島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 22 : 176−183, 1988

Studies on Host-Selective Infection Mechanism of *Pyricularia oryzae* Cavara (1) Usefulness of Rice cv. Sekiguchi-asahi as a Test Plant for Detection of Disease Determinant (s)*

Sakae ARASE**, Mikio KANO**, Kazumi KONDO**, Mikio NOZU**, Emiko TANAKA*** and Syoyo NISHIMURA***

イネいもち病菌の宿主選択的感染機構に関する研究 (1) 病害決定因子検索の検定植物としてのイネ品種関口朝日の有用性*

荒瀬　栄**・加納幹生**・近藤一美**・野津幹雄**・田中恵美子***・西村正暘***

Abstract

When rice cvs. Sekiguchi-asahi and Shin 2 were inoculated with *P. oryzae*, orange to orange brown lesions ("Sekiguchi" lesion) were formed only on cv. Sekiguchi-asahi. On cv. Shin 2, typical necrotic blast lesion was only formed. Size of the "Sekiguchi" lesions was larger than that of commonly visible blast lesions. The "Sekiguchi" lesions rapidly enlarged, gradually merged and finally occupied the entire plant. Significant difference in lesion formation was also observed among isolates of *P. oryzae* used. Typical "Sekiguchi" and blast lesions were formed on the leaves by inoculation of isolates Hoku 1, Ken 54-04, 0528-2 and Naga 69-150 of *P. oryzae*. In cases of isolates Ina 168 and SH 85-125, only "Sekiguchi" lesions were induced. Such difference in lesion formation was already recognized at cytological observation of fungal behaviors in detached rice sheath cells. Hyphal growth of *P. oryzae* isolates that produced two typical lesions on cv. Sekiguchi-asahi was very good into the rice sheath cells. However, that of isolates Ina 168 and SH 85-125 that produced only "Sekiguchi" lesions was very poor. When spores of isolate SH 85-125 ("Sekiguchi" lesion inducer) were mixed with those of isolate Hoku 1("Sekiguchi" and blast lesions inducer), and the spore mixed-pastes of two isolates were punch-inoculated onto the leaves of cv. Sekiguchi-asahi, there was difference in the numbers of typical "Sekiguchi" lesions between mixed- and single-inoculation of isolates Hoku 1 and SH 85-125.

INTRODUCTION

There are already many studies conducted on field development[1], resistance[2], fungal race and chemical control[3] in rice blast disease caused by *Pyricularia oryzae* Cavara; however, physiological or biochemical studies, particularly, with emphasis on the chemical nature of host specificity and role of disease determinants produced by host and/or pathogen as reported in some diseases[4),5),6], is limited to only few reports[7),8),9]. Previous studies[10),11),12] on induced susceptibility suggested that, in early infection

* This study was supported in part by Grant-in-Aid for Cooperative Research No. 62304015 from Ministry of Education, Science and Culture of Japan
** Laboratory of Plant Pathology, Faculty of Agriculture, Shimane University, Matsue 690, Japan
*** Laboratory of Plant Pathology, Faculty of Agriculture, Nagoya University, Nagoya 464. Japan

stage, rice blast fungus releases the essential factor(s), such as host-specific toxins[5] or suppressors[6] in determining suppression of resistance of rice plants. However, final proof of the identity of such factor(s) must await the isolation of the compounds in sufficiently large amounts of spore germination fluids and also the innovative bioassay for the detection. Following explanations may be offered for not having detected above factors in *P. oryzae*. First, although many rice cultivars used in our experiments have many blast resistant genes[3], they are not suitable in detecting the disease determinants. Second, although, phytotoxic metabolites of blast fungi have been isolated from culture filtrates[13),14),15], they were not noticed in the fluids of germinating spores at initial event of blast infection.

In 1965, Sekiguchi and Furuta[16] reported a mutant rice plant, cv. "Sekiguchi-asahi", derived from cv. Asahi which formed orange to orange brown lesions by infection of *P. oryzae* and *Cochliobolus miyabeanus* or by treatment of chemicals. This unique lesion was named the "Sekiguchi" lesion by Kiyosawa[17]. Marchetti *et al.*[18] also found mutants in American rice lines showing unique lesions similar to those described previously in Japan. In a previous report[19], we pointed out high susceptibility of cv. Sekiguchi-asahi to several fungi.

