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**

Hitoshi KUNOH**, Katsutoshi KURODA**, Kazuhiro TOYODA**,
Naoto YAMAOKA** and Issei KOBAYASHI**

Key words: *Erysiphe graminis*, induced accessibility.

Prior attack of plants by a nonpathogen or an incompatible race of pathogen often triggers a series of biochemical changes which lead to resistance to a compatible pathogen at the interaction site. Conversely, plants preliminarily attacked by a pathogen become susceptible to a nonpathogen or an incompatible race of the pathogen. These induced resistance and susceptibility are thought to help the exploitation of molecular mechanisms of mutual recognition between microbes and plants⁷⁾. In our laboratory, these induced phenomena at the cellular level have been investigated using barley coleoptile cell-*Erysiphe graminis* and *E. pisi* systems²⁻⁶⁾, and we reported that the accessible state of barley coleoptile cells induced by prior penetration of *E. graminis* was effective to enhance the subsequent infection of a challenging *E. pisi* at the same cells.

In this study, we elucidated whether such a cellular state induced by *E. graminis* would be effective to promote the subsequent infection by a separate, challenging *E. graminis* at the same cells. In this paper, the term accessibility is used to denote the acceptance state of cells, and the term inaccessibility for the rejection state of cells, according to the concept proposed by Ouchi *et al.*⁸⁾ and Kunoh *et al.*⁵⁾

Barley (*Hordeum vulgare* L. cv. Kobinkatagi) and *Erysiphe graminis* f. sp. *hordei* race I (compatible to Kobinkatagi) were used as a test plant and a fungus, respectively. The fungus was maintained on cv. Kobinkatagi in a chamber at 20°C, 70% relative humidity (RH) under 4,000 lux of fluorescent light for 12 hr per day. Coleoptiles were excised from barley seedlings 9 days after sowing, and single cell epidermal layers of partially dissected coleoptiles were prepared as previously described⁹⁾. Coleoptiles A and B were inoculated with 15-20 and about 50 freshly harvested conidia with a brush, respectively, and then were floated on 0.01 M CaCl₂ solution in petri dishes. The timing of the inoculation to each coleoptile was arranged to ensure that conidia reached the stages of penetration at the time intervals indicated below. Inoculated coleoptiles floated in petri dishes were incubated under the same conditions as above but in a separate growth chamber. Coleoptile A was mounted in 0.01 M CaCl₂ on a glass slide 5.0-5.5 hr after inoculation, and several germlings with appressorial germ tubes (G₁) were selected with a light microscope such that no other germlings were located within 5 host cells. Five hours

* Contribution No. 102 from the Laboratory of Plant Pathology, Mie University. This research was supported in part by a Grant-in-Aid for Co-operative Research Nos. 60304022 (1985, 1986), 62304015 (1987, 1988) and 01304014 (1989, 1990) from the Ministry of Education, Science and Culture of Japan.

** Faculty of Bioresources, Mie University, Tsu 514, Japan. 三重大学生物資源学部

after inoculating coleoptile B, conidia that had an appressorial germ tube (G_2) were individually transferred with a micromanipulator from coleoptile B to coleoptile A so that transferred G_2 could attempt to penetrate the same cell as an appressorium of an untransferred G_1 . By observing at $\times 200$ magnification every 15 min, the times of initiation of cytoplasmic aggregation (corresponding to those of attempted penetration) was recorded for the respective conidia of each pair. After the initiation of cytoplasmic aggregation had been observed, coleoptile A was incubated for additional 12 hr, and the rate of haustorium formation (penetration efficiency: PE) of G_2 was determined.

Using other pairs of coleoptiles, germinating conidia (G_3) from coleoptile B were transferred onto cells of coleoptile A which were laterally adjacent to cells on which G_1 had formed an appressorial apex. The following incubation and observation were carried out as above.

