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Effect of Preinfectional Treatment of Homogenates from Rice Leaves on Resistance of Rice Plants to Several *Pyricularia* spp.

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各種いもち病菌に対するイネ体の抵抗性に及ぼすイネ葉磨砕液の感染前処理の影響

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When rice leaf-sheaths were infiltrated with the homogenates from healthy rice leaves and then inoculated with the incompatible race of *Pyricularia oryzae*, remarkable hyphal growth in the tissues was observed. The homogenates increased hyphal growth indices of the incompatible races, regardless of whether the rice cultivars used to prepare the homogenates were resistant or susceptible to the races of the rice blast fungi inoculated after the homogenate treatment. Such an induced-susceptibility was also recognized in the rice leaf-sheaths pre-treated with the boiled(100 C-30 min)-homogenates. In case of post-inoculation with *Pyricularia* sp., non-pathogenic to rice plants, the hyphal growth in the leaf sheaths pre-treated with leaf homogenates was nearly the same values as that of pretreatment with distilled water, and the values of both the average and highest indices were also found to be very poor.

Introduction

Recently, a number of biochemical or physiological mechanisms have been proposed to explain interaction and its specificity between plant and pathogen. In some diseases[1-4], the research trends emphasized the chemical nature of host specificity and role of disease determinants produced by host and/or pathogen. In rice blast, caused by *Pyricularia oryzae*, however, the evidence is not available that disease resistance or susceptibility is determined by highly specific mechanisms or specific disease determinants unique in their role to contain the development of infectious pathogen. Previous reports[5,6] showed that rice plants preliminarily infected by a compatible race of *P. oryzae*, or immersed in hot water at 55 C for 10 or 15 min became susceptible not only to an incompatible race of *P. oryzae* but also to nonpathogens. This phenomenon has not been explained extensively as a process involving specific interaction between molecular constituents of the host tissues and the fungus. However, the accumulation of the studies on such an induced-susceptibility seem to provide some valuable information. The present study extends these observations by reporting the effects of the homogenates from rice leave on hyphal growth of several *Pyricularia* spp.

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Brief reports of portion of this study have been published previously[7,8].

Materials and Methods

Plant and fungus. Three rice cultivars, Shin 2, Aichi-asahi and Kanto 51 were used in this study. Seedlings of the three cultivars in the two-leaf age were planted in pots (1/5000 a) and were fertilized with 8 g of $(NH_4)_2SO_4$, 1.6 g of KCl and 12 g of $CaH_4(PO_4)_2H_2O$ per pot, as described previously[9].

Isolates Hoku 1 (race 007) and Naga 87 (race 131) of *P. oryzae* and two *Pyricularia* spp., non-pathogenic on rice plants, from Italian ryegrass (isolate L1-1) and from ragi (isolate Y2-6) were used. The relationships between rice plant and blast fungus were summarized in Table 1. The conidia to be used were prepared as described previously[9]. All of these isolates

Table 1. Relationships between rice plant and blast fungus used in this study

Cultivar	*P. oryzae*		*Pyricularia* sp.	
	Hoku 1	Naga 87	L1-1[a]	Y2-6[b]
Shin 2	S	S	I	I
Aichi-asahi	S	R	I	I
Kanto 51	R	S	I	I

a) Pathogenic to Italian ryegrass. b) Pathogenic to ragi.
S : Susceptible. R : Resistant. I : Immune.

were cultured on oatmeal medium for 10-14 days at 26 C. The cultured plates were incubated at 26 C for 2 days after aerial hyphae on the medium were washed away by distilled water. Thus, synchronously formed conidia were used as inocula.

Preparation of rice leaf homogenates from rice leaves. Rice leaves (5 g) of the above three cultivars were cut fine with scissor, then homogenized in distilled water (15 ml) with guartz sand at room temperatures. The homogenates were centrifuged at 3,500 rpm for 10 min. The supernatant was then filtrated on a filter paper (Toyo No. 2), and the filtrate was centrifuged again at 15,000 rpm for 30 min. The homogenate (0 fraction) was filtrated through a membrane filter (Toyo TM-2), and were dialyzed against distilled water for 24 hr with seamless cellulose tubing (Visking Company). The inner non-dialysate was used as a high molecular weight fraction (HM fraction) and outer dialysate concentrated and used as a low molecular weight fraction (LM fraction).

