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Protection of Broad Bean against Chocolate or Red Spot by Several *Pyricularia* spp.

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いもち病菌によるソラマメ赤色斑点病の交叉防御

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Effect of pre-inoculation with *Pyricularia* spp. on lesion formation of *Botrytis fabae* Sardina in broad bean was observed. When a spore suspension (10^6 spores/ml) of *Pyricularia oryzae* Cavara was previously inoculated to broad bean plant, the size and number of lesions by *B. fabae* in the pre-inocolated leaves were significantly reduced as compared with those in the uninoculated leaves (control). Such phenomenon was observed in broad bean leaves previously inoculated with isolates of *Pyricularia* sp. from rice, crabgrass, Italian ryegrass or mioga plants. However, protection was not induced in broad bean, when spores of *P. oryzae* (isolate Hoku 1) which had been heated at 100 °C for 60 hr were pre-inoculated. The fact that *Pyricularia* spp. can elicit highly effective protection against *B. fabae*, lead to the conclusion that *Pyricularia* spp. are useful tools for the practical control of plant diseases.

INTRODUCTION

It is well known that preliminary inoculation with non-pathogens, incompatible races of pathogens and pathogen induces systemic or local protection against pathogens[1−13,16]. This phenomenon was called "induced resistance" and was observed in many plant diseases. Particularly, many researches on induced resistance by Kuć and co-workers are well known[2,3,4,5,7,8]. They reported that infection of cucumber with *Colletotoricum lagenarium* induced systemic protection against disease caused by subsequent inoculation with the pathogen. Elucidation of this phenomenon is useful to understand the defence mechanism of plants to the pathogens. On the other hand, much information on such induced resistance offered the possibilty of establishment of new techniques for plant disease control. In fact, Ogawa *et al.*[14,15] established a technique of the practical control against Fusarium wilt of sweet potato by utilizing a non-pathogenic isolate of *Fusarium oxysporum* as a inducer of systemic protection.

In this paper, we report the resistance to *Botrytis fabae* Sarbina is induced by pre-inoculation of *Pyricularia* spp., which are non-pathoges, in leaves of broad bean plants.

MATERIALS AND METHODS

Plant. Seeds of broad bean plant (*Vicia fabae* L., cvs. Umami-nagazaya-soramame

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and Taitou) were immersed in distilled water for 24 hr and then germinated on moist papers. Germinated seeds were planted in pots. Plants were grown in greenhouse.

Fungi and inoculation. *Pyricularia grisea* (Cooke) Sacc., *P. oryzae* Cavara (isolates Hoku 1 and Naga 87), and *Pyricularia* sp. from Italian ryegrass and rice plants were cultured on oatmeal medium at 26 °C for about 14 days. After removal of aerial hyphae, cultured plates were kept under a BLB (FL 40 S, BLB 40 W, Toshiba) light irradiation for 2 days. Spores formed synchronously were used in this study. *Botrytis fabae* was cultured on V-8 juice medium at 15 °C for about 10 days irradiating a BLB light. Spores were then harvested by blushing the mycelial surface and flushing with distilled water. The resultant suspension was then filtrated through 4 layers of tissue paper to collect only spores and eliminate mycelial fragments. Spore suspension was then centrifuged at 2000 rpm for 5 min. The sediments from *Pyricularia* spp. and *B. fabae* were re-suspended in distilled water to give a final concentration of 10^6 and 10^5 spores/ml, respectively.

Detached leaf of broad bean was cut in half at midrib, and one was inoculated with a spore suspension (10^6 spores/ml) of several *Pyricularia* spp. on the under surface and the other was sprayed with distilled water as control. Both the uninoculated and inoculated broad bean leaves were maintained in the moist chambers at 26 °C for 24 hr, and then inoculated with a spore suspension (10^5 spores/ml) of *B. fabae* after the air-dryness of the leaf surface. Inoculated leaves were replaced in the moist chambers at 15 °C for 24 hr in the dark and then transferred under light irradiation at 15 °C. Lesion formation by *B. fabae* was observed 3 days after the secondary inoculation.

RESULTS AND DISCUSSION

There were large differences in the size and number of lesions between the pre-inoculated and uninoculated leaves. When the leaves of broad bean which had been sprayed with distilled water for 24 hr were infected by *B. fabae*, abundant necrotic

Table 1. Effect of pre-inoculation with isolate Hoku 1 of *P. oryzae* on lesion formation by *B. fabae* in broad bean leaves[a)]

Treatment or inoculation	Number of lesions per leaf[b)]
Water ——→*B. fabae*	122.3
P. oryzae ——→*B. fabae*	31.0

a) Detached broad bean leaves were inoculated with a spore suspension (10^6 spores/ml) of *P. oryzae* or treated with distilled water and both the leaves were kept at 26 °C in the moist chambers. After 24 hr, both the leaves were re-inoculated with *B. fabae* spores (10^5 spores/ml). The number of lesions (more 0.5 mm in diameter) was investigated 5 days after the secondary inoculation.

b) Average of three replications.

spots were formed and about 50% of those were more 1.0 mm in diameter. However, when the leaves which had been inoculated with a spore suspension (10^6 spores/ml)