To examine any deleterious effects that micromanipulation might have on the growth of the conidia, germinating conidia on coleoptile B were transferred onto uninoculated coleoptile C. After 20 hr incubation, the presence or absence of haustoria formed below appressoria was used to judge normality.

Data were accumulated from more than 100 separate, but replicate, experiments performed over a period of one year. During manipulation and observation, the microscope room was maintained at $20 \pm 2^\circ\text{C}$ and 70% RH. Differences in PE between groups were analyzed by the χ^2 test.

PE of transferred conidia on coleoptile C (control) was 62.8% on average. When G_1 had attempted penetration less than 5 hr earlier than G_2 and successfully formed haustoria in cells of coleoptile A, 122 out of 152 G_2 (80.3%) also produced haustoria in the same cells (Table 1). Moreover, when G_1 had attempted penetration more than 5.25 hr earlier than G_2 and formed haustoria, PE of G_2 was enhanced to 93.2% (110 out of 118). These PEs of G_2 were significantly higher than that of control ($P < 0.01$). These results evidently indicate that the accessibility in host cells induced by prior attack of an inducing *E. graminis* is effective to enhance the subsequent infection of another challenging *E. graminis* as well as that of *E. pisi*, a nonpathogen to barley^{2,5}. On the other hand, when G_1 had failed in penetration on coleoptile A, only 10 out of 177 G_2 (5.6%) formed a haustorium regardless of the time intervals between attempted penetrations of both fungi (Table 1). These results are consistent with previous results^{2,5} that

Table 1. Effects of time interval between initiation of cytoplasmic aggregates by an inducing (G_1) and a challenging *E. graminis* (G_2) in a single coleoptile cell on haustorium formation (penetration efficiency: PE) of G_2

Interval between initiation of cytoplasmic aggregates induced by G_1 and G_2 ^{a)}	No. of haustoria/Total No. of G_2 and PE (%) at the cells where G_1 had produced or induced:	
	Haustroria	Papillae
0.00- 1.00 (hr)	49/53	5/29
1.25- 2.00	21/28	0/13
2.25- 3.00	15/23	0/13
3.25- 4.00	20/27	0/9
4.25- 5.00	17/21	0/9
5.25- 6.00	14/15	0/8
6.25- 7.00	12/13	0/10
7.25- 8.00	11/12	0/5
8.25- 9.00	15/15	1/11
9.25-10.00	14/16	2/19
10.25-11.00	13/14	0/18
11.25-12.00	24/25	2/21
12.25-14.00	7/8	0/12

a) Penetration attempt by G_1 occurred earlier than that of G_2 .

Table 2. Effects of time interval between initiation of cytoplasmic aggregates induced by an inducing (G_1) and a challenging *E. graminis* (G_3) on penetration efficiency of G_3 on laterally adjacent coleoptile cells of barley

Interval between initiation of cytoplasmic aggregates induced by G_1 and G_3 ^{a)}	No. of haustoria/Total No. of G_3 and penetration efficiency (%) of G_3
0. 00–1. 00 (hr)	9/14
1. 25–2. 00	6/11
2. 25–3. 00	6/14
3. 25–4. 00	6/13
4. 25–5. 00	6/ 8
5. 25–6. 00	10/18
6. 25–7. 00	9/19
7. 25–8. 00	7/12
8. 25–9. 50	7/ 9

55.9%

a) Penetration attempt of G_1 occurred earlier than that of G_3 .

successful penetration in barley coleoptile cells by *E. pisi* occurred only when prior attack of *E. graminis* to the same cells had been successful and never occurred when papillae which were not accompanied by haustorial formation had been induced by the prior attack in the same cells. Thus, it seems likely that host cells are conditioned towards the highly inaccessible state when haustorial formation does not follow papilla formation induced by an inducer regardless of a pathogen and a nonpathogen.