In this paper, we report that rice cv. Sekiguchi-asahi may be extremely useful for the detection of disease determinant(s) in rice blast disease as a test plant.

MATERIALS AND METHODS

Plant. Rice cultivars, Sekiguchi-asahi and Shin 2, were used in this study. Seedlings of two cultivars at 2-3 leaf stage were planted in seedling case and were fertilized with 1g of $(NH_4)_2SO_4$, 0.2g of KCl and 1.5g of $CaH_4(PO_4)_2H_2O$ per case.

Fungus. Isolates Hoku 1, Ina 168, Ken 53-33, Ken 54-04, Ken 60-19, Naga 69-150, 0528-2 and SH 85-125 of *P. oryzae* were used. Each isolate was grown on oatmeal medium at 26 C for 14 days, and spores of each isolate were prepared as described previously[11]. Spores were harvested in water.

Inoculation. Following two methods were employed; I) A 20-ml suspension of spores which was adjusted to approximately 10^5 spores ml^{-1}, containing Tween 20 (0.05%), was sprayed on rice leaves at 4-5 leaf stage. II) The spore suspension was poured through 1g of powdered cellulose on filter paper (Toyo No. 2) in a funnel, and 0.3g of carboxymethyl cellulose was added to make a paste. The spore paste was placed on 7-9 pinched sites (each 1mm in diam. and about 2cm apart) per one leaf.

Spray- and punch-inoculated rice plants were kept in a moist chamber at 28 C for 24 hr. The number of lesions and its size were observed 7 to 8 days after inoculation.

Fungal behaviors. A spore suspension (10^5 spores ml^{-1}) was inoculated on inner surface of detached rice leaf-sheaths and fresh onions. Inoculated rice sheaths and onions were kept in a moist chamber at 28 C. After 24 and 48 hr, respectively, spore germination, appressorial formation, penetration and hyphal growth in the rice sheath and onion cells were investigated by using a light microscope. Penetration and

hyphal growth in rice sheath cells were determined by Takahashi's method[20].

Sporulation test on lesions. Leaves with lesions were kept in a moist chamber for 48 hr at 26 C. Spores on the lesions were adhered to a scotch tape. Then spores on this tape were observed by using a light microscope.

RESULTS

"Sekiguchi" lesion development on leaves of cv. Sekiguchi-asahi infected with *P. oryzae*

There was large difference in lesion type observed (*i. e.* color or size) between cvs. Sekiguchi-asahi and Shin 2 inoculated with *P. oryzae*. In cv. Sekiguchi-asahi at 4-5 leaf stage, orange to orange brown ("Sekiguchi" lesion) and typical blast lesions were formed on the leaves by inoculation of isolates Hoku 1, Ken 53-33, Ken 54-04, Ken 60-19, 0528-2 and Naga 69-150 of *P. oryzae*. However, in isolates Ina 168 and SH 85-125, only "Sekiguchi" lesions were induced on the leaves (Fig. 1 and Table 1). On the other hand, all isolates used produced only blast lesions on those of cv. Shin 2. Size

Table 1. The number of "Sekiguchi" and blast lesions formed on leaves of rice plants inoculated with *P. oryzae*

Isolate (race)	cv. Sekiguchi-asahi		cv. Shin 2	
	S lesion[a]	B lesion[b]	S lesion	B lesion
Ina 168(101)	35.3	0.0	0.0	7.0
Ken 53-33(137)	16.5	14.0	0.0	11.5
Ken 54-04(003)	16.0	22.7	0.0	11.5
Ken 60-19(037)	41.0	27.0	0.0	14.5
Naga 69-150(007)	33.3	40.3	0.0	24.0
0528-2(333)	15.7	5.7	0.0	19.5
SH 85-125(?)	31.3	0.0	0.0	23.0

Rice cvs. Sekiguchi-asahi and Shin 2 at 4-5 leaf stage were spray-inoculated with spore suspension of *P. oryzae*, and were kept in a moist chamber for 24 hr. The number of lesions was investigated 7 days after inoculation.
a) "Sekiguchi" lesion. b) Blast lesion.

