Induced Accessibility and Enhanced Inaccessibility at the Cellular Level in Barley

XVI. The Accessibility-associated Factor(s) Induced in Barley Coleoptiles Inoculated with *Blumeria graminis* f. sp. *hordei**

Masao ARAKAWA** and Hitoshi KUNOH***

Abstract

It was examined whether successful infection by *Blumeria* (syn. *Erysiphe*) *graminis* f. sp. *hordei* (a pathogen) induced production of some chemical factors which could explain the accessible state of coleoptile cells of barley. Water-soluble extracts were prepared from coleoptiles 0, 6, 12, 18 hr after inoculation with *B. graminis* and uninoculated coleoptiles. Coleoptiles whose lower surfaces were treated with these extracts, were inoculated with *Erysiphe pisi* (a nonpathogen), and its penetration efficiency (PE) was examined. This nonpathogenic fungus formed haustoria at a significantly high PE level only in the coleoptiles treated with extracts of the *B. graminis*-inoculated coleoptiles prepared at 18 hr after inoculation but not with those extracted earlier than 12 hr. These results suggested that some chemical factors might be produced in coleoptiles by 18 hr after inoculation of *B. graminis*, at the time of incipient development of its haustoria. In thin layer chromatography of the extracts, a bluish fluorescence under UV light and a ninhydrin-positive spot was detected at position $R_f = 0.61$. The fraction containing this spot and two other fractions which were not detected by UV or the ninhydrin reaction had accessibility-inducing activity, suggesting that some of these factors may associate mutually to induce accessibility in coleoptile cells.

(Received November 11, 1998 ; Accepted January 5, 1999)

Key words : accessibility-associated factor(s), barley, *Blumeria* (syn. *Erysiphe*) *graminis* f. sp. *hordei*, powdery mildew.

INTRODUCTION

Some plants preliminarily attacked by a pathogen may later become susceptible to a nonpathogen or an incompatible race of the pathogen^{4,6,9,10,14-16}. This phenomenon is called "acquired or induced susceptibility"^{14-16,21}. Ouchi *et al.*¹⁵ proposed the term accessibility to connote the acceptance-rejection relationship in parasite-plant interactions at the tissues, cellular and molecular level, and reserved the term susceptibility for host-parasite relations at the organ and whole plant levels. In previous papers^{1,6-11}, we demonstrated using the barley coleoptile cell-pathogen (*Blumeria graminis* = *Erysiphe graminis*) and -nonpathogen (*E. pisi*) systems that the preliminary formation of a haustorium by *B. graminis* in a coleoptile cell is a prerequisite for *E. pisi* to form a haustorium in the same cell, and suggested that *B. graminis* might release a suppressor to block the resistance of coleoptiles prior to its actual penetration. However, little information is available about the com-

ponents responsible for the induction of this accessible state in cells^{4,13,17}. This study aims at determining whether *B. graminis* induces the host to produce some chemical factors which could explain the accessible state in coleoptile cells of barley.

MATERIALS AND METHODS

Plant and fungi Barley, *Hordeum vulgare* L. cv. Kobinkatagi, was grown from seed in vermiculite in a growth chamber under fluorescent lights (*ca.* 11.8 Wm⁻²) and a 12-hr photoperiod at 20°C and 70% relative humidity (RH). The coleoptiles were excised from seedlings 8 days after sowing, and layers of the epidermal cells of partially dissected coleoptiles were prepared as described previously²⁰. These dissected coleoptiles are simply called "coleoptiles" hereafter. *Blumeria graminis* de Candolle f. sp. *hordei* (syn. *Erysiphe graminis* f. sp. *hordei*) Em. Marchal, race I (abbreviated *B. graminis* hereafter), maintained on leaves of a compatible barley cv. Kobinkatagi, and *E. pisi* de Candolle, race 1 (ab-

* Contribution No. 143 from the Laboratory of Plant Pathology, Mie University.

** Laboratory of Plant Pathology, Faculty of Bioresources, Mie University, Tsu 514-8507, Japan 三重大学生物資源学部

*** Author to whom correspondence should be addressed.

breviated as *E. pisi*), on the compatible pea, *Pisum sativum* L. cv. Midoriusui were incubated in separate growth chambers with the same environmental conditions.

