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Preinfectional Interactions between *Magnaporthe grisea* Spores and Rice Plants *

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Abstract Preinfectional interactions between *M. grisea* spores and resistant and susceptible rice plants were investigated by the scanning electron microscope. At 2 hr after inoculation, germ tubes emerged mostly from top cells of spores and elongated on cell junctures between the papillae. At this stage, mucilage-like substances which contacted with the cuticle surface were observed on both resistant and susceptible cultivars. Mucilages were observed on the undersides of germ tubes and were often attached to papillae on the leaf surface. Most of immuture appressoria of *M. grisea* were formed 6 hr after inoculation and secreted the mucilages. The wax crystals around appressoria of *M. grisea* disappeared. Above phenomena were observed regardless of the compatibility between *M. grisea* and rice cultivars used. The present study showed that the mucilage and enzyme productions might enable the fungus to attach to the leaf surface and facilitate.

Key words: *Magnaporthe grisea*; rice plant; rice blast; SEM.

Introduction

The ascomycete *Magnaporthe grisea* (Hebert) Barr (imperfect stage; *Pyricularia oryzae* Cavara) causes the devastating plant disease called rice blast. The physiology and pathology of this fungus has been studied by a number of workers (Ou, 1985). Resistance and susceptibility of rice plants to *M. grisea* are determined by race-cultivar combination (Yamada *et al.*, 1979). Evidences on induced resistance and induced susceptibility in rice blast disease suggest that rice cells recognize the pathogen before penetration (Arase and Itoi, 1981; Arase *et al.*, 1990; Arase and Fujita, 1992). Recognition factors in rice blast are not yet fully understood, even though the molecules, such as host-specific toxins, suppressors and elicitors, were demonstrated in the other diseases. So, we started to investigate the early stages of infection process to detect fungal components of recognition factors. One of the early steps in any host-parasite interaction is the attachment to the hosts. However, preinfectional behaviors of *M. grisea* are not yet fully observed on the rice leaf surface, although there are many observations of them on the artificial surfaces (Bourett and Howard, 1989; Howard *et al.*, 1991).

In this study, we report preinfectional interaction between *M. grisea* spores and rice plants.

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Materials and Methods

Plant: Rice cvs. Asahi (resistance gene *Pi-a*) and Kanto 51 (resistance gene *Pi-k*) were grown in the greenhouse as reported previously (Arase *et al.*, 1993).

Fungus: Isolate Naga 69-150 (race 007) of *M. grisea* was grown on oatmeal agar medium for 14 days at 26°C. Spores were synchronously formed by the method reported previously (Arase *et al.*, 1990). This isolate is compatible to rice cv. Asahi, but incompatible to cv. Kanto 51.

Electron microscope: Synchronously-formed spores were inoculated to the leaves of cvs. Asahi and Kanto 51. Inoculated leaves were kept in the moist chambers at 26°C. After 2, 6, 12 and 24 hr, small pieces of leaf tissues (about 3×2 mm) were cut out from inoculated leaves and pre-fixed at 4°C over night in 2.5% glutaraldehyde solution buffered at pH 7.4 with 0.1 M phosphate buffer. After rinsing with a buffer solution, specimens were successively post-fixed at 4°C for 3 hr in 1% OsO_4 solution buffered at pH 7.4 with 0.1 M phoshate buffer. They were washed in the buffer after fixation. The speciemens were dehydrated with ethyl alcohol and dried in a critical-point drying apparatus (Hitachi, model MCP-2) with 3-methylbutyl acetate as the intermediate fluid and liquid carbon dioxide as the transition fluid. All specimens were then mounted on stubs with gold paste and coated with gold-palladium in a sputter coater (Eiko Engineering Co., Ltd., Model IB-3). Examination and micrography were done with an Akashi MSM-30 scanning electron microscope.