Bioassy of rice leaf homogenate. 0, LM and HM fractions prepared as above described were infiltrated into detached rice leaf-sheath (2.5 cm in length) at reduced pressure (approximately 50 mm Hg) for 5 min. This infiltration for 5 min was repeated 3 times. The treated leaf-sheaths were rinsed with distilled water, and inoculated with several *Pyricularia* spp. Hyphal growth index into the sheath cells was estimated 40 hr after inoculation by Takahashi's method[10].

Result

Effect of rice leaf homogenates on hyphal growth of the incompatible race of *P. oryzae*

Table 2. Effect of preinoculation infiltration of rice leaf homogenates on hyphal growth of the incompatible race of *P. oryzae* in rice leaf-sheath

Preinoculation treatment of leaf-sheath	Average index of hyphal growth in leaf-sheath of :	
	cv. Aichi-asahi[a)]	cv. Kanto 51[a)]
Infiltration with rice leaf homogenate of :		
cv. Shin 2	4.61	3.86
cv. Aichi-asahi	2.69	3.57
cv. Kanto 51	—[b)]	2.78
Infiltration with distilled water	0.78	0.77

a) After preinoculation treatment, the cv. Aichi-asahi was inoculated with an incompatible race 131 (isolate Naga 87), and the cv. Kanto 51 was with an incompatible race 007 (isolate Hoku 1). Hyphal growth in leaf-sheath determined 40 hr after inoculation.
b) Not determined.

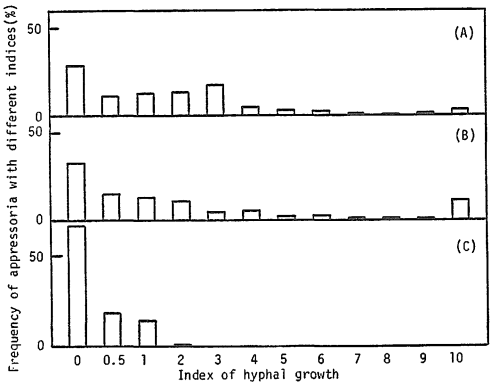

Fig. 1. Effect of preinoculation treatment of rice feaf homogenates on hyphal development of the incompatible race of *P. oryzae* in leaf-sheath. Leaf-sheaths of the cv. Kanto 51 were infiltrated with rice leaf homogenates of the cv. Kanto 51(A) and Shin 2(B) or distilled water(C) and then were inoculated with the incompatible race 007(isolate Hoku 1) of *P. oryzae*. Indices of hyphal growth were determined 40 hr after inoculation.

Leaf-sheaths of the cvs. Aichi-asahi and Kanto 51 were infiltrated with rice leaf homogenates (0 fraction) from the cvs. Shin 2, Aichi-asahi and Kanto 51, respectively, and then inoculated with the incompatible races (isolates Hoku 1 and Naga 87) of *P. oryzae*. The hyphal growth of two races in the homogenate-treated rice sheaths increased remarkably (Table 2). The highest index value in the treated sheaths was more than 10 (Fig. 1). When leaf-sheaths were pretreated with distilled water as a control and then infected by the incompatible races, hyphal growth and host responses seen in leaf-sheaths were almost similar to those[11)] reported earlier involving the incompatible rice blast fungi. The average index value of the incompatible races in the cvs. Aichi-asahi and Kanto 51 was 0.87 and 0.77, respectively. The results given in Table 2 and Fig. 1 show that all rice leaf homogenates increase hyphal growth indices of the incompatible races, regardless of whether the rice plants used to prepare homogenates were resistant or susceptible to the races of the rice blast fungi inoculated after the homogenate treatment.

The above induced-susceptibility was also observed by pretreatment with boiled homogenates at 100 C for 30 min (Fig. 2). Although, as responses to an incompatible race of *P. oryzae*, fine granulation of cytoplasm accompanying uncolored change, or if any, only pale yellow discoloration was observed, the infiltration with homogenates tented to suppress such morphological changes in the infected host cells.

Comparision of hyphal growth among the isolates of *Pyricularia* spp.