Table 2. Comparison of size in Sekiguchi and blast lesions formed on leaves of cvs. Sekiguchi-asahi and Shin 2

Isolate	cv. Sekiguchi-asahi		cv. Shin 2
	S lesion[a]	B lesion[b]	B lesion
Ina 168	6.2	—[c]	2.6
Ken 53-33	6.2	3.2	3.0
Ken 54-04	5.9	3.5	2.8
Ken 60-19	6.0	2.6	3.0
Naga 69-150	5.3	3.7	3.1
0528-2	2.5	2.2	2.3
SH 85-125	4.0	—[c]	2.1

Rice cvs. Sekiguchi-asahi and Shin 2 were spray-inoculated with spore suspension of *P. oryzae*, and were kept in a moist chamber for 24 hr. Lesion size was measured 7 days after inoculation.
a) "Sekiguchi" lesion. b) Blast lesion. c) Blast lesions were not formed.

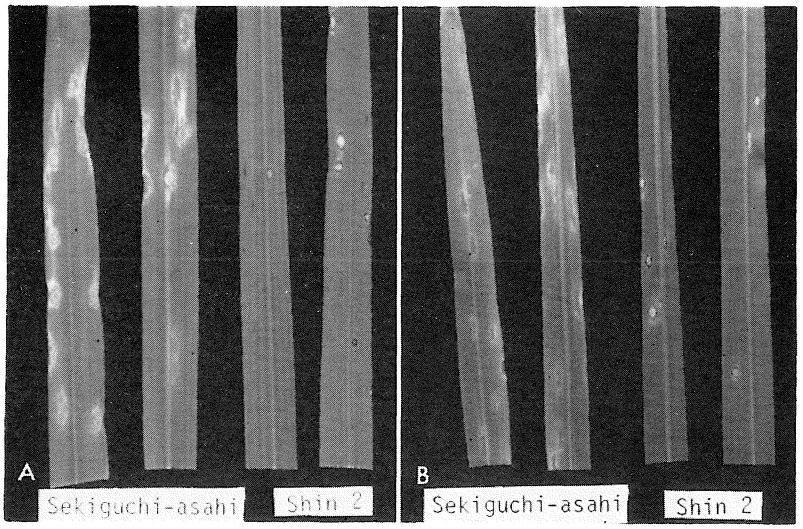

Fig. 1. Comparison of lesion formed on the leaves of cvs. Sekiguchi-asahi and Shin 2 inoculated with isolates Hoku 1 and Ina 168 of *P. oryzae*.
A : The "Sekiguchi" and blast lesions on the leaves of cv. Sekiguchi-asahi inoculated with isolate Hoku 1 of *P. oryzae*.
B : The "Sekiguchi" lesions on the leaves of cv. Sekiguchi-asahi inoculated with isolate Ina 168 of *P. oryzae*.

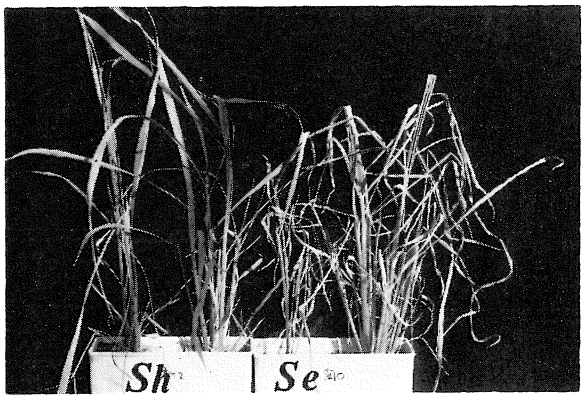

Fig. 2. Comparison of susceptibility of cvs. Sekiguchi-asahi(Se) and Shin 2(Sh) to *P. oryzae*.

of the "Sekiguchi" lesions were larger than that of blast lesions (Table 2). The incidence of the "Sekiguchi" lesions was faster than that of blast lesions, and they enlarged rapidly, gradually merged and occupied the entire plant. Some plants died (Fig. 2).

Sporulation on the" Sekiguchi" and blast lesions was tested. Although many spores were produced on blast lesions regardless of whether the lesions used were from cvs. Sekiguchi-asahi and Shin 2, no sporulation was observed on the "Sekiguchi" lesions.