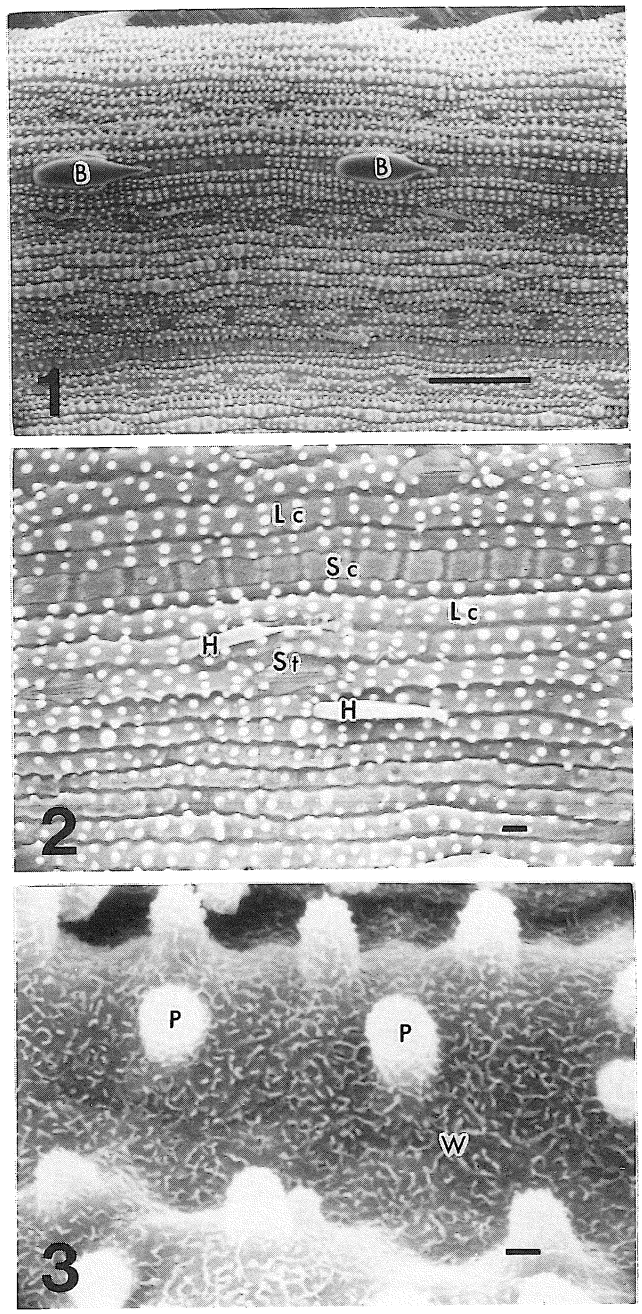
Results and Discussion

Morphology of rice leaf surface

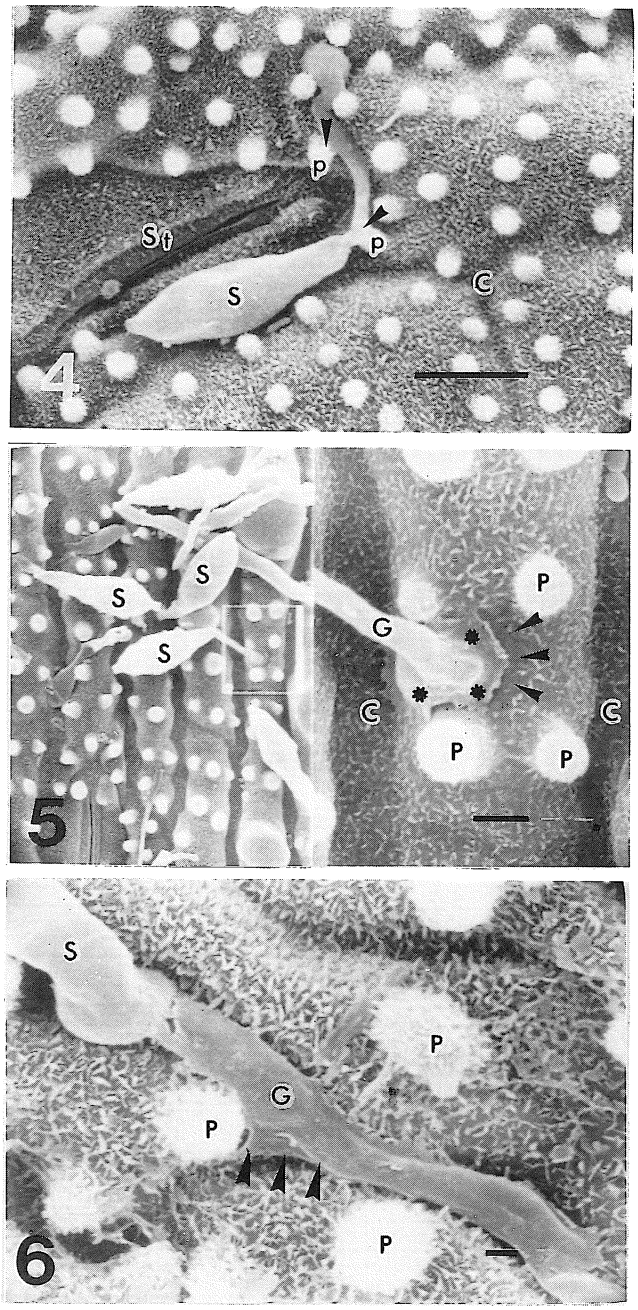
The rice leaf has a typical gramineous epidermis composed of long cells (Lc), short cells (Sc) (Figs. 1 and 2). The rice leaf surface is characterized by the presence of papillae (P). The papillae covered with the wax crystals (W) were observed on the both sides of the leaves (Fig. 3). These are epidermal cell projections arranged in rows along the long axis of the leaf.

Pre-penetration behaviors of M. grisea on the resistant and susceptible rice plants

The early infection process consisted of spore germination, germ tube elongation, formation of immature appressoria and maturation of appressoria. At 2 hr after inoculation, germ tubes emerged mostly from top cells of spores and elongated on cell junctures between the papillae (Figs. 4 and 5). At this stage, mucilage-like substances were observed in both resistant (cv. Kanto 51) and susceptible (cv. Asahi) cultivars. These substances contacted with the cuticle surface (Figs. 4-6). These were observed on the tips (Fig. 5, asterisks) and the undersides (Fig. 6, arrows) of germ tubes where they emerged from the spores were often attached to papillae. Such substances were also observed by interactions between *Cochliobolus miyabeanus* and rice plant (Hau and Rush, 1992). Hamer *et al.* (1988) demonstrated that dormant



Figs. 1-3. Scanning electron micrographs of rice leaf surface. 1: Under surface of cv. Asahi. Bar=100μm. 2: Under surface of cv. Kanto 51. Bar=100μm. 3: Papillae and wax crystals on under surface of cv. Asahi. Bar=1μm. B: Bristle, Lc: Long cell, Sc: Short cell, St: Stoma, H: Hair, P: Papilla, W: Wax crystal.



Figs. 4-6. Germination and germ tube elongation of *M. grisea* on rice leaf surface with papillae and wax crystals. 4: The mucilage-like substance (arrows) was produced around germ tubes and the germ tubes attached to the rice leaf surface with papillae. Bar=100μm. 5: The tip of the germ tube was attached to the papillae with the mucilage-like substance (asterisks) and the wax crystale around the mucilage disappeared. Bar=10μm. 6: The germ tube was attached to the papillae by the mucilages (arrows). Bar=1μm. C: Cell juncture, P: Papilla, S: Spore, G: Germ tube.