As indicated in Table 2, PE of G_3 which was transferred onto cells laterally adjacent to the target cells in which G_1 had attempted penetration, was 55.9% on average (66 out of 118). This PE was not significantly different from that of control, suggesting that the accessibility induced by *E. graminis* in one target cell might not be transferred to its adjacent cell at earliest before 9.5 hr after the fungus attempts penetration in the target cell. These results are consistent with the case of the accessibility induced by *E. graminis* which enhanced the subsequent infection by *E. pisi*⁵⁾.

The present results obviously indicate that the accessibility induced by *E. graminis* is effective to the same fungus as well as a nonpathogen, *E. pisi*. However, it is still unknown whether such an accessibility detected at the cellular level is effective also to other genera of powdery mildew as proved by Ouchi *et al.*⁷⁾ Recently, we obtained cytological evidence that *E. graminis* releases a suppressor(s) which blocks the enhancement of the inaccessibility caused by *E. pisi*¹⁾. It is most likely that the suppressor released from *E. graminis* effectively diminishes the native, inaccessible state of barley cells, rendering another *E. graminis* to form haustoria more easily.

Literature cited

1. 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 a suppressor of host inaccessibility expression from *Erysiphe graminis*. *Physiol. Mol. Plant Pathol.* 37: (in press).
2. Kunoh, H., Hayashimoto, A., Harui, M. and Ishizaki, H. (1985). Induced susceptibility and enhanced resistance at cellular level in barley coleoptiles. I. The significance of timing of fungal invasion. *Physiol. Plant Pathol.* 27: 43–54.
3. 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. Plant Pathol.* 33: 81–93.
4. Kunoh, H., Komura, T., Yamaoka, N. and Kobayashi, I. (1988). Induced accessibility and en-

- hanced inaccessibility at cellular level in barley coleoptiles. IV. Escape of the second lobe of *Erysiphe graminis* appressorium from inaccessibility enhanced by the previous attack of the first lobes. Ann. Phytopath. Soc. Japan 54: 577-583.
5. Kunoh, H., Kuroda, K., Hayashimoto, A. and Ishizaki, H. (1986). Induced susceptibility and enhanced resistance at cellular level in barley coleoptiles. II. Timing and localization of induced susceptibility in a single coleoptile cell and its transfer to an adjacent cell. Can. J. Bot. 64: 889-895.
 6. 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.
 7. Ouchi, S., Hibino, C., Oku, H., Fujiwara, M. and Nakabayashi, H. (1979). The induction of resistance or susceptibility. In Recognition and Specificity in Plant Host-parasite Interactions (Daly, J.M. and Uritani, I. eds.). Japan Sci. Soc. Press, Tokyo/Univ. Park Press, Baltimore. pp. 49-65.
 8. 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.
 9. Takamatsu, S., Ishizaki, H. and Kunoh, H. (1978). Cytological studies of early stages of powdery mildew in barley and wheat. V. Effect of calcium on the infection of coleoptile of barley by *Erysiphe graminis hordei*. Can. J. Bot. 56: 2544-2549.

和 文 摘 要

久能 均・黒田克利・豊田和弘・山岡直人・小林一成：オオムギ子葉鞘細胞における誘導受容性および拒否性 IX *Erysiphe graminis* の二次接種菌の感染を促進する一次接種菌誘導受容性

Erysiphe graminis の分生胞子をオオムギ子葉鞘細胞に接種した（一次菌）。さらに同一細胞上に、別の子葉鞘上で発芽させた同菌の発芽胞子を細針で移植接種した（二次菌）。一次菌が二次菌よりも 0~5 時間早く侵入を開始すると二次菌の吸器形成率は約 80%，またこの時間差が 6 時間以上になると約 93% に上昇した。針移植した対照区の吸器形成率は約 63% であったので、一次菌の侵入によって誘導される受容性は同じ菌の二次菌にも有効であると結論された。この受容性は一次菌が吸器形成に成功した細胞でのみ誘導された。一細胞で誘導された受容性は、一次菌が侵入を開始してから少なくとも 9.5 時間までには隣接細胞に移行しなかった。

(Received July 6, 1990)