Evaluation of accessible states of individual cells of coleoptiles induced by preinoculation of *B. graminis* Coleoptiles (A and B coleoptiles) were paired for this experiment. Inner surfaces of A coleoptiles were inoculated, using a paint brush, with about 20 freshly harvested conidia of *B. graminis*. They were then floated with outer surfaces down on deionized water or 0.01 M CaCl₂ solution and incubated at 20±2°C, 70% RH in the dark. B coleoptiles were inoculated similarly with about 50 freshly harvested conidia of *E. pisi* 13 hr after inoculation of the A coleoptiles and incubated for 2 hr under the same conditions as described above. This timing of inoculation on B coleoptiles ensured that germlings of *E. pisi* would reach a stage suitable for transfer to A coleoptile which had been incubating for 15 hr after its inoculation with *B. graminis*. Both A and B coleoptiles were mounted on a glass slide 15 hr after inoculation with *B. graminis*. Germlings of *E. pisi* with an immature appressorial germ tube were individually transferred by micro-manipulation^{1,5-11)} onto cells of the A coleoptile in which *B. graminis* had formed an incipient haustorium (Fig. 1). In this case, only those germlings of *B. graminis* on the A coleoptiles were selected that had no other germlings located within a radius of five host cells. After a further 22 hr of incubation, the A coleoptiles were mounted on a glass slide, and haustorium formation by *E. pisi* in the targeted cells was observed with a light microscope. Penetration efficiency (PE: percentage of haustorium formation) of the transferred *E. pisi* was determined to evaluate the accessible state of coleoptile cells as follows:

PE = (No. of haustoria / Total no. of mature appressoria) × 100 (%). PE of the transferred *E. pisi* in separate coleoptile cells which had not been pre-inoculated with *B. graminis* was determined as a control.

Determination of inoculum density to allow *B. graminis* to form haustoria in individual cells of a single coleoptile

As reported in previous papers^{1,6,9,10)}, when *B. graminis* forms haustoria in coleoptile cells, the cells are directed towards the accessible state. For effectively extracting accessibility-associated factors (AAF) from coleoptiles of these accessible cells, it is desirable to obtain coleoptiles in which *B. graminis* conidia have formed haustoria in as many cells as possible. Thus, the most suitable inoculum density of *B. graminis* was determined prior to extraction of AAF as follows.

Coleoptiles were inoculated with *B. graminis* conidia at densities ranging from 1-150 conidia/mm² with a paint brush. After floating the coleoptiles on deionized water or 0.01 M CaCl₂ and incubating as described for 24 hr, the number of conidia and haustoria formed per mm² of coleoptile were determined by light microscopy.

Extraction of accessibility-associated factor(s)

Coleoptiles were inoculated with a paint brush with conidia of *B. graminis* at a density of about 100 conidia/mm², and incubated on 0.01 M CaCl₂ for 0-18 hr as described. This inoculum density was most suitable for allowing *B. graminis* to form haustoria in the entire coleoptile cells, and 0.01M CaCl₂ was a better medium to induce formation of abundant haustoria than deionized water. At the end of the respective incubation times, 30 inoculated coleoptiles were homogenized in liquid nitrogen. The homogenate was suspended in a mixture of 1 ml deionized water and 2 ml ethyl acetate, and the suspension was centrifuged at 1000×*g* for 10 min. The water phase was recovered, mixed with 1 ml ethyl acetate and centrifuged as before. The final collected water phase was ultrafiltrated (type LCC, <5000, Millipore Co.), and the filtrate was lyophilized as a crude extract of low molecular-weight materials. Dry weight of the extract was determined and kept at -20°C until use.

The activity of the crude extract to induce accessibility in coleoptiles was examined as follows. Three

Fig. 1. Schematic illustration of inoculation, transfer and evaluation of the accessible state of coleoptile cells. *E. pisi* was transferred onto coleoptile cells where *B. graminis* had formed an incipient haustorium. The penetration efficiency of *E. pisi* was determined 37 hr after inoculation with *B. graminis*. ha, haustorium; app, appressorium.

coleoptiles were inoculated with about 50 conidia of *E. pisi* with a paint brush, respectively. They were floated on 300 μ l of various concentrations (0–1.0 mg/ml) of the crude extract dissolved in 0.01 M CaCl_2 and incubated for 24 hr as already described. The PE value of *E. pisi* for each concentration of extract was expressed as a mean of PEs observed on more than 12 coleoptiles. Statistical analysis of PEs was performed by Student's *t*-test.