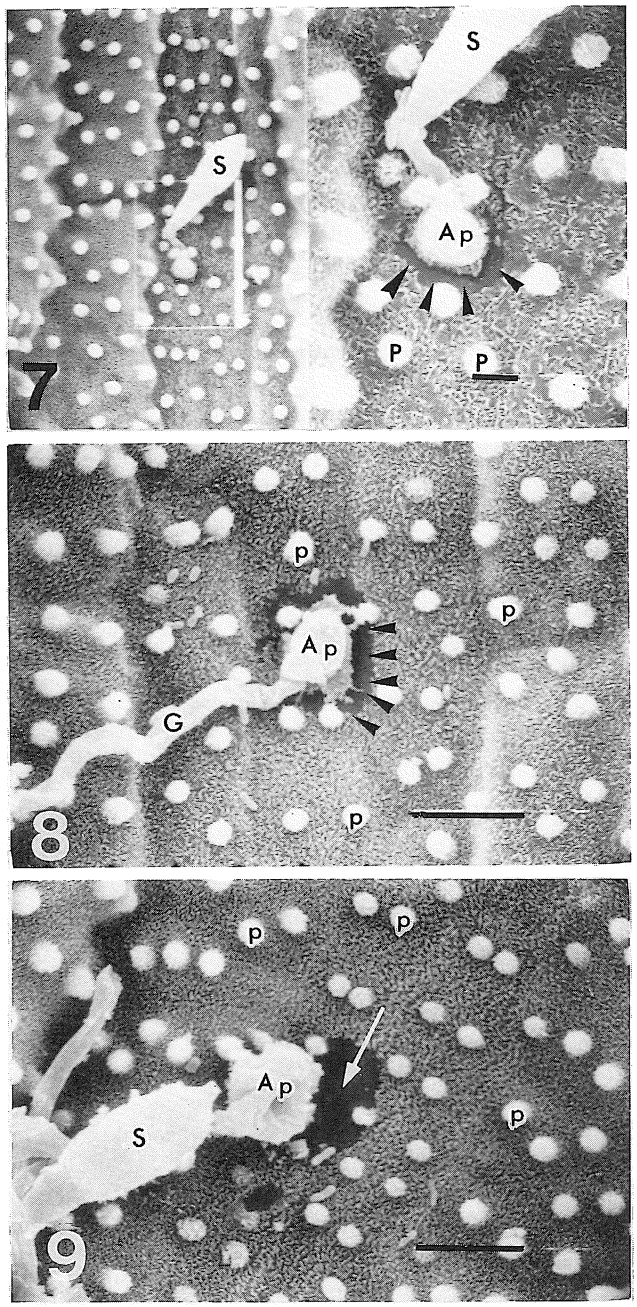


Fig. 7-9. Disapearance of wax crystals around appressoria of *M. grisea* (arrows). G: Germ tube, P: Papilla, S: Spore, Ap: Appressorium. Bar=10μm.

spores of *M. grisea* contained a prominent deposit of material in the periplasmic space at the spore apex for the attachment to a rice leaf surface prior to germ tube emergence and infection structure formation. However, it was not yet demonstrated whether the mucilage at the spore apex and sheath around germ tubes were same or not. Most of immuture appressoria of *M. grisea* were formed 6 hr after inoculation and secreted the mucilages. In the previous studies (Hashioka *et al.*, 1967a, b; Hashioka and Kitano, 1971), an extracellular mucilaginous sheath was observed on the germ tubes and appressria of *M. grisea* by the transmission electron microscope (TEM). Substances we found correspond to the ones on the germ tubes or appressoria at the TEM level. Further, this mucilage production on these fungal structure was observed on the surface of nonhost plants (Hashioka *et al.*, 1967; Hashioka and Ando, 1975) or on the artificial surfaces (Uchiyama *et al.*, 1979). This observation suggests that the mucilage is an essential substance for the attachement to the host surface, although it is not a specific one in rice cultivar-*M. grisea* interaction.

