

# Resistant Strains of Rice Blast Fungus (*Pyricularia oryzae* Cav.) against Fungicides in Japan

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It has been a serious problem that the effectiveness of antibiotics (kasugamycin and blasticidin S) and an organo-phosphorus compound (IBP) against rice blast disease broke down in some districts in Japan since 1972. It was confirmed that the appearance and multiplication of rice blast fungus strains resistant to these fungicides caused the break down of the controlling effect<sup>13)</sup> of these fungicides. In the 1960's, blast fungus strains resistant to phenyl-mercury acetate, copper sulphate and blasticidin S (BcS) were found on the artificial media by an adaptation culture test, but resistant strains were not obtained *in vivo* tests. In 1971 some isolates taken from blast-infested fields, in which the effectiveness of kasugamycin (KSM) broke down apparently, were proved to be resistant to KSM in Yamagata Prefecture<sup>6)</sup>. Thereafter KSM resistant (KSM-R) strains and IBP resistant (IBP-R) strains were found in Niigata Prefecture, in 1974 and 1975, respectively. In 1977, IBP-R strains were found in Toyama Prefecture too. The appearance of KSM-R and IBP-R strains was recognized subsequently in many prefectures by a nationwide survey of resistant strains<sup>13)</sup>. In this paper, the present situation of researches on resistant strains of rice blast fungus against fungicides and the countermeasures to be taken are reviewed.

## The appearance of fungicide-resistant strains in paddy fields

### 1) *Kasugamycin resistant strains*

Difference in the leaf blast occurrence and the fungicide application practice to control the disease between two districts in Yamagata Prefecture is shown in Table 1. In Shonai district a KSM-R strain appeared in 1971 for the first time, but not in Murayama. In the latter district the proportion of KSM to all the fungicides used for blast control was lower than 60%, while in the former district it was higher than 90% in 1969. In addition, the frequency of fungicide application in Shonai was 2-3 times more than in Murayama due to the severe outbreak of rice blast in Shonai. The severe outbreak of rice blast, high frequency of fungicide application and predominant use of KSM seem to trigger the appearance of the KSM-R strain of blast fungus. In many other prefectures such as Fukushima, Miyagi, Nagano, Iwate, Toyama, Ibaraki and Hyogo, KSM-R strains have also been found. However, the blast-control effect of KSM was expressed in the field, because appropriate countermeasures such as no use of KSM or alternate use with other fungicides were adopted after the finding of KSM-resistant strains.

### 2) *IBP resistant strains*

The use of organophosphorus compounds (IBP and ediphenphos) increased in 1974 after the multiplication of KSM-R strains in Uonuma district, Niigata Prefecture, and in

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**Table 1. Difference in the leaf blast occurrence and the fungicide application practice to control the disease between two districts in Yamagata Prefecture (Miura 1984)**

Year	Shonai district			Murayama district		
	Application frequency of fungicides	Rate of KSM to the all fungicides applied	Rate of leaf blast areas to the total field area	Application frequency of fungicides	Rate of KSM to the all fungicides applied	Rate of leaf blast areas to the total field area
1967	2.7	36 %	26 %	1.3	28 %	30 %
1968	3.5	54	29	1.9	46	17
1969	4.6	94	40	2.7	56	26
1970	4.2	90	31	2.0	57	14
1971*	5.0	87	79	2.4	60	61

\* The effect of kasugamycin broke down in 1971.

1975, lowering of the controlling effect of IBP was observed in several fields where IBP had consecutively been used. The break down of controlling effect of IBP and the appearance of IBP-R strains was recognized in 45 fields among 87 fields surveyed in 1976 in Toyama Prefecture. The similar phenomena were observed in 1979 in Akita and Miyagi Prefectures.

## Multiple resistance

### 1) Cross resistance against kasugamycin and blasticidin S

KSM-R strains of blast fungus show generally the resistance against BcS, but some isolates have not cross resistance against KSM and BcS<sup>2,6)</sup>. KSM-R strains show resistance against BcS on the agar plate media, but they are sensitive to BcS when they are inoculated to rice seedlings after spraying with BcS<sup>9)</sup>. KSM and BcS have the same action mechanism inhibiting the synthesis of protein, but action loci on the ribosome are different between two fungicides. Therefore cross resistance against KSM and BcS has to be investigated more precisely.

