

オオムギ子葉鞘細胞における誘導受容性および拒絶性(4)

Erysiphe graminis付着器第一突起侵入失敗と第二突起からの侵入成否

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Induced Accessibility and Enhanced Inaccessibility at the Cellular Level in Barley Coleoptiles IV Escape of the Second Lobe of the *Erysiphe graminis* Appressorium from Inaccessibility Enhanced by the Previous Attack of the First Lobe*

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Abstract

Appressoria of *Erysiphe graminis* conidia produced a second lobe within 2~3 hr after the first lobe failed to penetrate a host cell. How penetration from the second lobe was affected by the inaccessible state of host cells enhanced by the prior-attack of the first lobes of the same appressoria was examined in this study. When the second lobes attempted penetration of host cells in which a papilla had been formed by the previous attack of the first lobes of the same appressoria, penetration efficiency (rate of haustorium formation) of the second lobes was prominently low (about 9% on average). Moreover, penetration efficiency of the second lobes in cells adjacent to the cells where a papilla had been induced by the first lobes of the same appressoria was about 30%, when the second attempted penetration was less than 9.00 hr after the first attempted penetration. When the interval between the times of penetration attempt of both lobes was more than 9.00 hr, the penetration efficiency of the second was reduced drastically (about 7%). These results led us to assume that the cellular condition that results from the attempted penetration of the first lobe is transferred to the adjacent cells after an interval of about 9.00 hr. This assumption was confirmed by transferring germinating conidia onto cells adjacent to the cells previously attacked by the first lobes of untransferred conidia. These results suggest that the second lobes consequently escape the highly inaccessible state enhanced by the prior-attack of the first lobes simply by attempting penetration of adjacent cells before they turn highly inaccessible.

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Key words: *Erysiphe graminis*, second lobe of appressorium, inaccessibility.

INTRODUCTION

Conidia of *Erysiphe graminis* usually attempt penetration from the hook-shaped lobes of appressoria near their apical end. However, only 70-80% of the first attempts result in successful haustorium formation. In the remaining cases, a papilla forms below the first lobe without occurrence of a haustorium. Then, a second lobe is formed near the middle part of the same appressorium for the second attempt of penetration^{1-3,5,10}.

In a series of our previous works⁶⁻⁸, we have studied accessibility induced by a prior-attack

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of a pathogen and inaccessibility enhanced by a previous attack of a non-pathogen at the cellular level in barley coleoptiles. We found that when penetration failure occurred in one cell attacked by either a non-pathogen or a pathogen, the inaccessible state of the host cell was enhanced prominently enough to reject even the subsequent penetration attempt of a compatible race of the pathogen^{6,9)}. This enhanced inaccessibility was always associated with papilla formation in the host cell^{6,9)}. These results led us to believe that the inaccessible state of one coleoptile cell which had been enhanced by the penetration failure of the first lobes of appressoria of *E. graminis* conidia must have some effect on the subsequent penetration attempt by the second lobe of the same appressorium.

In this paper, we demonstrated the association of penetration attempts by both lobes with regard to timing and location to determine how the second lobes could escape from the inaccessible state enhanced by the first.

MATERIALS AND METHODS

Test plant and fungus. Barley (*Hordeum vulgare* L. cv. Kobinkatagi) and *Erysiphe graminis* de Candolle f. sp. *hordei* Em. Marchal race 1 were used, respectively, as the host plant and the pathogen. *Erysiphe graminis* was maintained on the host cultivar grown at 20 C, 70% relative humidity under 4,000 lux of fluorescent light for 12 hr per day.

Specimen preparation. Barley was grown from seed under 4,000 lux fluorescent light for 14 hr per day. Coleoptiles were excised from barley seedlings 8 days after sowing and single cell thick epidermal layers of partially dissected coleoptiles were prepared, as previously described¹¹⁾. The coleoptiles were floated on 0.01 M CaCl₂ solution in petri dishes.