Fractionation of AAF by thin layer chromatography (TLC) The crude extracts prepared from *B. graminis*-inoculated coleoptiles were dissolved in ultrapure water (0.2 mg/10 μ l), spotted on a silica gel plate (Silicagel 70 FM, Wako Co.) and developed using a solvent system of *n*-butanol : acetic acid : water (3 : 1 : 1, v/v). After drying, the plate was irradiated with ultraviolet light (UV) of 290–320 nm UV-B wavelength light or sprayed with the ninhydrin reagent (Wako Co.) for qualitative assay.

To isolate the AAF, 3 mg of the crude extract prepared from approximately 1000 coleoptiles 18 hr after inoculation with *B. graminis* was developed similarly by TLC. After drying, the plate was cut into 10 parts of equal length between the front and the base line. These parts were designated as fractions 1 to 10 from the base to the front. The silica gel on each part was scratched into separate microtubes and 1 ml of ultrapure water was added to elute the components. After the silica gel was removed by filtration with a Millipore filter (Millipore Co., pore size: 0.22 μ m), the filtrate was lyophilized and kept at -20°C until use after the dry weight was determined. The activity of these fractions to induce accessibility in coleoptiles was determined the PE of *E. pisi* in coleoptiles treated with these fractions as described previously.

RESULTS

Induction of accessibility in coleoptile cells by infection of *B. graminis*

When coleoptiles inoculated with *E. pisi* were floated on deionized water or 0.01 M CaCl_2 , none or only 0.8% appressoria formed haustoria, respectively. However, in

Table 1. Penetration efficiency (PE) of transferred *E. pisi* in coleoptile cells in which preinoculated *B. graminis* had produced haustoria. Coleoptiles were floated on water or 0.01 M CaCl_2

Incubation solution	PE of <i>E. pisi</i> (%)	
	Preinoculated ^{a)}	Control ^{b)}
Deionized water	50.6 $\pm$ 6.0	0.0 $\pm$ 0.0
0.01 M CaCl_2	57.5 $\pm$ 4.0	0.8 $\pm$ 0.7

a) PE of *E. pisi* in coleoptile cells in which the preinoculated *B. graminis* had formed haustoria.

b) PE in coleoptile cells with no previous inoculation of *B. graminis*.

cells of coleoptiles where pre-inoculated *B. graminis* had formed haustoria, *E. pisi* formed haustoria at PEs of 50.6% and 57.5% when the coleoptiles floated on deionized water and 0.01 M CaCl_2 , respectively (Table 1). These results are consistent with those of previous papers^{1,6,9,10}.

Inoculum density of *B. graminis* suitable for haustoria formation in individual cells of a single coleoptile

When coleoptiles inoculated with *B. graminis* were floated on deionized water, no definite correlation was observed between inoculum density and haustoria formation. The density of haustoria was, at most, 40/mm² regardless of the inoculum densities (Fig. 2). On the other hand, when inoculated coleoptiles were floated on 0.01 M CaCl_2 solution, a positive correlation was obtained between the inoculum density and haustorial density ($r=0.95$), and in coleoptiles inoculated with 100 conidia/mm², one or two haustoria were formed in almost all infected cells. Inoculation with more than 100 conidia/mm² increased not only the density of haustoria in the whole area of the coleoptile but also the number of coleoptile cells with more than two haustoria. Such an enhancement of haustorium formation by CaCl_2 was reported previously²⁰.

A further transfer inoculation of *E. pisi* onto the coleoptile cells in which *B. graminis* had formed haustoria proved that more than 50% of the transferred *E. pisi* were able to form a haustorium, regardless of the inoculum density of *B. graminis*, indicating that these cells had been conditioned towards the accessible state (data not shown).

Fig. 2. Relationships between inoculum density and haustoria density of *B. graminis* in cells of coleoptiles floated on deionized water (A) or 0.01 M CaCl_2 (B).

Fig. 3. Penetration efficiency of *E. pisi* in coleoptiles floated on solutions of crude extract prepared from *B. graminis*-inoculated coleoptiles at various times after inoculation. Control, 0.01 M CaCl_2 ; 18u, crude extract prepared from uninoculated coleoptiles after 18 hr incubation.