### 2) Cross resistance against IBP and other fungicides

IBP-R strains obtained by *in vitro* tests showed cross resistance against ediphenphos (EDDP<sup>11)</sup> and isoprothiolane (IPT)<sup>4)</sup>. IBP-

R strains isolated from the field showed cross resistance against EDDP and IPT by *in vitro* tests, but this cross resistance is not clear by the spray and inoculation test using rice seedlings (*in vivo* test). Since IBP-R strains occurring in the field are generally medium-resistant, their reaction to EDDP and IPT *in vivo* tests may be recognized as sensitive to EDDP and IPT which have higher effectiveness against blast disease than IBP.

Joint action of IBP, IPT and phosphoramidate (PA) was estimated by a cross paper technique<sup>4)</sup>. A strip of filter paper impregnated with a test fungicide was placed on PSA plate media inoculated uniformly with conidia of a test strain. Another paper strip impregnated with the other fungicide was placed at a right angle to form a filter paper cross. As shown diagrammatically in Fig. 1, A: two test fungicides having entirely different fungicidal action do not affect each other in the inhibitory zones, B: those having a similar mechanism of fungicidal action may form a round corner at the crossing of the inhibitory zones of the two fungicides, C: a fungicide produces a synergistic effect upon another fungicide and expands the inhibitory zone, and D: two fungicides which are antagonistic in effect each other cause a narrowing of the inhibitory zone. With wild type strains the growth inhibitory zone around crossed paper strips impregnated, respectively, with IPT and PA widened at the crossing

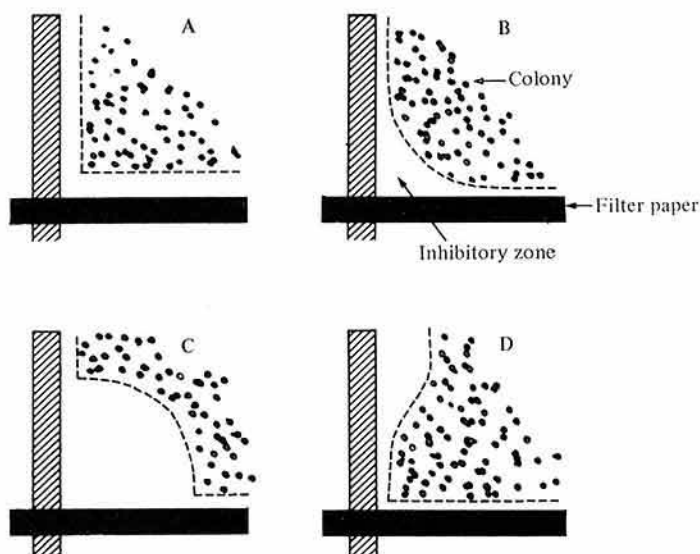


Fig. 1. Four typical joint actions as demonstrated by the crossed-paper technique (Katagiri and Uesugi, 1977). Paper strips each impregnated with each test fungicide are shown by solid lines and the resultant growth inhibitory zone is shown by dotted lines.  
A: Independent action, B: Additive action, C: Synergism, D: Antagonism.

synergism in fungicidal action. The inhibitory zone of IBP and IPT showed rounding of corners (B) which is common for the additive effect of fungicides that have the same mode of action. The pattern of synergism by PA and IPT as well as by PA and IBP was not observed with IBP-R mutants or IPT-R mutants. A perfect correlation of cross resistance between IBP and IPT was shown in the above mentioned experiments. Furthermore all mutants resistant to IPT and IBP were more sensitive to PA than their parent wild type strains. Synergistic fungicidal action between IBP and PA was found in wild type strains, but not in IBP-R and IPT-R mutants. Although mutants resistant to both IPT and PA would probably be expected, they were not observed after selection with IPT from a large number of conidia of wild type strains. All mutants obtained in this experiment were IPT resistant and PA sensitive. Thus the

negatively correlated cross resistance between PA and IPT or IBP seems to follow a consistent pattern. There is a close relationship among IPT, IBP and PA fungicides in their mode of action in *P. oryzae*. The chemical structure of IPT is seemingly different from that of IBP but the thio-ether linkages of IPT probably resemble those of a thio-ester. This similarity in chemical structure may be the basis of similarity between IPT and IBP fungicides in their action on *P. oryzae*.