Observation of attempted penetration from the second lobes of appressoria. Coleoptiles were inoculated with 15–20 freshly harvested conidia of *E. graminis* by a brush and incubated on CaCl₂ solution at 20 C in the dark. After 9 hr incubation, the coleoptiles were mounted with CaCl₂ solution on a glass slide. Germinating conidia with a mature appressorium were selected so that no other germlings were located within five cells from the selected conidia. Subsequently, fungal growth and host cell response were observed using an Olympus interference contrast microscope BH-2 at $\times 200$ every 15 min. For each conidium, the time of initiation of cytoplasmic aggregates induced by the first appressorial lobe was recorded. After coleoptiles were again incubated for an additional 5 hr, they were mounted on a glass slide as above. Among the conidia whose attempted penetration from the first lobe had been observed previously, only those which had failed in penetration to induce papilla formation were selected for the subsequent observation at 15 min intervals. After the time of initiation of cytoplasmic aggregates below the second lobe of appressorium was recorded for each of the selected conidia, the coleoptiles were incubated as above until 36 hr after inoculation. Finally, haustorium formation from the second lobes was recorded (Fig. 1c). When both lobes attempted penetration on the same host cells, the distance between the attempted penetration sites of both lobes was measured using a micrometer set in the microscope. However, when both lobes attempted penetration on adjacent host cells, the distance between the attempted penetration site and cell walls bordering the cells attacked by the respective lobes was measured, since it was proved that the entire cells turned highly inaccessible within 2 hr after either pathogen or non-pathogen had failed in penetration within the cells⁹⁾. As mentioned below, the second lobes attempted penetration more than 8 hr later than the first lobes. Thus, we assumed that when the second lobes attempted to penetrate cells adjacent to the cells previously attacked by the first lobes, the entire area of the latter cells had been highly inaccessible. Therefore, to access the effects of location of penetration sites on adjacent cells, the above measurement was thought reasonable.

Transfer of enhanced inaccessibility to an adjacent cell. One pair of coleoptiles was prepared for each experiment. One coleoptile (coleoptile A) was inoculated with 15–20 freshly harvested conidia. Another coleoptile (coleoptile B) was inoculated similarly with about 100

conidia 4 hr before the conidia were transferred. These coleoptiles were incubated in the same incubator as above for given times. Between 9 and 12 hr after inoculation, coleoptile A was observed to record the time of initiation of cytoplasmic aggregates which were induced by the first lobes of appressoria. After 2–3 hr incubation, the conidia whose first lobes had induced a papilla at the penetration sites in host cells were selected. Subsequently, at proper times germinating conidia with an appressorial germ tube were individually transferred with a micromanipulator from coleoptile B to coleoptile A so that the appressoria of transferred conidia attempted to penetrate cells laterally adjacent to cells of which had been attacked by the first lobes of untransferred conidia. Transfer was made to the adjacent cells which would not be attacked by the second lobes of untransferred conidia. Transfer of conidia was completed within 1 hr after initiation of the manipulation. Immediately after the transfer was completed, coleoptile A was returned to the petri dish and incubated for 4 hr. Subsequently, growth of transferred conidia and host cell response on coleoptile A were observed every 15 min. After the time of initiation of cytoplasmic aggregates induced by the first lobes of appressoria of the transferred conidia was recorded, coleoptile A was incubated again as above for additional hours. Finally, haustorium formation from these lobes was examined 24 hr after inoculation of coleoptile B. In this experiment, penetration attempt of only the first lobes was concerned for transferred conidia.

Data were accumulated from more than 50 separate, but replicated, experiments performed over a period of six months. During manipulation and observation, the microscope room was maintained at 20 ± 2 C and 70% relative humidity. Differences in penetration efficiency (rate of haustorium formation) between groups were analyzed by the χ^2 test.

RESULTS

General feature of fungal growth and host response

Appressorial germ tubes generally emerged from conidia 4–5 hr after inoculation. Their apical portion became gradually swollen to form the hook-shaped first lobe near the apical end (Fig. 1b). The first lobes usually attempted to penetrate, resulting in cytoplasmic aggregates below them between 10 and 12 hr after inoculation (Fig. 1b). Between 3–4 hr after the first lobes failed in penetration (*i.e.* induced papilla formation alone without haustorium formation), the second lobes emerged from the middle part of the same appressorium. When the second lobes attempted penetration in the same cells as the first lobes of the same appressorium had done, cytoplasmic aggregation below the second lobes occurred 8.75 ± 0.20 hr on average after initiation of cytoplasmic aggregates below the first lobes. On the other hand, when the second lobes attempted penetration in adjacent cells (Fig. 1c), that time was 8.13 ± 0.12 hr on average. Thus, attempted penetration from the second lobes occurred significantly earlier when the second lobes penetrated an adjacent cell than when attempted penetration was in the same cells ($P = 0.005$). The second lobes never formed when the first lobes succeeded in penetration to form a haustorium. The penetration attempt from the second lobe was sometimes successful in forming a haustorium (Fig. 1a) but other times unsuccessful (Fig. 1d).