Fungal behaviors of *P. oryzae* in rice leaf-sheath and fresh onion tissues

All isolates of *P. oryzae* used germinated well, formed appressoria and penetrated into the sheath cells 24 hr after inoculation. However, hyphal growth into the sheath cells differed among isolates of *P. oryzae*. Hyphal growth of isolates that produced two typical lesions on cv. Sekiguchi-asahi was very good into the sheath cells of the two cultivars. However, that of isolates Ina 168 and SH 85-125 that produced only "Sekiguchi" lesions on cv. Sekiguchi-asahi was very poor (Table 3). On the other

hand, in case of the sheath cells of cv. Shin 2, that of all isolates was very good.

On fresh onion tissues, all isolates of *P. oryzae* used have germinated well, formed appressoria, and penetrated into the tissues. There was no difference in infection

Table 3. Infection behaviors of *P. oryzae* in detached rice sheaths of cv. Sekiguchi-asahi

Isolate	Germination (%)		Appressorial formation (%)		Penetration[a] (%)		Hyphal growth[a]	
	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
Ina 168	92.9	93.8	80.8	92.6	26.1	33.2	1.4	1.7
Ken 54-04	87.4	83.8	97.6	98.1	26.4	42.9	1.5	3.0
Naga 69-150	91.7	89.5	93.0	95.9	66.4	86.7	2.5	8.5
0528-2	86.1	96.0	96.0	96.8	30.1	55.8	1.3	3.1
SH 85-150	81.7	96.4	98.5	99.5	25.6	37.6	1.6	1.8

a) Penetration and hyphal growth of *P. oryzae* in rice sheath cells were determined by Takahashi's method[20].

Table 4. Infection behaviors of *P. oryzae* on fresh onion tissues

Isolate	Germination (%)		Appressorial formation (%)		Infection hypha formation (%)	
	24hr	48hr	24hr	48hr	24hr	48hr
Ina 168	98.4	98.5	95.5	97.9	46.0	59.9
Ken 54-04	100.0	100.0	100.0	100.0	69.6	73.9
Naga 69-150	98.8	99.5	97.8	99.1	74.4	96.7
0528-2	95.9	95.9	88.7	91.4	86.5	87.7
SH 85-125	90.6	97.3	86.3	91.0	51.6	91.8

Table 5. Effect of spore mixed-inoculation with different isolates of *P. oryzae* on the "Sekiguchi" and blast lesion formation

Inoculated isolate	Total number of lesions observed	Type of lesions formed		
		B[a]	B+S[b]	S[c]
Hoku 1	135 (100)[d]	18 (13.3)	115 (85.2)	2 (1.5)
SH 85-125	139 (100)	5 (3.6)	23 (16.5)	111 (79.9)
Hoku 1	141 (100)	19 (13.5)	109 (77.3)	13 (9.2)
Hoku 1+ SH 85-125	163 (100)	6 (3.7)	108 (66.2)	49 (30.1)
Hoku 1+SH 85-125	127 (100)	6 (4.7)	73 (57.5)	48 (37.8)
SH85-125	116 (100)	3 (2.6)	28 (24.1)	85 (73.3)

The spore pastes prepared from isolates Hoku 1 and SH 85-125 of *P. oryzae* were placed on pinched sites of rice leaves. Inoculated rice plants were kept in a moist chamber for 24 hr. Type of lesion formed on the leaves were determined 8 days after inoculation.

a) Typical blast lesions were formed.
b) The "Sekiguchi"(S) and blast (B) lesions were simultaneously formed around a punch-inoculated site.
c) Typical "Sekiguchi" lesions were formed.
d) Numbers in parentheses show the ratio(%) of each lesion type to total lesions observed.

hypha formation among isolates of *P. oryzae*, and invading hyphae developed well into the onion cells at 48 hr after inoculation (Table 4).

Effect of spore mixed-inoculation with different isolates of *P. oryzae* on the "Sekiguchi" and blast lesions development in leaves of cv. Sekiguchi-asahi

From the results of spray-inoculation test, it was demonstrated that *P. oryzae* possessed the "Sekiguchi" lesion-inducing ability. However, its inducing-ability was different among isolates of *P. oryzae* used. To elucidate more clearly its lesion-inducing ability, the spore mixed-pastes of isolates Hoku 1 (two typical lesions inducer) and SH 85-125 ("Sekiguchi" lesion inducer) were punch-inoculated on the leaves of cv. Sekiguchi-asahi. After 8 days, mixed-lesions with "Sekiguchi" and blast types were simultaneously formed around a punch-inoculated site, although, in case of single isolate, lesion formation showed similar tendency to those in spray-inoculation test (Table 5).

