as on Fukei 69.

Another factor that would influence the racial distribution of races C-1 and C-3 is the low frequence of reisolation of C-3 compared to C-1 on Tatsumimochi when this variety was inoculated with a mixture of spores of the two races. When the variety is inoculated with a mixture of spores of two races, each lesion usually contains only one of the two races rather than a mixture of both races(H. Yaegashi, unpublished results). It seems likely that on Tatsumimochi there were actually few lesions containing race C-3 compared race C-1. This may be due to the phenomenon of racial competition as found for races C-1 and C-8 by Iwano and Yamada[3]. These authors[4] believe that the prevalence of race C-8 compared to race C-1 is due to suppression of spore germination of race C-1 by chemical substances produced when the mixture of races C-1 and C-8 is placed on rice leaves. Yamanaka[14] pointed out that the relative survival ability among isolates might be changed by antagonistic effects that are closely related to the rate of lesion enlargement. In the case of races C-1 and C-3 on Tatsumimochi, it is not clear whether there is a suppression of spore germination or an inhibition of lesion development of race C-3 by race C-1

The disadvantage of race C-3 in multiplication on Tatsumimochi seems to be due to a factor in this variety which specifically inhibits multiplication of race C-3. Race C-3 has fewer genes for pathogenicity than race C-1 and according to the stabilizing selection theory of Van der Plank[16] this race should become the prevalent race in the absence of race-specific factors in the varieties. Also race C-3 has an advantage over race C-1 in time required for infection. This may be important in the field when short dew periods or other environmental factors may limit the time available for the fungus to infect the plant. In mixed inoculation of these two races, however, the advantageous character of race C-3 seems to be masked. It is probably due to some effects caused by mixing the two races.

It appears that, even in varieties that have no major genes for resistance to the prevalent races of the rice blast fungus, there may be race-specific factors in these varieties, that influence the distribution of the races. Antagonism and competition among races may also play a role in racial distribution.

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和 文 摘 要

イネいもち病菌のレース分布支配要因

II　レースC-1，C-3の関東51号型品種における増殖比較

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関東51号型品種のタツミモチとふ系69号，イネいもち病菌のレースC-1とC-3を用い，親和性のある品種とレース間にレース分布を支配する要因があるかどうかを検討した。

レース増殖に関与すると考えられる要因，すなわち，感染に要する時間，形成病斑数，病斑の大きさおよび胞子形成量，レース間の競合のうち，特に病斑当たり胞子形成量および混合接種した場合のレース分離割合の点で，品種とレースとの間に特異的関係が認められた。レースC-1，C-3の病斑当たり胞子形成量は，ふ系69号ではほぼ同等であるのに対し，タツミモチではC-1に比較してC-3の胞子形成量がきわめて少なかった。この差は，おもにタツミモチでのC-3の病斑がC-1に比較して著しく小さい事による。これは菌株の組合せいかんにかかわらず認められる一定の傾向で，タツミモチにC-3の病斑拡大を抑える要因のある事が推察される。これは培地上における胞子形成量とは無関係なもので，品種とレースとの特異的関係においてのみ認められるものと考えられる。また，レースC-1，C-3の混合液を両品種に同時に噴霧接種すると，ふ系69号では両レースほぼ同じ割合で分離されたのに対し，タツミモチではC-3の分離割合がC-1に比較してかなり低かった。なお，両品種におけるレースC-3の感染に要する時間は，C-1に比較してやや短い傾向がみられた。

親和性のある品種においても，レースの増殖比率が特異的に変わるなど，レース分布に影響を及ぼす要因のある事が明らかにされた。また，レース間の競合現象も，レース分布支配要因のひとつと考えられる。