### The cause of occurrence of fungicide-resistant strains

The resistant strain of blast fungus against copper sulphate appears as a sector in the mycelial colonies which have been cultured on the synthetic media containing gradually increased concentration of copper sulphate. It was presumed that the resistant strain occurs

from mutation induced by consecutive cultures on media containing copper sulphate<sup>11</sup>). But in the other experiment resistant strains against antibiotics and organophosphorus compounds were obtained from old isolates collected in the years when these fungicides had not been developed<sup>11</sup>). Therefore it was presumed that the resistant strains against antibiotics and organophosphorus compounds occur from natural mutation regardless of fungicidal application, and they multiply under the selection pressure of fungicides. Frequencies of emergence of KSM-R mutants are different by isolates, showing 2-246 per  $10^6$  spores scattered on PSA media<sup>7</sup>). Kata-giri and Uesugi (1978)<sup>5</sup>) reported frequencies of emergence of resistant mutants against several fungicides in *in vitro* tests as follows, KSM: 100/10<sup>7</sup>, IBP: 34/10<sup>7</sup>, EDDP: 26/10<sup>7</sup>, IPT: 31/10<sup>7</sup>, and Benomyl: 1.1/10<sup>7</sup>. *Pyricularia oryzae* has a high frequency of emergence of mutants against KSM or organophosphorus compounds, but has a low frequency against benomyl.

The high frequency with KSM was regarded reasonable from the fact that the antibiotic practically tends to cause resistance problem in fields. The low frequency with benomyl seems somewhat curious, because the occurrence of resistance in various plant pathogenic fungi to benomyl has often been reported. The frequency of the occurrence of resistant mutants against KSM in the IBP-R strain was nearly the same as in the case of selection for KSM-resistance from a wild strain. The similar mutants, i.e. IBP-resistant mutants of a KSM-R strain, were obtained at the same frequency as in the selection for resistance against IBP from a wild strain.

Blast lesions produced on rice leaves by the inoculation of the wild strain sensitive to KSM were sprayed with KSM solution. The frequency of emergence of resistant mutants of fungus spores formed on the lesions were examined. The result was as follows, no application of KSM:  $0-7/9 \times 10^5$ , 2 times of application:  $0-4/10^6$ , and 4 times of application:  $0-8/8 \times 10^5$ . These frequen-

cies shown by the *in vivo* test are almost the same as shown by the *in vitro* test.

## Heredity of resistance to fungicide

The KSM resistance of individual ascospores obtained from the crossing of *Pyricularia* strains was tested. The one strain is isolates from finger millet (*Eleusine coracana*) and the other is isolates from weeping love grass (*Eragrostis curvula*). Both the strains consisted of KSM-sensitive wild type strain and KSM-resistant mutants<sup>12</sup>). Two different genes responsible for KSM resistance were found. They are located on different chromosomes. The KSM-R isolates having two genes were phenotypically indistinguishable from the one gene resistant isolates. Some of the KSM-resistant isolates indicated resistance to blastidicin S, and the trait of these isolates resistant against both fungicides was not segregated by hybridization. It indicates that it might be due to pleiotropy of a single gene or close linkage between genes for KSM- and BcS-resistance.

It was revealed as a result of a mating test with sensitive and resistant strains against KSM that the resistance obtained naturally or artificially is controlled by an independent major gene on a chromosome<sup>10</sup>). Three resistant genes, kas-1, kas-2, kas-3, which have no linkage, were identified, and their mode of manifesting the resistance against KSM and BcS was different each other. The kas-3 controls cross resistance against KSM and BcS. These three genes do not show synergistic or additive action and any interaction with each other.

Taga (1982) made clear that there are two types of resistance in IBP-R strains, namely highly and medium resistant types, and the former may be controlled by a major gene and the latter by multiple minor genes. And also one gene ib-P was identified in a strain highly resistant to IBP as a result of an allelism test. Furthermore it was suggested that the cross resistance against IBP, EDDP and IPT may be controlled by the ib-P gene alone.

## Characters of strains resistant to fungicides

The blast fungus strains resistant to fungicides, obtained as a sector in the mycelial colony, which had been cultured consecutively on the media containing fungicides, were characterized by the weaker potential of sporulation and pathogenicity compared with the wild strains sensitive to fungicides. On the contrary resistant strains obtained as a mutant grown from the spores scattered on the plate media have the same characters as those of the wild sensitive strains. The yearly change of population density of KSM-R strains and the protective values of KSM during five years starting from the emergence of the KSM-R strains followed by the suspension of using KSM in Yamagata Prefecture is shown in Fig. 2<sup>8)</sup>.