When leaf-sheaths were infiltrated with the homogenates boiled and not boiled from rice

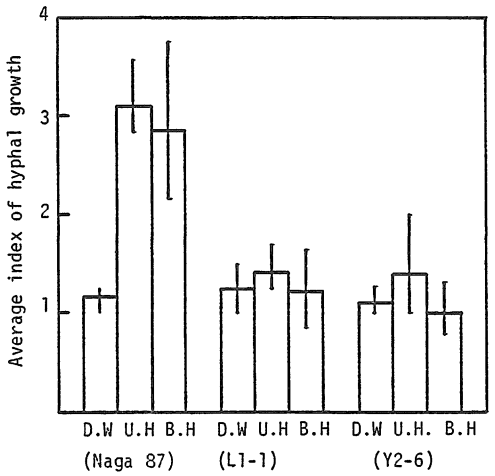

Fig. 2. Effect of boiled (100 C-30 min) rice leaf homogenates on hyphal growth of several *Pyricularia* spp. Detached leaf-sheaths (cv. Aichi-asahi) were infiltrated with unboiled (U. H.), boiled (B. H.) homogenates (cv. Shin 2) and distilled water (D. W.), and then were inoculated with an incompatible race (isolate Naga 87) of *P. oryzae* and *Pyricularia* spp., non-pathogenic on rice plants, from Italian ryegrass (isolate L1-1) and from ragi (isolate Y2-6).

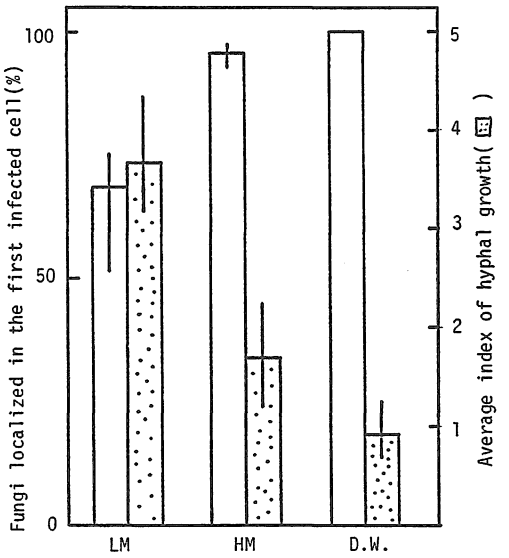

Fig. 3. Effeet of low and high molecular weight fractions of rice leaf homogenates (cv. Shin 2) on hyphal growth of incompatible race 131 (isolate Naga 87) of *P. oryzae*.
LM : Low molecular weight fraction.
HM : High molecular weight fraction.
D. W. : Distilled water.

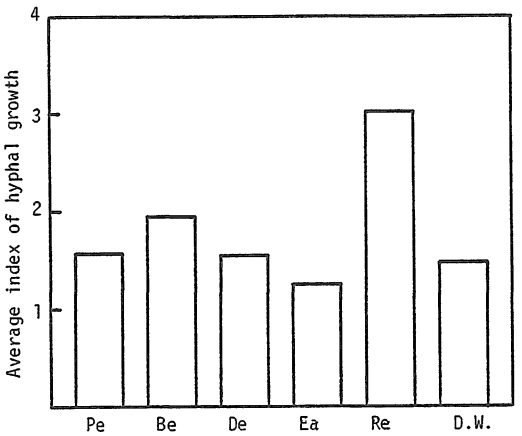

Fig 4. Effect of solvent extracts from rice leaf homogenates on hyphal growth. Homogenates of rice leaves (cv. Shin 2) were extracted with petroleum ether, benzene, diethyl ether and ethyl acetate, successively. Each extract was infiltrated in detached sheath (cv. Aichi-asahi). D. W. : Distilled water. Pe : Petroleum ether. Be : Benzene. De : Diethyl ether. Ea : Ethyl acetate. Re : Residue.

leaves of the cv. Shin 2 and then inoculated with the incompatible race of *P. oryzae*, average index of hyphal growth in leaf-sheath of the cv. Aichi-asahi increased remarkably (Fig. 2). However, when non-pathogenic *Pyricularia* spp. (isolates L1-1 and Y2-6) were inoculated into homogenate-treated leaf-sheath, hyphal growth was very poor, similar to that of control.