Fig. 4. TLC-UV profiles of crude extracts prepared from coleoptiles inoculated with *B. graminis*. Inoculated coleoptiles were incubated for indicated times, then subjected to water extraction. 18u, crude extract prepared from uninoculated coleoptiles. Water extracts were developed on TLC with *n*-butanol:acetic acid:water (3:1:1, v/v) solvent and the developed TLC plate was exposed to UV light.

Accessibility-inducing activity of crude extracts from coleoptiles inoculated with *B. graminis*

Figure 3 shows PE of *E. pisi* conidia in coleoptiles treated with crude extracts prepared from coleoptiles various hours after inoculation with *B. graminis*. In coleoptiles inoculated with *E. pisi* and floated on 0.01 M CaCl_2 , only 0.7% of appressoria formed a haustorium (control). When the inoculated coleoptiles were floated on 0.01 M CaCl_2 containing 0.01–1.0 mg/ml of the crude extracts from coleoptiles incubated for 0–12 hr after inoculation with *B. graminis*, PEs of *E. pisi* ranged only from 0.5–1.7%. These values were not significantly different from the PE of the control ($p > 0.05$). However, when *E. pisi*-inoculated coleoptiles were floated on 0.01 M CaCl_2 containing 0.05–1.0 mg/ml of the crude extracts from coleoptiles incubated for 18 hr after inoculation with *B. graminis*, the PE of *E. pisi* increased significantly ($p < 0.05$). Incubation in 0.5 mg/ml gave a PE of 16.4%, the highest induction of accessibility. However, the crude extracts prepared from *B. graminis*-uninoculated coleoptiles after an 18-hr incubation on 0.01 M CaCl_2 did not affect PE of *E. pisi*. These results indicated that AAF was produced in coleoptiles inoculated with *B. graminis* by 18 hr after inoculation but not earlier than 12 hr.

Accessibility-inducing activities of fractionated extracts

Figure 4 shows a UV-detected TLC profile of crude extracts prepared from *B. graminis*-inoculated

Fig. 5. TLC-ninhydrin profiles (A) of crude extracts prepared from *B. graminis*-inoculated coleoptiles, and penetration efficiencies of *E. pisi* in coleoptiles which were incubated with fractionated extracts (B). Extracts were prepared from coleoptiles inoculated with *B. graminis* and incubated for 18 hr. 18u, crude extract prepared from uninoculated-coleoptiles after 18 hr incubation.

coleoptiles at various times after inoculation. R_f values of spots were determined by image analysis (NIH Image, USA). As evident from Fig. 4, several spots with R_f values higher than 0.4 were detected in all extracts regardless of inoculation and uninoculation. Three or more characteristic spots with $R_f=0.3-0.4$ were detected only in the crude extracts prepared from coleoptiles incubated for 14, 16 and 18 hr after inoculation. A bluish fluorescent spot was detected at $R_f=0.61$, specifically in the extract prepared from coleoptiles incubated for 18 hr after inoculation.

The ninhydrin test applied to the same TLC plate revealed a positive reaction in this unique spot ($R_f=0.61$ by UV) (Fig. 5-A). Several other positive spots were detected commonly in crude extracts from uninoculated and inoculated coleoptiles, regardless of the time after inoculation.

Subsequently, ten fractions which had been extracted from evenly cut pieces of the TLC plate were subjected to the test for accessibility-inducing activities. In this test, only the crude extract (0.1 mg/ml) prepared from coleoptiles inoculated with *B. graminis* and incubated for 18 hr were used. As indicated in Fig. 5-B, fractions 2, 7 and 8 had significant activity in promoting *E. pisi* infection, although PEs (4.7-5.9%) were much lower than those shown in Fig. 3. Fraction 4 and 7 included the spots of $R_f=0.3-0.4$ and $R_f=0.61$ which were detected by UV irradiation, respectively (Fig. 3).