In 1971 population density of KSM-R strains reached 80% when the protective value of KSM against leaf blast had dropped lower than 40%. However, in late 1972 population density of the KSM-R strains decreased to lower than 40%, and it further decreased with years due to the suspension of using KSM, reaching finally lower than 20% in 1975. This fact indicates that the population density of KSM-R strains tends to decrease under a field condition. Some properties con-

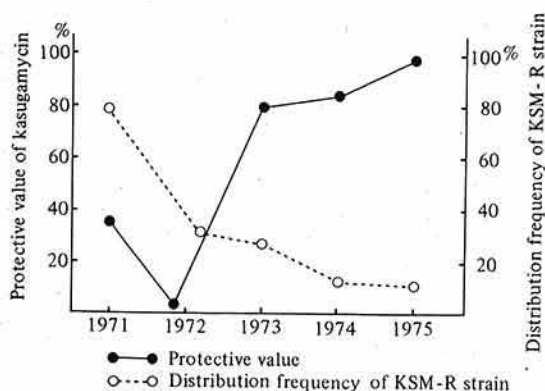


Fig. 2. Yearly change in population density of KSM-R strains of blast fungus and in protective value shown by KSM in Shonai district of Yamagata Pref. (Miura 1984)

cerning pathogenicity and competence for survival were compared between sensitive and resistant isolate groups. No significant difference was found between the two groups in regard to mycelial growth and sporulation on agar media, and number and length of lesions on infected leaves<sup>7)</sup>. Therefore, these properties are not attributable to the predominance in competition between these two groups. Then the difference of sensitive and resistant strains in their process of infection into rice seedlings was investigated to elucidate the cause of the decline of population density of resistant strains<sup>3)</sup>. Each lesion formed by a spray inoculation with a mixture of an equal number of sensitive and resistant spores was found to consist of either the sensitive or the resistant strain alone, and the lesions colonized by resistant strains were less than those by sensitive strains. It shows that the resistant strain is generally less competitive in respect of infection ability than the sensitive strain. There was no significant difference in the number of lesions produced per leaf between sensitive and resistant strains when spore suspension

Table 2. Percent infection of KSM-sensitive and resistant strains of rice blast fungus to rice leaves after 8, 10, and 12 hr of incubation in an inoculation chamber (Ito & Yamaguchi 1979)

Prefecture	Sensitivity	Isolate	Percent infection <sup>a)</sup>		
			8	10	12
Yamagata	Sensitive strain	YS <sub>1</sub>	0.5	16.5	43.7
		YS <sub>2</sub>	0	3.5	53.8
	Resistant strain	YR <sub>1</sub>	0	3.3	43.6
		YR <sub>2</sub>	0	2.4	34.0
Niigata	Sensitive strain	NS <sub>1</sub>	0	2.3	58.1
		NS <sub>2</sub>	0	7.5	38.1
	Resistant strain	NR <sub>1</sub>	0	1.3	15.1
		NR <sub>2</sub>	0	3.6	11.9

a): Percent infection observed after 8, 10, and 12 hr of incubation indicates the number of lesions as percentage of that observed after 24 hr of incubation.



of each strain was separately inoculated to rice seedlings which were then kept in an incubation chamber for 24 hr. But as shown in Table 2, percent infection by resistant strains was lower than that by sensitive strains when the incubation periods were 10 or 12 hr. And also the rate of appressorium formation of resistant spores after 8 hr was lower than that of sensitive spores. It seemed that the time required for appressorium formation and colonization by sensitive strains is shorter than that by resistant strains, and this time lag may cause less competitiveness of the resistant strain in the infection to rice seedlings. Such an inferior competitive ability of resistant strains regarding the process of infection to rice seedlings must have caused the decline of population density of the resistant strains after the suppression of KSM application.

Inoculation tests with a mixture of IBP-sensitive and resistant strains, obtained naturally or artificially, and the reisolation of spores formed on lesions were conducted. After the successive inoculations and isolations the rate in the number of IBP-R strains among the isolates decreased gradually<sup>1)</sup>. Therefore, IBP-R strains are also inferior as to survival ability under natural conditions or competition with sensitive strains in the infection process, like the KSM-R strain.