Fractionation of susceptibility inducer from rice leaf homogenates

The effects of LM and HM fractions on hyphal growth of the incompatible race of *P. oryzae* were shown in Fig. 3. When LM fraction from the homogenates of the cv. Shin 2 was infiltrated in leaf-sheath of the cv. Aichi-asahi, average index of the incompatible race (isolate Naga 87) of *P. oryzae* increased more than those in sheaths infiltrated with HM fraction. In contrast, the

infection hyphae localized in the first infected cells was about 70 % in the sheaths pretreated with LM fraction, in comparison with the data which gave almost 100 % in the sheaths pretreated with HM fraction or water (Fig. 3). The susceptibility inducer in LM fraction could not be extracted with petroleum ether, benzene, diethyl ether and ethyl acetate (Fig. 4).

Discussion

The preinfectional treatment of the homogenates from the rice leaves promoted significantly hyphal growth of the incompatible race of *P. oryzae* in rice leaf-sheaths. This phenomenon suggests that inducer for hyphal growth exists in the homogenates of rice leaves. The inducer seems to be stable to boil at 100 C for 30 min. Ohata *et al.*[12] reported that hyphal growth of the compatible race in the sheath cells was promoted by the preinfectional treatment of amino acids, sugars and organic acids. As shown in Figs. 3 and 4, active substances could not be extracted by several organic solvents, and were dialyzed by cellulose tubing. The active substance might be low molecular weight substances, such as sugars, amino acids and organic acids in rice leaf homogenates.

Following explanations may be offered for the finding of the effectiveness of rice leaf homogenates to hyphal growth in leaf sheaths. i) The functions of rice plants concerning the blast resistance may be suppressed by the homogenates. According to Tanaka *et al.*[13], host metabolic pathway concerning the resistance could be affected when amino acids and their amides were accumulated abnormally into the rice tissues. On the other hand, it is well known that the application of ammonium sulphate always reduces the puncture resistance of the leaf epidermis as well as the resistance to blast fungus[14]. ii) Oku and Sumi[15] reported that the boiling water extracts of rice plants increased the ability of the mechanical penetration of rice blast fungus. Recently, we demonstrated that *P. oryzae* releases the toxic substances with infection-inducing ability in spore-germinating fluids[16,17]. Therefore, the possibility that a stimulation of the pathogenicity expression of *P. oryzae* (*e.g.* ability of the mechanical penetration or toxin-productivity) occurs as a result of leaf homogenates infiltration is not excluded. iii) A final and perhaps more likely explanation, as observed by Ohata *et al.*[12] for rice blast infection, could be that the homogenates from rice leaves serve as a nutrient for supporting the growth of the invading hyphae.

On the other hand, our earlier reports[5,6,18] demonstrated that the incompatible race of *P. oryzae* and nonpathogens colonized in leaf-sheaths which had been treated with cycloheximide or heat-shock, and inoculated with the compatible race of *P. oryzae*. In general, it is well known that susceptibility of plant tissues induced by the infection of pathogens or abiotic treatments were effective in promoting additional development not only of avirulent isolates of the pathogens but also of different non-pathogenic fungus[19-24]. However, the results described in this paper show that the preinfectional treatment of the rice leaf homogenates promoted significantly hyphal growth of the incompatible race of *P. oryzae*, but not in non-pathogenic blast fungus. Phenomenon observed in this study somewhat differs from those on induced-susceptibility reported previously[5,6,18]. The present investigation suggests that there

is difference in susceptibility induced in the rice cultivars by rice leaf homogenates between host and non-host interactions with *Pyricularia* spp. Therefore, although above discussion can elucidate the hyphal growth of *P. oryzae*, but not poor hyphal growth of non-pathogenic *Pyricularia* spp. Many investigations are as yet necessary for elucidation of this phenomenon. This study may be useful for elucidation of specific susceptibility of host plants in rice blast disease.