DISCUSSION

Our previous studies^{1,6,9,10)} demonstrated that once *B. graminis* formed haustoria in cells of coleoptiles, these cells were conditioned towards the accessible state, enabling challenging *E. pisi* to form haustoria. However, these cytological works did not reveal qualitative and quantitative characters of some accessibility-associated factor(s) (AAF). In the present study we demonstrated that some AAF of low molecular weight (<5000) were produced in barley coleoptiles which had been inoculated with *B. graminis* and incubated for 18 hr to secure haustorial formation of the fungus (Fig. 3). The water-soluble crude extracts containing AAF permitted more than 16% of *E. pisi* to form haustoria in coleoptiles of barley. Several fractions of the extracts on TLC significantly promoted successful infection by *E. pisi* (Fig. 5). Ouchi *et al.*^{15,16)} demonstrated that within 18 hr after inoculation a compatible race of *B. graminis hordei* conditioned barley leaves to be accessible not only to incompatible races of the fungus but also to wheat and melon powdery mildew fungi. Hence, accessibility might be induced immediately after the formation of primordial haustoria, perhaps after the translocation of the fungal nucleus into the haustoria. In a preliminary experiment, we confirmed that in coleoptiles *B. graminis* attempted to breach cell walls by 10-11 hr after inoculation, formed spherical primordia of haustoria by 14 hr and initiated short finger-like projections from these

primordia by 16-18 hr on average. Thus, the induction of AAF in *B. graminis*-infected coleoptiles may be related to incipient development of haustoria. Several spots having $R_f=0.3-0.4$ were specifically detected under UV light on TLC plate which had been developed with the crude extract prepared from *B. graminis*-inoculated coleoptiles 14-18 hr after inoculation (Fig. 4). In the crude extract prepared at 18 hr, only the spot at $R_f=0.61$ showed a unique, bluish fluorescence under UV light. At the same position, an orange, ninhydrin-positive spot was detected (Fig. 5). Such a unique spot was detected only in the crude extracts prepared at 18 hr after inoculation, but not in those prepared earlier. As indicated in Fig. 3, the AAF that enhanced the *E. pisi* infection was present in the crude extracts prepared from coleoptiles at 18 hr after inoculation. Among the fractions including UV-detected specific spots of $R_f=0.3-0.4$ and 0.61 on TLC plate in which the crude extracts prepared at 14-18 hr after inoculation were developed, only fraction 7 containing the $R_f=0.61$ spot had accessibility-inducing activity (Fig. 5-B). Thus, the component(s) contained in this unique spot might be a candidate for the AAF. Fractions 2 and 8 which did not contain the UV- and/or ninhydrin-detectable spots also had accessibility-inducing activity (Fig. 5). Therefore, some components related to these fractions may have a mutual association with the components in fraction 7 to the induction of accessibility. Further studies are required for identification of the AAF molecules.

Heath⁴⁾ extracted intercellular fluids from leaves of French bean, cowpea and sunflower inoculated with the respective pathogenic rust fungi, injected the respective fluids to leaves of these three plants, then inoculated the leaves with the respective nonpathogenic rust fungi. Injection of extracts from susceptible rust-infected leaves of French bean had no effect on subsequent haustorium formation by the sunflower rust fungus in French bean or cowpea or by the cowpea rust fungus in sunflower. However, these extracts did increase haustorium production by the bean rust fungus in cowpea and by the cowpea rust fungus in bean; the effective components were of low molecular weight and partially heat labile. These data demonstrated that some components that enhanced infection by nonpathogenic fungi were released into the intercellular spaces of susceptible rust-infected plants. These components were assumed to be produced by the fungi in their host plants. Similarly, Ouchi *et al.*¹³⁾ floated abaxial epidermis-detached barley leaves on water, which had been inoculated with a compatible race of *B. graminis hordei* on the adaxial surface, and demonstrated the diffusion of low molecular-weight factors from the leaves into water, which significantly increased the infection frequency of *B. graminis tritici* on barley leaves floated on the diffusion extract solution. The present results were similar to these previous reports in many respects, but it was impossible to determine whether the AAF originated from the fungus and/or coleoptile cells, because the

crude extracts containing the AAF were prepared from *B. graminis*-infected coleoptiles. A further study is needed to clarify this question. Shiraishi *et al.*¹⁷⁾ extracted an infection-enhancing factor (IEF) from healthy barley seedlings, and demonstrated that penetration efficiency of the compatible and incompatible races of *B. graminis* markedly increased on barley leaves treated with this IEF. However, this IEF had no activity in allowing the nonpathogen *E. pisi* to form haustoria in leaves of barley. Thus, their IEF and our AAF are probably different when their specificity to host and nonhost plants are considered.