DISCUSSION

When the leaves of cvs. Sekiguchi-asahi and Shin 2 were inoculated with *P. oryzae* spores, typical "Sekiguchi" lesions were formed on cv. Sekiguchi-asahi. The Sekiguchi lesions enlarged rapidly, gradually merged and finally occupied the entire plant. Eventually, some plants died. On cv. Shin 2, typical necrotic blast lesions were formed, plants didn't die by inoculation test in laboratory. The "Sekiguchi" lesion was formed by all isolates of *P. oryzae* used. This result indicated that *P. oryzae* possessed the "Sekiguchi" lesion-inducing ability on the leaves of the cv. Sekiguchi-asahi and that rice cv. Sekiguchi-asahi is a highly susceptible cultivar to rice blast as compared to previous rice cultivars. At present, our most interest concentrated to some isolates such as Hoku 1, Ken 53-33, Ken 54-04, Ken 60-19, Naga 69-150 or 0528-2 that produced typical blast lesions besides the "Sekiguchi" lesions. Others such as Ina 168 or SH 85-125 induced only the "Sekiguchi" lesions. Although blast lesions were observed 4-5 days, the "Sekiguchi" lesions were already observed 3 days after inoculation, respectively. Sporulation was not recognized on the "Sekiguchi" lesions. Such large difference in lesion formation on the leaves among isolates of *P. oryzae* was already recognized in cytological level at an early stage of infection. Hyphal growth of isolates that produced only "Sekiguchi" lesion was significantly inhibited into the sheath cells of cv. Sekiguchi-asahi, although others showed similar tendency to that in the compatible race-cultivar interaction. Our pathological studies suggested that cv. Sekiguchi-asahi has resistant gene(s) to isolates Ina 168 and SH 85-125 of *P. oryzae* used in this study, and the "Sekiguchi" lesion formation was induced in incompatible interaction. According to Kiyosawa[17], the "Sekiguchi" lesion is controlled by a recessive gene, designated as *sl*, and cv. Sekiguchi-asahi has *Pi-a* gene with *sl* gene. Further, he described that there is a close relationship between formation of the "Sekiguchi" lesions and that of resistant lesions. Our results fitted in his genetic results. However, there are still some important questions as follows: 1) Size of the "Sekiguchi" lesions was larger than that of blast lesions. 2) The "Sekiguchi" lesions enlarged,

merged, and finally occupied the whole leaves, and some plants died. 3) In a previous report[19], we demonstrated that even *Helminthosporium* spp., *Alternaria* sp. and *Curvularia* sp. induced the "Sekiguchi" lesions and sporulated on the leaves. These results suggest that the "Sekiguchi" lesions caused by *P. oryzae* are induced independently regardless of resistant response of rice plant controlled by *Pi-a* gene. Marchetti *et al.*[18] reported that the "Sekiguchi" lesion is a manifestation of a flow in the biochemical mechanism that regulate the hypersensitive reaction of the plant.

When spores of *P. oryzae* (isolates Hoku 1 and SH 85-125) were mixed and were punch-inoculated to the leaves of cv. Sekiguchi-asahi, mixed-lesions differed somewhat from that formed by single isolate. The mixed-spores of these two isolates germinated well on the glass slide. In case of cultivation on oatmeal medium, antagonistic phenomenon was not observed between the two *P. oryzae* isolates. From results of infection behaviors on onion tissues, blast fungi used must possess an equal aggressiveness to penetrate the tissues. These results suggest that pathogenicity of *P. oryzae* concern in the "Sekiguchi" and blast lesions formation on cv. Sekiguchi-asahi. It seems that all virulent *P. oryzae* produce not only the "Sekiguchi" lesion-inducing factor(s) but also some blast fungus release the inhibitor to the "Sekiguchi" lesion-inducing factor(s) during spore germination.

In conclusion, cv. Sekiguchi-asahi is a useful test plant for detection of disease determinant(s), not only concerning the basic compatibility but also race-cultivar interaction, of the pathogen in rice blast disease.