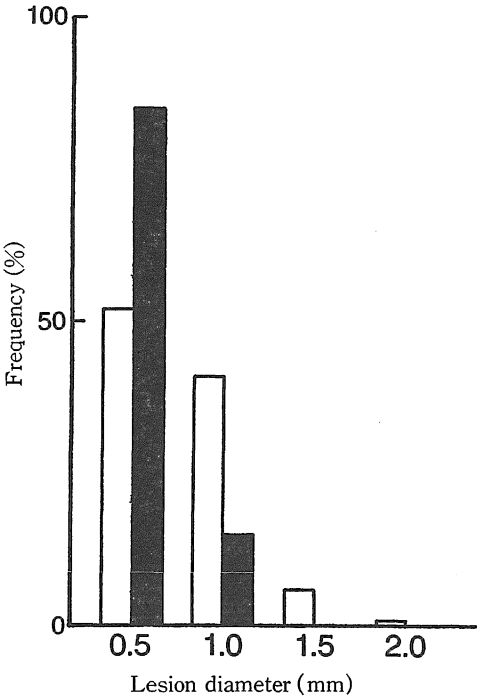

Fig. 1. Effect of pre-inoculation with *P. oryzae* on lesion development by *B.fabae* on broad bean leaves. Detached broad bean leaves were inoculated with *P. oryzae* (■) or treated with distilled water (□). After 24 hr, both the infected and uninfected leaves were re-inoculated with *B. fabae*. Lesion size was investigated 5 days after the reinoculation.

of *P. oryzae* (isolate Hoku 1) for 24 hr were secondarily inoculated with *B. fabae* (10^5 spores/ml), the size and number of lesions were significantly reduced as compared with those in the uninoculated leaves (control) (Fig. 1 and Table 1). Such phenomenon was observed in broad bean leaves previously inoculated with isolates of *Pyricularia* sp. from rice, crabgrass, Italian ryegrass or mioga plants (Table 2). Spores of *B. fabae* germinated and formed appressoria well on the pre-inoculated broad bean. No antibiosis was also observed in confronting plate culture (PSA) between *P. oryzae* and *B. fabae*. Therefore, a reduction in the size and number of lesions not caused by each other's antagonism, but by the resistance induced in the broad bean leaves. We should determine whether protection induced in broad bean is local or systemic in the future. On the other hand, such protection was not induced in broad bean, when spores of *P. oryzae* (isolate Hoku 1) which had been heated at 100 °C for 60 hr were pre-inoculated. This data suggest that physical

Table 2. Effect of pre-inoculation with several *Pyricularia* spp. on lesion formation by *Botrytis fabae* in broad bean leaves

Fungus (original host or isolate)	Number of lesions (spots/cm² leaf)
P. grisea	
(crabgrass)	19.2 ab*)
P. oryzae	
(isolate Hoku 1)	12.3 a
(isolate Naga 87)	8.6 a
Pyricularia sp.	
(mioga)	9.8 a
(Italian ryegrass)	10.2 a
(rice)	11.6 a
Control	30.1 b

Detached broad bean leaf was cut in half at midrib, and one was sprayed water (control) on the under surface and the other was inoculated with a spore suspension (10^6 spores/ml) of several *Pyricularia* spp. Both the inoculated and control leaves were kept in the moist chambers at 26 °C for 24 hr. Each leaf was inoculated with a spore suspension (10^5 spores/ml) of *B. fabae* after air-the dryness of bean surface. Inoculated leaves were re-kept in the moist chambers at 15 °C in the dark and then transferred under light irradiation at 15 °C. Lesion formation by *B. fabae* was observed 3 days after secondary inoculation.

*) Numbers followed by different letters are significantly different ($p<0.05$).

or chemical stimulation from the living pathogen are necessary for induction of protection.

It is well known that the resistance is induced when rice leaves or leaf-sheaths which had been preliminarily inoculated with the incompatible races of *P. oryzae* were re-inoculated with the compatible races[17-20]. Recently, Arase *et al.*[21] demonstrated that pre-inoculation with *P. grisea* induced resistance against *P. oryzae* in rice leaf-sheath cells. Our results and those of others suggest that all the fungi belonging to the *Pyricularia* species seems to possess resistance-inducing ability against not only host plants, but also non-host plants.

The fact that *Pyricularia* spp. can elicit highly effective protection against *B. fabae*, lead to the conclusion that *Pyricularia* spp. are useful tools available for protection of plant diseases.

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摘　　要

ソラマメ葉における赤色斑点病菌（*Botrytis fabae*）の病斑形成に及ぼす *Pyricularia* spp. の前接種の影響を調査した．ソラマメ葉に病原性を示さないイネいもち病菌北1菌株の分生胞子を前接種しておくと，後接種した *B. fabae* による病斑の大きさ及び数が対照区に比べ著しく抑制された．この様な抵抗性の誘導現象は，前接種菌にメヒシバ，イタリアンライグラス及びミョウガから分離したいもち病菌を用いても認められ，前接種葉では赤色斑点病斑の形成及び拡大は，著しく抑制された．しかし，熱処理（60 °C, 1時間）分生胞子を前接種したソラマメ葉では *B. fabae* に対する抵抗性は誘導されなかった．各種いもち病菌がソラマメに *B. fabae* に対する効果的交叉防御を誘導した事実は，いもち病菌が病害防除の有用な材料になり得ることを示した．

Explanation of Plate I

Effect of pre-inocution with several *Pyricularia* spp. on lesion formation of *Botrytis fabae* in broad bean. Broad bean leaves were inoculated with several *Pyricularia* spp. (left) or treated with distilled water (right). Both the inoculated and treted leaves were re-inoculated with *B. fabae* 24 hr after the primary inoculation or treatment. Lesion formation by *B. fabae* was observed 3 days after the secondary inoculation. 1: isolate Naga 87 of *P. oryzae*. 2: isolate Hoku 1 of *P. oryzae*. 3: *P. grisea*. 4-6: *Pyriculara* sp. from mioga, rice and Italian ryegrass, respectively.