Penetration efficiency of the second lobes in host cells which were attacked previously by the first lobes of the same appressoria

When the second lobes attempted to penetrate the cells in which a papilla had been formed by the first lobes of the same appressoria, their penetration efficiency was prominently reduced. As indicated in Table 1, only 3 out of 18 (16.7%) succeeded in penetration when the second lobes attempted penetration within 8 hr after the first induced cytoplasmic aggregates. When the time interval between the attempted penetration from both lobes was 8.25–9.00 hr, haustorium formation occurred from only 2 out of 21 second lobes (9.5%). However, when the second lobes attempted penetration more than 9.00 hr later than the first, haustorium formation never occurred below the second. These tendencies of penetration efficiency were not affected by the distance between penetration sites of both lobes.

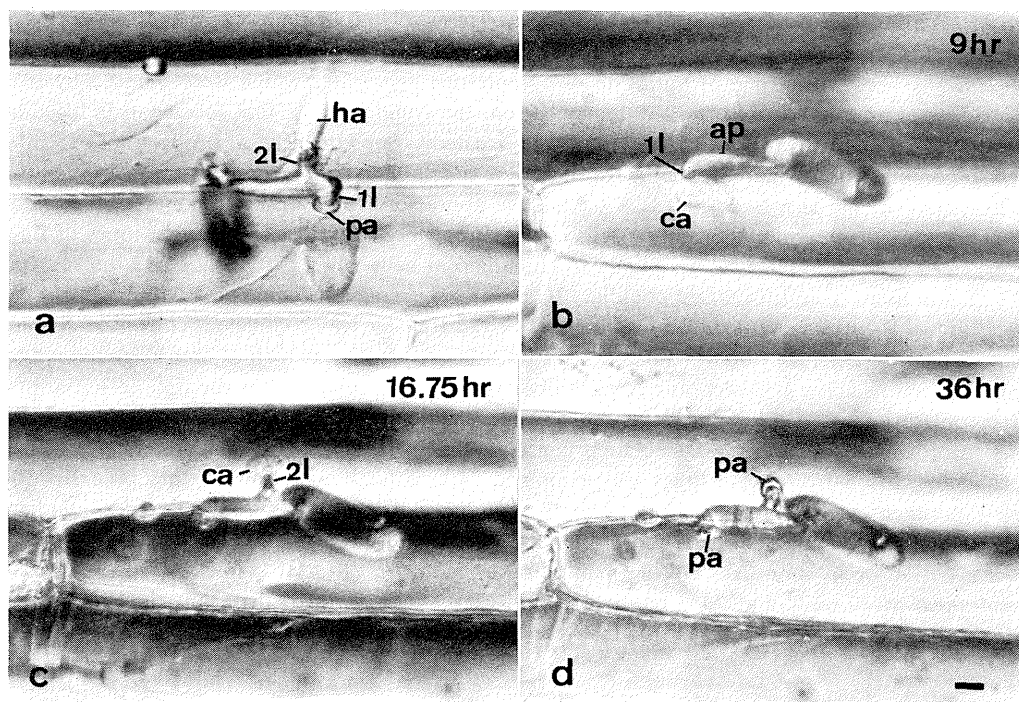


Fig. 1. Micrographs showing growth patterns of *Erysiphe graminis* on barley coleoptile cells. (a) An appressorium of *E. graminis* with the first and the second lobes. The first lobe (1l) failed in penetration to induce a papilla (pa) in one cell and the second lobe (2l) succeeded in penetration to form a haustorium (ha) in the adjacent cell. (b-d) A series of micrographs showing successive growth patterns of *E. graminis*. A cytoplasmic aggregate (ca) was induced below the first lobe of the appressorium (ap) 9 hr after inoculation. At 16.75 hr another cytoplasmic aggregate occurred below the second lobe which had been produced as the result of penetration failure of the first lobe. When the same conidium was observed at 36 hr, a papilla had been produced below the second lobe. Scale=10 μ m.

Table 1. Effects of relative time of initiation of cytoplasmic aggregates induced by the 1st and the 2nd lobes of the same appressoria of *Erysiphe graminis* on penetration efficiency (PE) of the 2nd lobes on the same coleoptile cells of barley

Difference between times of initiation of cytoplasmic aggregates induced by the 1st and the 2nd lobes	2nd lobe	
	A/B*	PE
less than 8.00 hr	3/18	16.7%
8.25-9.00	2/21	9.5
more than 9.25	0/18	0.0

* A: No. of the 2nd lobes with a haustorium, B: Total No. of the 2nd lobes observed.