A part of the wax crystals around the hyphal tips of *M. grisea* disappeared 2 hr after inoculation (Fig. 5, arrows). Such phenomenon was also observed around the immature apressoria (Fig. 7, arrows). After 24 hr, mature appressoria with varied size and shapes were observed on cell junctures. Rarely, appressoria were formed on the stomata. The wax crystals around (Fig. 8, arrows) and under (Fig. 9, arrow) the appressoria also disappeared. Above phenomenon were observed regardless of the compatibility between *M. grisea* and rice cultivars used.

These observations suggested that *M. grisea* released the enzymes resolving the wax crystals. It is well known that cuticle surface of rice leaves are covered by waxes and are resistant to wetting. Such nature of rice leaf surfaces is convenient for removing the rain droplets with fungal spores in the fields. The present studies showed that the mucilage and enzyme productions might enable the fungus to attache to the leaf surface and facilitate the infection process.

References

ARASE, S. and K. FUJITA, Induction of inaccessibility to *Pyricularia oryzae* by pre-inoculation of *P. grisea* in rice leaf-sheath cells. J. Phytopathology, **134**: 97-102, 1992.

——— and S. ITOI, Growth of *Alternaria alternata* and incompatible race of *Pyricularia oryzae* CAVARA in rice leaf-sheath previously infected with compatible race. Ann. Phytopath. Soc. Japan, **47**: 269-271, 1981.

———, M. KANO, K. KONDO, M. NOZU, E. TANAKA and S. NISHIMURA, Studies on host-selective infection mechanism of *Pyricularia oryzae* CAVARA (2) Production of susceptibility-inducing factor(s) from germinating spores and their phytotoxicity. Ibid., **56**: 322-330, 1990.

———, K. MIYAKE, Y. HONDA and M. NOZU, Production of anti-fungal substance(s) in the "Sekiguchi lesion" induced by *Pyricularia oryzae* infection on rice cv. Sekiguchi-asahi. Bull. Fac. Agr. Shimane Univ., **27**: 7-14, 1993.

BOURETT, T. M. and R. J. HOWARD, *In vitro* development of penetration structures in the rice blast fungus *Magnaporthe grisea*. Can. J. Bot., **68**: 329-342, 1990.

HAMER, J. E., R. J. HOWARD, F. G. GHUMLEY and B. VALENT, A mechanism for surface

attachment in spores of a plant pathogenic fungus. Science, **239**: 288-290, 1988.

HASHIOKA, Y., H. IKEGAMI, O. HORINO and T. KAMEI, Fine structure of the rice blast. I. Electronmicrographs of *Pyricularia oryzae* and the epidermal cell wall of rice plants. Res. Bull. Fac. Agr. Gif Univ., **24**: 66-77, 1967a.

———, H. IKEGAMI, O. HORINO and T. KAMEI, Fine structure on the rice blast. II. Electronmicrographs of the initial infection. Ibid., **24**: 78-90, 1967b.

——— and N. KITANO, Fine structure of the rice blast. VIII. Structure and ultrastructure of the initial infection of neck-blast. Ibid., **31**: 83-97, 1971.

——— and N. ANDO, Fine structure on the rice blast. 5 XIII. Ultrastructure of *Pyricularia oryzae*-nonhost interfaces. Ibid., **38**: 39-47, 1975.

HAU, F. C. and M. C. RUSH, Preinfectional interactions between *Helminthosporium oryzae* and resistant and susceptible rice plants. Phytopathology, **72**: 285-292, 1982.

OU, S. H., Rice Diseases (2nd ed). Commonwealth Mycological Institute, Kew, England, 380 pp., 1985.

YAMADA, M., S. KIYOSAWA, T. YAMAGUCHI, T. HIRANO, T. KOBAYASHI, K. KUSHIBUCHI and S. WATANABE, Proposal of a new method for differentiating races of *Pyricularia oryzae* CAVARA in Japan. Ann. Phytopath. Soc. Japan, **42**: 216-219, 1976.