The authors are grateful to Mr. J. Isota, Shimane Prefecture Agricultural Experiment Station, for supplying isolate of *P. oryzae*.

Literature cited

1. Ou, S. H.: *In* Rice Diseases. Commonwealth Mycological Institute, 1985, pp. 109-201.
2. Suzuki, H.: *In* Rice Blast Disease (Yamanaka, S. and Yamaguchi, T. *eds.*). Youkendou, Tokyo. 1987, pp. 100-123.
3. Yamada, M.: *In* Rice Blast Disease (Yamanaka, S. and Yamaguchi, T. *eds.*). Youkendou, Tokyo. 1987, pp. 189-216.
4. Heath, M. C.: Phytopathology **70**: 356-360, 1979.
5. Nishimura, S. and Kohmoto, K.: *In* Toxins and Plant Pathogenesis (Daly, J. M. and Deverall, B. J. *eds.*). Academic Press, Australia Sydney, 1983, pp. 137-157.
6. Shiraishi, T., Oku, H., Yamashita, M. and Ouchi, S.: Ann. Phytopath. Soc. Japan **44**: 659-665, 1978.
7. Akatsuka, T., Kodama, O., Kato, H., Kono, Y. and Takeuchi, S.: Agric. Biol. Chem. **47**: 445-447, 1983.
8. Iwata, M., Iwamatsu, H., Suzuki, Y., Watanabe, T. and Sekizawa, Y.: Ann. Phytopath. Soc. Japan **48**: 267-274, 1982.
9. Matsuyama, N.: *Ibid.* **49**: 270-273, 1983.
10. Arase, S. and Itoi, S.: *Ibid.* **47**: 269-271, 1981.
11. Arase, S., Nitta, H. and Itoi, S.: *Ibid.* **48**: 578-584, 1982.

12. ARASE, S., TANISHIKI, M. and ITOI, S.: *Ibid.* 48: 544-546, 1982.
13. IWASAKI, S., MURO, H., NOZOE, S., OKUDA, S. and SATO, Z.: Tetrahedron Letters **1**: 13-16, 1972.
14. IWASAKI, S., NOZOE, S., OKUDA, S., SATO, Z. and KOZAKA, T.: *Ibid.* **45**: 3977-3980, 1969.
15. NUKINA, S., SASSA, T., IKEDA, M., UMEZAWA, T. and TASAKI, H.: Agr. Biol. Chem. **45**: 2161-2162, 1981.
16. SEKIGUCHI, Y. and FURUTA, T.: Ann. Phytopath. Soc. Japan **30**: 71-72(Abstr.), 1965.
17. KIYOSAWA, S.: Bull. Nat. Inst. Agr. Sci. Ser. D, **21**: 61-71. 1970.
18. MARCHETTI, M. A., BOLLICH, C. N. and UECKER, F. A.: Phytopathology **73**: 603-606, 1983.
19. KURIHISA, H. and ARASE, S.,: Bull. Assoc. Pl. Prot. Shimane **11** : 32-35, 1986.
20. TAKAHASHI, Y.: Hokkaido Pref. Agr. Exp. Sta. Rept. **3**: 1-61, 1951.

摘　　要

イネいもち病菌をイネ品種関口朝日と新2号に接種すると，関口朝日上には橙黄一橙黄褐色病斑（関口病斑）が形成されたが，新2号には典型的いもち病斑だけが形成された．関口病斑はいもち病斑よりも大型であった．形成された関口病斑は，急速に拡大し，次第に融合し，植物体全体に広がった．また，関口朝日上の病斑形成には供試いもち病菌株間で顕著な違いがみられ，北1，研54-04，0528-2，長69-150菌株は関口及びいもち両病斑を形成した．稲168とSH85-125菌株は関口病斑のみを形成した．このような病斑形成の違いは，イネ葉鞘細胞でのいもち病菌の侵入行動においてすでに認められた．即ち，前者の葉鞘細胞内での菌糸伸展は良好であったが，後者のそれは非常に貧弱であった．SH85-125（関口病斑形成菌株）及び北1（関口及びいもち病斑形成菌株）の両菌株分生胞子を混合後，関口朝日葉にパンチ接種すると，形成関口病斑数はこれら菌株の単独接種の場合とは異なっていた．