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Literature cited

1) DOKE, N., TOMIYAMA, K. and FURUICHI, N. : *In* Plant Infection : The Physiological and Biochemical Basis (Asada *et al. eds.*). Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin, 1982, pp. 79-96.
2) HEATH, M.C. : Phytopathology, **70** : 356-360, 1979.
3) 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.
4) SHIRAISHI, T., OKU, H., YAMASHITA, M. and OUCHI, S. : Ann. Phytopath. Soc. Japan, **44** : 659-665, 1978.
5) ARASE, S. and ITOI, S. : *Ibid.*, **47** : 269-271, 1981.
6) ARASE, S., TANISHIKI, M. and ITOI, S. : *Ibid.*, **48** : 544-546, 1982.
7) ARASE, S. and ITOI, S. : *Ibid.*, **49** : 385-386 (Abstr.), 1983.
8) ARASE, S., KOHZU, Y. and ITOI, S. : *Ibid.*, **50** : 125 (Abstr.), 1984.
9) ARASE, S., KATSUTA, M. and ITOI, S. : *Ibid.*, **49** : 698-703, 1983.
10) TAKAHASHI, Y. : Hokkaido Pref. Agr. Exp. Sta. Rept., **3** : 1-61, 1951.
11) TAKAHASHI, Y. : Yamagata Univ. Agric. Sci. Bull., **2** : 37-51, 1956.
12) OHATA, K., GOTO, K. and KOZAKA, T. : Bull. Natl. Inst. Agric. Ser. C, **20** : 1-65, 1966.
13) TANAKA, S. : *In* The Rice Blast Disease (Hopkins, J. *eds.*). The Johns Hopkins Press, Baltimore, Maryland, 1963, pp. 23-33.
14) SUZUKI, N. : *In* The Rice Blast Disease (Hopkins, J. *eds.*). The Johns Hopkins Press, Baltimore, Maryland, 1963, pp. 277-301.
15) OKU, H. and SUMI, H. : Ann. Phytopath. Soc. Japan, **34** : 250-254, 1968.
16) ARASE, S., KINOSHITA, S., NOZU, M., TANAKA, E. and NISHIMURA, S. : *Ibid.*, **52** : 129 (Abstr.), 1986.
17) KINOSHITA, S., ARASE, S., NOZU, M., TANAKA, E. and NISHIMURA, S. : *Ibid.*, **52** : 529 (Abstr.), 1986.
18) ARASE, S. and OKA, T. : *Ibid.*, **51** : 490-493, 1985.
19) CHAMBERLAIN, D.W. : Phytopathology, **62** : 645-646, 1972.
20) CHAMBERLAIN, D.W. and GERDEMAN, J.W. : *Ibid.*, **56** : 70-73, 1966.
21) HEATH, M.C. : Physiol. Plant. Pathol., **15** : 211-218, 1979.
22) KUNOH, H., HAYASHIMOTO, A., HARUI, M. and ISHIZAKI, H. : *Ibid*, **27** : 43-54, 1985.
23) OUCHI, S. and OKU, H. : *In* Plant Infection : The Physiological and Biochemical Basis (Asada, T. *et al. eds.*), Japan Sci. Soc. Press, Toyo/Springer-Verlag, Berlin, 1982, pp. 117-136.
24) TANI, T., OUCHI, S.. ONOE, T. and NAITO, N. : Phytopathology, **65** : 1190-1193, 1975.

摘　　要

イネ品種愛知旭と関東51号の葉鞘にイネ品種新2号と愛知旭の葉身磨砕液をあらかじめ減圧浸透処理しておくと，非親和性イネいもち病菌レースの菌糸伸展度は著しく増加した．しかし，イネ葉鞘に蒸留水を処理後非親和性イネいもち病菌レースを接種すると平均伸展度，最高伸展度共に非常に低い値であった．磨砕液はそれを調製するために用いたイネ品種が磨砕液処理後に接種するイネいもち病菌に対して抵抗性であるか，罹病性であるかに関係なく，非親和性イネいもち病菌レースの菌糸伸展度を増加させた．このような誘導感受性は磨砕液を熱処理（100℃，30分）したものを用いても認められた．一方，熱処理した磨砕液としない磨砕液を葉鞘に処理後，イネに病原性のないいもち病菌を接種すると菌糸伸展度は蒸留水処理した葉鞘における値とほぼ同じで，平均，最高伸展度共に低い値であった．