Penetration efficiency of the second lobes in host cells laterally adjacent to cells in which a papilla was induced by the first lobes of the same appressoria

As shown in Table 2, when the second lobes attempted penetration less than 9.00 hr later than the first had done, 23 out of 78 second lobes (29.5%) succeeded in penetration. However, during the time interval was 9.00-9.75 hr, the penetration efficiency of the second lobes was

reduced to 10.0%, and when the time interval was more than 10.00 hr, none of the second lobes penetrated successfully. These tendencies of penetration efficiency were independent of location of penetration sites of the second lobes within the cells.

Table 2. Effects of relative time of initiation of cytoplasmic aggregates induced by the 1st and the 2nd lobes of the same appressoria of *Erysiphe graminis* on penetration efficiency (PE) of the 2nd lobes on the adjacent coleoptile cells of barley

Difference between times of initiation of cytoplasmic aggregates induced by the 1st and the 2nd lobes	2nd lobe		
	A/B*	PE**	Average PE
less than 6.75 hr	5/18	27.8% ^a	29.5%
7.00-7.75	10/28	35.7 ^a	
8.00-8.75	8/32	25.0 ^a	
9.00-9.75	2/20	10.0 ^b	
more than 10.00	0/10	0.0 ^c	

* A: No. of the 2nd lobes with a haustorium, B: Total No. of the 2nd lobes observed.

** Percentages within a column differ significantly ($P=0.01$) if followed by a different letter, as determined by the χ^2 test.

Difference between times of initiation of cytoplasmic aggregate induced by the inducer (▽) and the challenger (▼)	Penetration efficiencies of the challenger	
▽ 0.00-1.00 ▼	12/17 (70.6)%	18/26 69.2 %
▽ 1.25-2.00 ▼	6/9 (66.7)	
▽ 2.25-3.00 ▼	4/11 (36.4)	
▽ 3.25-4.00 ▼	6/15 (40.0)	54/163 33.1 %
▽ 4.25-5.00 ▼	6/17 (35.3)	
▽ 5.25-6.00 ▼	7/18 (38.9)	
▽ 6.25-7.00 ▼	6/14 (42.9)	
▽ 7.25-8.00 ▼	6/20 (30.0)	
▽ 8.25-9.00 ▼	6/20 (30.0)	
▽ 9.25-10.00 ▼	7/28 (25.0)	
▽ 10.25-11.00 ▼	6/20 (30.0)	1/68 1.5 %
▽ 11.25-12.00 ▼	1/20 (5.0)	
▽ 12.25-13.00 ▼	0/14 (0.0)	
▽ 13.25-14.00 ▼	0/14 (0.0)	

Fig. 2. Effect of the relative time of initiation of cytoplasmic aggregates induced by the first lobe of appressoria of untransferred conidia (inducer) in one cell and the first lobe of appressoria of transferred conidia (challenger) in the adjacent cell on penetration efficiency of the challenger. The times of initiation of cytoplasmic aggregates were recorded only for the inducers whose penetration attempt had failed resulting in papilla formation.

Intensity of inaccessibility in host cells laterally adjacent to cells in which a papilla was induced previously by the first lobes

Penetration efficiency of appressoria of transferred conidia varied in host cells laterally adjacent to cells where a papilla had been induced previously by the first lobes of untransferred conidia, depending on intervals between times of initiation of cytoplasmic aggregates induced by both penetration attempts (Fig. 2). When appressoria of transferred conidia attempted penetration 0.00–2.00 hr later than the first lobes of appressoria of untransferred conidia, the penetration efficiency of the former was 69.2%. However, when the time intervals were 2.25–11.00 hr, the mean penetration efficiency of transferred conidia was reduced to 33.1%. Furthermore, when the time intervals were more than 11.25 hr, almost no penetration of transferred conidia occurred. These tendencies were not affected by location of penetration sites of transferred conidia within the host cells.