### Countermeasures to the occurrence of fungicide-resistant strains

(1) Emphasis must be placed on the use of disease-resistant varieties of rice and agronomic control measures for avoiding the increase of fungicide application frequency. Economic and effective fungicide application has to be done by making use of disease forecasting information.

(2) Attention must be paid not to apply consecutively the same fungicide or fungicides which have the same mode of action. The mechanism of action of each blast fungicide is not yet clear, but they will be grouped into five types, namely (i) antibiotics (KSM, BcS), (ii) organophosphorus compounds (IBP,

EDDP, IPT), (iii) fthalide, (iv) probenazole, and (v) tricyclazole, piroquilon. The rotational application or mixed application of these fungicides are recommended.

(3) It is necessary to monitor the appearance, distribution, and population density of resistant strains in each district. When the population density of a resistant strain was lower than 20%, rotational application or mixed application of fungicides including the causal fungicide should be recommended. When it becomes higher than 20%, the application of causal fungicides has to be suspended immediately, and rotational application or mixed application of fungicides except causal fungicides should be recommended<sup>13)</sup>.

### References

- 1) Iijima, A.: Studies on drug resistant strains of rice blast fungus, *Pyricularia oryzae* Cav. *Proc. Kanto Pl. Prot. Soc.*, 32, 22-23 (1985) [In Japanese].
- 2) Ito, I. & Yamaguchi, T.: Occurrence of kasugamycin resistant rice blast fungus influenced by the application of fungicides. *Ann. Phytopathol. Soc. Jpn.*, 43, 301-303 (1977) [In Japanese with English summary].
- 3) Ito, I. & Yamaguchi, T.: Competition between sensitive and resistant strains of *Pyricularia oryzae* Cav. against kasugamycin. *Ann. Phytopathol. Soc. Jpn.*, 45, 40-46 (1979) [In Japanese with English summary].
- 4) Katagiri, M. & Uesugi, Y.: Similarities between the fungicidal action of isoprothiolane and organophosphorus thiolate fungicides. *Phytopathology*, 67, 1415-1417 (1977).
- 5) Katagiri, M. & Uesugi, Y.: *In vitro* selection of mutants of *Pyricularia oryzae* resistant to fungicides. *Ann. Phytopathol. Soc. Jpn.*, 44, 218-219 (1978).
- 6) Miura, H. et al.: Occurrence of resistant strains of *Pyricularia oryzae* Cav. against kasugamycin in Yamagata Prefecture. *Ann. Phytopathol. Soc. Jpn.*, 39, 239 (1973) [In Japanese].
- 7) Miura, H. et al.: Mode of occurrence of kasugamycin resistant rice blast fungus. *Ann. Phytopathol. Soc. Jpn.*, 42, 117-123 (1976).
- 8) Miura, H.: Ecological study on the kasugamycin resistant rice blast fungus. *Spec. Bull. Yamagata Agr. Exp. Sta.*, 14, 1-44 (1984) [In Japanese with English summary].
- 9) Sakurai, H. et al.: Cross resistance to anti-

- biotics in kasugamycin resistant strains of *Pyricularia oryzae* Cav.. *Bull. Agr. Chem. Inspect. Sta.*, 15, 82-91 (1975) [In Japanese with English summary].
- 10) Taga, M.: Genetical studies on fungicide resistance of blast fungus. *Ann. Phytopathol. Soc. Jpn.*, 48, 260 (1982) [In Japanese].
  - 11) Uesugi, Y. et al.: Resistance in *Pyricularia oryzae* to antibiotics and organophosphorus fungicides. *Bull. Nat. Inst. Agr. Sci.*, C23, 93-112 (1969) [In Japanese with English summary].
  - 12) Yaegashi, H.: Studies on the perfect stage of *Pyricularia* species. *Bull. Tohoku Nat. Agr. Exp. Sta.*, 63, 49-125 (1981) [In Japanese with English summary].
  - 13) Yamaguchi, T.: Present situation of the emergence of fungicide resistant strains of rice blast fungus. *Shokubutsu Bōeki*, 33, 471-476 (1979) [In Japanese].
  - 14) Yamasaki, Y. et al.: Studies on drug resistance of the rice blast fungus, *Pyricularia oryzae* Cav.. *Bull. Nat. Inst. Agr. Sci.*, D11, 1-110 (1964) [In Japanese with English summary].

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