To date various components which direct plant tissues and organs towards the susceptible or accessible conditions have been reported: an ethyl acetate-soluble fraction of spore germination fluids of *Pyricularia oryzae*^{2,3)}, a suppressor of *Mycosphaerella pinodes*^{18,19)} and AK-toxin of *Alternaria kikuchiana*¹²⁾. These factors are all fungal products of low molecular weight. When plant tissues are treated exogenously with these factors, the tissues are directed towards the susceptible condition to accept infection by nonpathogens and incompatible races of pathogens. Thus, they may be called "the accessibility- or susceptibility-inducing factors (AIF or SIF)". The putative components in the current study, however, were extracted from coleoptiles inoculated with *B. graminis*. At present we cannot determine whether the accessible condition was newly induced in coleoptiles treated with these extracts or the accessible factors which had been induced in the *B. graminis*-infected coleoptiles were simply transferred into the coleoptiles by treatment with the extracts. Thus, we tentatively call the putative components AAF instead of AIF in this study.

Literature cited

1. Arakawa, M., Suzuki, S. and Kunoh, H. (1997). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. XV. Interference of AOA and AOPP with the establishment of accessibility. *Physiol. Mol. Plant Pathol.* 51: 227-241.
2. Arase, S. and Itoi, S. (1981). Growth of *Alternaria alternata* and incompatible race of *Pyricularia oryzae* Cavara in rice leaf-sheath previously infected with compatible race. *Ann. Phytopathol. Soc. Jpn.* 47: 269-271.
3. Arase, S., Kinoshita, S., Kano, M., Nozu, M., Tanaka, E. and late Nishimura, S. (1990). Studies on host-selective infection mechanism of *Pyricularia oryzae* Cavara (2). Production of susceptibility-inducing factor(s) from germinating spores and their phytotoxicity. *Ann. Phytopathol. Soc. Jpn.* 56: 322-330.
4. Heath, M.C. (1980). Effects of infection by compatible species or injection of tissue extracts on the susceptibility of nonhost plants to rust fungi. *Phytopathology* 70: 356-360.
5. Komura, T., Kobayashi, I., Yamaoka, N. and Kunoh, H. (1990). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. VIII. Cytological evidence for suppressor(s) of host inaccessibility from *Erysiphe graminis*. *Physiol. Mol. Plant Pathol.* 37: 409-416.
6. Kunoh, H., Hayashimoto, A., Harui, M. and Ishizaki, H. (1985). Induced susceptibility and enhanced resistance at the cellular level in barley coleoptiles. I. The significance of timing of fungal invasion. *Physiol. Plant Pathol.* 27: 43-54.
7. Kunoh, H., Katsuragawa, N., Yamaoka, N. and Hayashimoto, A. (1988). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. III. Timing and localization of enhanced inaccessibility in a single coleoptile cell and its transfer to an adjacent cell. *Physiol. Mol. Plant Pathol.* 33: 81-93.
8. Kunoh, H., Komura, T., Kobayashi, I. and Yamaoka, N. (1990). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. VII. Enhancement of inaccessibility at the prepenetration stage of a nonpathogen. *Physiol. Mol. Plant Pathol.* 37: 399-407.
9. Kunoh, H., Kuroda, K., Hayashimoto, A. and Ishizaki, H. (1986). Induced susceptibility and enhanced resistance at the cellular level in barley coleoptiles. II. The timing and localization of induced susceptibility in a single coleoptile cell and its transfer to an adjacent cell. *Can. J. Bot.* 64: 889-895.
10. Kunoh, H., Kuroda, K., Toyoda, K., Yamaoka, N. and Kobayashi, I. (1991). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. IX. Accessibility induced by *Erysiphe graminis* which promotes the subsequent infection of challenging *E. graminis*. *Ann. Phytopathol. Soc. Jpn.* 57: 57-60.
11. Kunoh, H., Toyoda, K., Yamaoka, N. and Kobayashi, I. (1989). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. V. Duration of stimulus by a non-pathogen in relation to enhanced inaccessibility. *Physiol. Mol. Plant Pathol.* 35: 507-518.
12. Otani, H., Nishimura, S., Kohmoto, K., Yano, K. and Seno, T. (1975). Nature of specific susceptibility to *Alternaria kikuchiana* in Nijisseiki cultivar among Japanese pears (V) Role of host-specific toxin in early step of infection. *Ann. Phytopathol. Soc. Jpn.* 41: 467-476.
13. Ouchi, S., Inamori, R., Shimizu, A., Shiraishi, T. and Oku, H. (1980). Accessibility inducing factors in powdery mildew-infected barley leaves. *Ann. Phytopathol. Soc. Jpn.* 46: 382 (Abstract in Japanese).
14. Ouchi, S. and Oku, H. (1982). Physiological basis of susceptibility induced by pathogens. In *Plant Infection: The Physiological and Biochemical Basis* (Asada, Y. *et al.* eds.), Japan Scientific Societies Press, Tokyo, pp. 117-136.
15. Ouchi, S., Oku, H., Hibino, C. and Akiyama, I. (1974). Induction of accessibility and resistance in leaves of barley by some races of *Erysiphe graminis*. *Phytopathol. Z.* 79: 24-34.
16. Ouchi, S., Oku, H., Hibino, C. and Akiyama, I. (1974). Induction of accessibility to a nonpathogen by preliminary inoculation with a pathogen. *Phytopathol. Z.* 79: 142-154.