DISCUSSION

Kunoh *et al.*^{6,8)} demonstrated that when *E. pisi*, a non-pathogen of barley, was inoculated onto barley coleoptiles, attempted penetrations always resulted in papilla formation within host cells, and that once a papilla formed in these cells, they turned to a perfectly inaccessible state enough to reject a subsequent penetration attempt of even a compatible race of *E. graminis* inoculated as a challenger. In another report, Kuroda *et al.*⁹⁾ presented a similar evidence that when a compatible race of *E. graminis* failed in penetration to form a papilla, the inaccessibility of the entire host cells became highly enhanced to reject the subsequent attack by appressoria of other conidia of the same fungus. As indicated in Table 1, penetration efficiency of the second lobes was prominently low regardless of the intervals between times of penetration attempt by both lobes. Judging from the results in Table 2, the second lobes should have a potential of successful penetration at least 30%. Therefore, it is likely that inaccessibility of the cells was enhanced by the penetration failure of the first lobes up to the level high enough to disturb the subsequent penetration attempt by the second lobes of the same conidia. In the cells adjacent to cells in which the previous penetration attempt from the first lobes was unsuccessful, the second lobes formed a haustorium at an average rate of about 30%, when they attempted penetration less than 9.00 hr after the first lobes. However, when the second lobes attempted penetration 9.00–9.75 hr later than the first, penetration efficiency was reduced prominently, and finally the second lobes were not allowed to produce a haustorium even in the adjacent cells when the second attempted penetration occurred more than 10.00 hr after the first. These results led us to assume that inaccessibility of one cell enhanced by the penetration failure of the first lobe may be transferred to laterally adjacent cells by 10.00 hr after the first induced a cytoplasmic aggregate. This assumption was supported by another experiment using transferred conidia. As indicated in Fig. 2, penetration efficiency of transferred conidia was about 69%, when their appressoria attempted penetration 0.00–2.00 hr later than the first lobes of appressoria of untransferred conidia in the adjacent cells. This penetration efficiency is approximately equal to that of *E. graminis* when used as the sole inoculum on Kobinkatagi coleoptiles. However, the penetration efficiency of transferred conidia was reduced to almost half when their penetration attempt was 2.25–11.00 hr behind that of the first lobes in adjacent cells. When the time intervals were more than 11.25 hr, appressoria of transferred conidia were not allowed to form a haustorium. Thus, it appears that the inaccessible condition induced by the penetration failure of the first lobes is transferred to adjacent cells beginning at 2.25 hr after the first attempted penetration. However, it appears that with time the adjacent cells become progressively more inaccessible and finally at periods greater than 11.25 hr they are completely inaccessible and no successful penetrations occur from challenge inoculations.

The transfer experiment confirmed that penetration failure of the first lobes of appressoria in one cell evidently affected the condition of the adjacent cell. When *E. graminis* conidia are

inoculated on coleoptiles or leaves of barley, most appressoria tend to form a second lobe so that it attempts to penetrate a cell adjacent to the cell where the previous penetration attempt occurred. This growth pattern consequently allows the second lobes to escape the inaccessible state of the host cell enhanced by the first lobes of the same appressorium and provides these lobes a greater chance for successful penetration. Furthermore, since about 70% of the second lobes attempted penetration before 9.00 hr after the first lobes had induced cytoplasmic aggregates, it also provided a greater chance for successful penetration because adjacent cells would not as yet become totally inaccessible.

Gold *et al.*⁴⁾ demonstrated that penetration attempt of *E. graminis* occurred later in tetracycline-treated barley coleoptiles than in untreated ones. Their results and the present observation that the second lobes attempted penetration earlier in the adjacent cells than in the cells where the first lobes of the same conidia had previously induced a papilla suggests that the fungus may recognize the condition of host cells prior to actual penetration.

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和 文 摘 要

久能 均・小村朋三・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絶性 *IV Erysiphe graminis* 付着器第一突起侵入失敗と第二突起からの侵入成否

Erysiphe graminis の付着器は第一突起からの侵入に失敗すると、第二突起を生じて再び侵入を試みる。第一突起からの侵入に失敗すると、侵入をうけた子葉鞘細胞の拒絶性が急速に高まる。高揚したこの細胞拒絶性と第二突起からの侵入成否について検討した。第二突起の侵入は、通常第一突起の侵入開始 8~9 時間後におこる。第一突起と第二突起が同一子葉鞘細胞に侵入を試みた場合には、第二突起の侵入成功率（吸器形成率）は約 9% であり、第一突起の侵入失敗の影響を受けていた。第一突起が侵入を失敗した細胞の隣の細胞に第二突起が侵入を試みた場合には、両突起の侵入時間差 9 時間を境にして第二突起の侵入成功率に有意差が現われた。すなわち、第一突起が侵入してから 9 時間以内に第二突起が侵入を試みた場合には、第二突起の侵入成功率は 29.5% であったが、9~9.75 時間では 10%、10 時間以上では 0% となった。この結果から、第一突起の侵入失敗によって高められた細胞の拒絶性は、約 9 時間後に隣接細胞に移行すると推定された。この仮説は第一突起からの侵入が失敗した細胞に隣接する細胞に別の胞子の付着器を移植し、その侵入行動を観察することによって裏づけられた。以上の結果は、第一突起が侵入に失敗した細胞の隣の細胞に第二突起が侵入を試みることによって、第一突起によって高められた細胞拒絶性が結果的に回避されていることを示している。