SENSITIVITY DISTRIBUTION OF PHYSIOLOGIC RACES OF *PYRICULARIA ORYZAE* CAVARA TO KASUGAMYCIN

HISASHI SAKURAI, MASATAKA IWANO*, NAOTOSHI GOH**, TSUNEO YAOITA**
KAZUO AOYAGI and HISASHI NAITO

Agricultural Chemicals Inspection Station, Ministry of Agriculture and Forestry, Kodaira-shi, Tokyo 187, Japan

(Received for publication March 28, 1977)

The shifts in population of physiologic races and drug-resistant strains of rice blast fungus, *Pyricularia oryzae* CAVARA, has been important for a long period of time since the fungus causes serious disease of rice in Japan. The isolates of pathogenic rice blast fungus in Japan were differentiated into T, C and N race groups[1,2]. Information on the distribution of physiologic races of rice blast fungus is important in forecasting which races might become prevalent in the future, thus helping farmers decide which varieties should be planted. It would also help farmers select sources of rice-blast resistant varieties in their breeding programs.

MIURA[3] reported that the kasugamycin-resistant strains of some physiologic races of *P. oryzae* were isolated from diseased plants. We also reported[4] the isolation strains resistant to antibiotics is frequent among the isolates of rice blast fungus obtained from diseased plants.

In the present study, epidemiological surveys of physiologic races and kasugamycin-resistant strains of *P. oryzae* in the field with random systematic sampling method were carried out.

The isolates of *P. oryzae* were obtained from diseased plants collected in various districts of Niigata Prefecture. In addition, 80 isolates were obtained from stock cultures in several different Agricultural Experiment Stations.

The procedure of GOTO *et al*[1,2] for differentiating Japanese rice blast fungus races was used.

Twelve varieties were classified to the following 3 groups:

A) Three races of T race group consisting of varieties having the resistance genes of *indica* rice against rice blast.

B) Nine races of C race group consisting of varieties having the resistance genes of Chinese varieties.

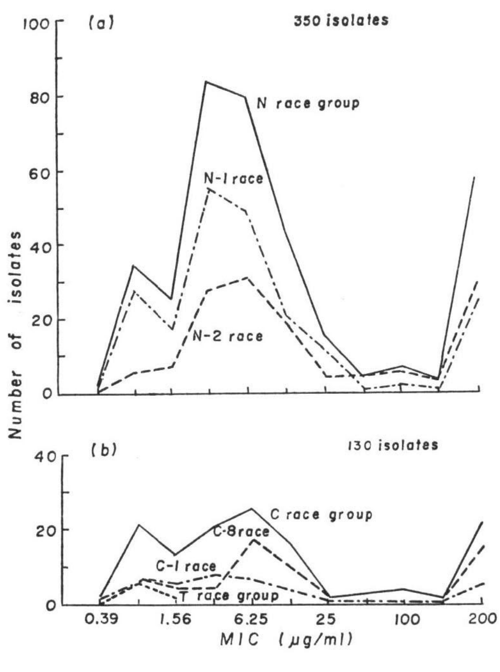
C) Six races of N race group consisting of other Japanese varieties.

Sensitivity test was done by the two-fold agar dilution method described in a previous paper[4]. For the determination of minimal inhibitory concentration (MIC), mycelial fragments of a test isolate was inoculated on agar media containing a given amount of drugs and then incubated for 48 hours at 28°C.

The following method was adopted to determine the correlation between the Japanese rice blast fungus races and kasugamycin-resistant strains of *P. oryzae*. To minimize the viability effect of various conditions of preservation, the MIC values of isolates were pooled for over 3 years. Each sensitivity distribution of physiologic races of *P. oryzae* to kasugamycin was plotted in a single figure (Fig. 1-a and -b).

The MIC value of 50 μg/ml kasugamycin was used for grouping of isolates into sensitive and resistant groups, as shown in Fig. 1-a and -b. The results clearly demonstrate that the relative

Fig. 1. Sensitivity distribution of the Japanese rice blast fungus races to kasugamycin.



* Hokuriku National Agricultural Experiment Station, Joetsu-shi, Niigata, Japan

**The Niigata-ken Agricultural Experiment Station Nagaoka-shi, Niigata, Japan frequency of kasugamycin-resistant strains of *P. oryzae* is not much different between N race and C race groups of the Japanese rice blast fungus races. The frequency of kasugamycin-resistant strains in N race and C race groups of the Japanese rice blast fungus was about 20% of both race groups.