17. Shiraishi, T., Miyazaki, T., Yamada, T., Oku, H. and Ouchi, S. (1989). Infection enhancing factor for *Erysiphe graminis* prepared from healthy barley seedlings. Ann. Phytopathol. Soc. Jpn. 55 : 357-360.
18. Shiraishi, T., Yamada, T., Ichinose, Y., Kiba, A. and Toyoda, K. (1997). The role of suppressors in determining host-parasite specificities in plant cells. Int. Rev. Cytol. 172 : 55-93.
19. Shiraishi, T., Yamada, T., Oku, H. and Yoshioka, H. (1991). Suppressor production as a key factor for fungal pathogenesis. In Molecular Strategies of Pathogens and Host Plants (Patil, S. S. *et al.* eds.), Springer-Verlag, New York, pp. 151-162.
20. Takamatsu, S., Ishizaki, H. and Kunoh, H. (1978). Cytological studies of early stages of powdery mildew in barley and wheat. V. Effects of calcium on the infection of coleoptile of barley by *Erysiphe graminis hordei*. Can. J. Bot. 56 : 2544-2549.
21. Yarwood, C.E. (1959). Predisposition. In Plant Pathology, Vol. 1, The Diseased Plant (Horsfall, J.G. and Dimond, A.E. eds.), Academic Press, New York, pp. 521-562.

和 文 摘 要

荒川征夫・久能 均：オオムギ子葉鞘細胞における誘導受容性および拒否性 (XVI) うどんこ病菌 *Blumeria graminis* f. sp. *hordei* 接種子葉鞘から抽出された受容性関連因子

Blumeria (syn. *Erysiphe*) *graminis* f. sp. *hordei* (病原菌) がオオムギ子葉鞘細胞に感染する過程で起こる、受容性誘導に関連する化学的因子について検討した。*B. graminis* 接種後 0, 6, 12, 18 時間目および非接種子葉鞘から低分子水溶性の粗抽出物を調製した。異なる濃度の粗抽出物液で処理した子葉鞘における *E. pisi* (非病原菌) の吸器形成率を調べ、それぞれの抽出物の受容性誘導活性を検定した。その結果、*B. graminis* 接種後 18 時間目の粗抽出物で処理した子葉鞘でのみ 16% 以上の *E. pisi* が吸器を形成し、高い活性が認められた。この結果から *B. graminis* 接種後 18 時間目までに子葉鞘中に受容性誘導に関連する化学的因子が生産されていることが示された。粗抽出物を薄層クロマトグラフィーで分画したところ、接種後 18 時間目の抽出物中に、紫外線照射およびニンヒドリンに陽性反応を示す特異的な因子 ($R_f=0.61$) が存在することが明らかになった。この因子を含む画分およびその他 2 画分からの溶出物には受容性誘導活性が認められた。これらの画分に含まれるいくつかの因子が受容性誘導に複合的に関与していると考えられた。