We have previously reported[5] that kasugamycin-resistant strains of *P. oryzae* were cross-resistant to blasticidin S. A comparison of the MIC values of kasugamycin and blasticidin S in physiologic races of *P. oryzae*, demonstrated that the resistance to kasugamycin in N-1, N-2, C-3 and C-8 races coincides with resistance to blasticidin S.

The relation between MIC of kasugamycin to physiologic races of *P. oryzae* and the therapeutic effect of the application of kasugamycin on rice blast was investigated. A method of determining the therapeutic effect of the antibiotic on rice blast was described in a previous paper[6]. As shown in Table 1, the results indicated that the relation between MIC values of kasugamycin to physiologic races of *P. oryzae* and the therapeutic effect of rice blast were correlated as described in the previous paper.[4,5]

Epidemiological surveys of physiologic races and kasugamycin-resistant strains of *P. oryzae* in the fields were made with random systematic sampling method. As shown in Table 2, the data indicate that there are factors which limit the isolation frequencies of physiologic races and kasugamycin-resistant strains of *P. oryzae* to the variety grown, and to a given fungicide in a particular area, such as a prefecture, district or field.

Genetic characters of fungal resistance to the antibiotic which govern the rate of mutation to resistance and those characters which govern pathogenicity, infectivity and parasitic ability, play a large role in the acquisition of resistance, *i.e.*, they increase the chance of contact with agricultural chemicals and affect the distribution of the resistant strains among the host. The isolation frequency of the resistant strains is governed by the amount of agricultural chemicals used,[3] duration of their use[3] and also by the genetic characters of rice blast fungus itself. The epidemiological surveys of physiologic races and drug-resistance of *P. oryzae* by random systematic sampling method might contribute to information that would be useful for agrotechnical and chemical control of rice blast in the field.

Table 1. The relation between MIC of antibiotic to isolates of the Japanese rice blast fungus races and the therapeutic effect of kasugamycin on rice blast in the rice seedlings

Designation of isolates	Physiologic races	MIC of kasugamycin	Therapeutic effect of rice blast*
P-2	N-2	0.78 μg/ml	S
Hoku 373	N-1	0.78	S
H 49-3	C-8	12.5	S
H 49-4	N-1	25	S
N 49-50A	N-1	25	S
N 49-118A	N-2	25	S
N 49-88A	N-2	50	S
N L-110	C-8	50	R
H 25-4	N-2	50	R

* Sensitivity (S=sensitive, R=resistant) was decided by percent decrease in number of lesions per leaf by application of 20 μg/ml solution of kasugamycin.

References

1) GOTO, K.; T. KOZAKA, M. YAMADA, S. MATSUMOTO, S. YAMANAKA, K. SHINDO, T. NARITA, T. IWATA, M. SHIMOYAMA, T. ENDO, I. NAKANISHI, M. NISHIOKA, Y. KUMAMOTO, M. KONO, T. FUJIKAWA, Z. OKADA & T. TOMIKU: Jointwork on the race of rice blast fungus, *Pyricularia oryzae*. Fasc. 1. Plant Disease & Insect Forecast. Spec. Bull. 5: 1~89, 1961
2) GOTO, K.; T. KOZAKA, M. YAMADA, S. MATSUMOTO, S. YAMANAKA, K. SHINDO, T. NARITA, T. IWATA, M. SHIMOYAMA, T. ENDO, I. NAKANISHI, M. NISHIOKA, Y. KUMAMOTO, M. KONO, T. FUJIKAWA, Z. OKADA & T. TOMIKU: Jointwork on the race of rice blast fungus, *Pyricularia oryzae*. Fasc. 2. Plant dis. & Insect Forecast. Spec. Bull. 18: 1~132, 1964
3) MIURA, H.: The incidence of kasugamycin-resistant strains of rice blast fungus and its prevention (in Japanese). Shokubutsuboheki 29: 29: 183~186, 1975
4) SAKURAI, H.; H. NAITO & S. FUJITA: Sensitivity distribution of phytopathogenic bacteria and fungi to antibiotics. J. Antibiotics 29: 1230~1236, 1976
5) SAKURAI, H. & H. NAITO: A cross-resistance of *Pyricularia oryzae* CAVARA to kasugamycin and blasticidin S. J. Antibiotics 29: 1341~1342, 1976
6) SAKURAI, H.; H. NAITO & K. YOSHIDA: Studies

on cross resistance to antifungul antibiotics in kasugamycin-resistant strains of *Pyricularia oryzae* CAVARA. Bull. Agr. Cem. Inspect. Stn. 15: 82~91, 1975

Table 2. The distribution of physiologic races and MIC values of kasugamycin of *Pyricularia oryzae* in the fields

No. of fields	Varieties	No. of sampling points							
		1		2		3		4	
		Races	MIC	Races	MIC	Races	MIC	Races	MIC
a-1	Gohyakumangoku	C-1	3.12	N-1	3.12	N-1	3.12	N-2	1.56
a-2	Koganemochi	C-8	0.78	C-8	100	C-8	>200	C-8	>200
a-3	Koshihikari	N-2	6.25	N-2	6.25	N-2	3.12	N-2	3.12
a-4	Todorokiwase	C-8	>200	C-8	0.78	C-8	>200	C-8	200
a-5	Koganemochi	N-2	3.12	N-1	3.12	C-1	1.56	C-8	1.56
a-6	Koshihikari	N-1	1.56	N-1	12.5	N-1	6.25	N-1	0.78
a-7	Hatsunemochi	N-2	>200	N-1	3.12	N-1	1.56	N-1	3.12
a-8	Todorokiwase	N-1	0.78	N-1	1.56	N-1	12.5	N-1	1.56
a-9	Koshihikari	N-2	3.12	N-2	12.5	N-1	6.25	N-2	3.12
a-10	Koshihomare	N-1	3.12	N-1	3.12	N-1	3.12	N-1	6.25
a-11	Koganemochi	N-1	0.78	C-1	3.12	N-2	1.56	N-1	0.78
a-12	Koganemochi	N-1	3.12	N-2	>200	N-1	12.5	N-2	3.12
a-13	Gohyakumangoku	N-1	3.12	N-1	0.78	N-1	12.5	N-1	3.12
a-14	Todorokiwase	N-1	1.56	N-1	0.78	N-1	3.12	N-1	0.78
a-15	Koganemochi	C-1	0.78	N-2	>200	N-2	>200	C-8	6.25
a-16	Koshihomare	N-2	3.12	N-1	1.56	N-1	0.78	N-1	3.12
a-17	Koshihikari	N-1	0.78	N-1	3.12	C-3	3.12	N-2	6.25
a-18	Gohyakumangoku	N-1	3.12	N-1	3.12	N-1	3.12	C-1	3.12
a-19	Koshihikari	N-1	12.5	C-8	100	C-8	>200	N-2	>200
a-20	Gohyakumangoku	N-1	0.78	N-1	3.12	N-1	3.12	C-1	3.12
a-21	Koshihikari	C-8	1.56	N-1	0.78	N-2	>200	C-1	1.56
a-22	Hohnenwase	N-1	3.12	N-1	3.12	N-1	3.12	N-1	0.78
a-23	Hohnenwase	N-2	100	N-2	3.12	N-2	3.12	N-2	50
b-1	Koganemochi	N-1	0.78	N-2	>200	N-2	>200	N-1	>200
b-2	Koganemochi	N-2	50	N-2	6.25	N-2	>200	N-2	>200
b-3	Koshihikari	N-1	3.12	N-1	3.12	N-2	3.12	N-1	>200
b-4	Todorokiwase	N-1	>200	N-1	3.12	N-1	6.25	N-1	>200
b-5	Hohnenwase	N-2	200	C-3	0.78	N-1	6.25	C-8	6.25
b-6	Koganemochi	N-2	6.25	N-2	6.25	N-2	6.25	C-8	6.25
b-7	Koshihikari	C-3	3.12	C-3	3.12	N-2	0.78	C-1	0.78
b-8	Koshihomare	N-1	3.12	N-2	6.25	N-1	>200	C-1	3.12
b-9	Koshihikari	C-3	3.12	N-1	6.25	N-1	>200	C-1	3.12
b-10	Koshijiwase	N-1	6.25	N-2	3.12	C-8	3.12	N-2	0.78
b-11	Koshihikari	C-3	3.12	N-2	6.25	N-2	>200	N-1	0.78