Cytological Studies of Early Stages of Powdery Mildew in Barley and Wheat (X) : Fluorescence at Penetration Sites of Fungi on Barley, Wheat and Rice

Cytological Studies of Early Stages of Powdery Mildew in Barley and Wheat (X)

Fluorescence at Penetration Sites of Fungi on Barley, Wheat and Rice*

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Introduction

Fungi frequently induce common responses of plants, when they attempt penetration. Halo and papilla formation have been observed in a number of parasite-host systems: *Erysiphe graminis*—barley and wheat[4,5,11], *E. polygoni*—red clover[9], *Helminthosporium avenae*—reed canarygrass[13], *Colletotrichum dematium* f. *circinans* or *Botrytis cinerea*—onion[10,12]. Cytoplasmic aggregation likewise occurs at penetration sites of various fungi[1,3,8,14,15]. Similarly silicon accumulation was reported to occur at penetration sites of *E. graminis*, *Alternaria kikuchiana*, *Colletotrichum lagenarium*, and *Cochliobolus miyabeanus* inoculated on primary leaves of barley and wheat, and cotyledons of cucumber and morning glory[7].

In a previous paper, we reported that autofluorescence occurred at sites of attempted penetration from appressoria of *E. graminis hordei* in barley coleoptiles prior to initiation of cytoplasmic aggregation, suggesting that the fluorescence is perhaps the first visible response in a living *E. graminis*—barley cell system. Furthermore, occurrence of the fluorescence was reported to be strikingly enhanced by the presence of divalent cations such as Ca^{++}, Mg^{++} and Mn^{++} [15].

The present study was undertaken to elucidate whether the fluorescence is a specific response in barley coleoptile cells infected with *E. graminis hordei*.

Meterials and Methods

Host plants. Barley (*Hordeum vulgare* L. cv. Kobinkatagi), wheat (*Triticum aestivum L.* cv. Norin 4) and rice (*Oryza sativa* L. cv. Jukkoku) were used as host plants. Barley and wheat were grown from seed under 4000 lx of fluorescent light for 14 h per day in a chamber maintained at 20°C and 70% relative humidity (r. h.). Coleoptiles were excised from seedlings 7 days after sowing and single cell layers of partially dissected coleoptiles were prepared, as previously described[14]. Rice was grown from seed in a green house. Sheaths were excised from rice plants 40 days after sowing and one or two cell layers of partially dissected sheaths were prepared using a dissecting microscope as similarly as the above coleoptiles.

Fungi. *Erysiphe graminis hordei* (race 1) and *E. graminis tritici* (race t_2) were, respectively, maintained on compatible Kobinkatagi and Norin 4 in similar chambers kept at 20°C and illuminated with 4000 lx of fluorescent light for 12 h per day. *E. pisi* de Candolle (race 1) was maintained on a compatible pea cultivar Alaska in a similar chamber as that conditioned for maintaining *E. graminis*. *Alternaria kikuchiana* Tanaka (A1-3, the stock No. of Mie Univ.) was grown on a potato-sucrose-agar medium at 28°C for 1 week.

Inoculation, incubation and observation. Dissected coleoptiles of barley were inoculated with one of *E.*

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graminis hordei, *E. graminis tritici* and *E. pisi* with a brush or *A. kikuchiana* by spraying. Simiarly, coleoptiles of wheat and sheaths of rice were inoculated with *E. graminis hordei*. Plants thus-inoculated were floated over 0.01 M $CaCl_2$ solution in petri dishes covered with lids, and incubated in the above chambers each for maintaining the respective fungus.

At various times after inoculation, the coleoptiles and sheaths were examined with an Olympus fluorescence microscope with a Type B exciter filter (maximum transmission 410 nm) and O 530 absorption filter. Micrographs were taken as either × 100 or × 70 on Kodak Ektachrome 400.

Results

1. *E. graminis hordei* — barley coleoptile.

Inoculated conidia produced appressorial germ tubes 4–5 h after inoculation. At 10–11 h the appressoria attempted penetration from their hooks and induced cytoplasmic aggregates in the underlying coleoptile cells (Fig. 1a). A few minutes before initiation of cytoplasmic aggregates, the circular area beneath the appressorium became fluorescent, and thereafter the area of fluorescence increased in diameter to reach nearly twice as much as the full length of appressorium (Fig. 1b). Once papillae formed beneath the appressorial hooks, they gave continuously an intense fluorescence, as shown in Fig. 1b.

2. *E. graminis tritici* — barley coleoptile.

Growth patterns of conidia and timing of events related to the conidia were similar to those of *E. graminis hordei* described above. An intensely fluorescent area appeared around penetration sites of appressoria (Figs. 2a, b). Intensity of fluorescence varied with appressoria.

3. *E. pisi* — barley coleoptile.

Around 4 h after inoculation, germ tubes emerged from conidia and their apices swelled to form typical appressoria at 6 h. Around 10 h after inoculation, cytoplasmic aggregates and fluorescence occurred below and around appressorial apices that attempted penetrations (Figs. 3a, b). Intensity of fluorescence was a little less than that induced by *E. graminis hordei and E. graminis tritici*. Papillae always gave an intense fluorescence, when present.

4. *Alternaria kikuchiana* — barley coleoptile.

Conidia initiated germination 4 h after inoculation. Germtube apices slightly swelled to become appressorium-like structures (Fig. 4a). Whenever cytoplasmic aggregates were seen below the appressorium-like structures, intense fluorescence was visible around the structures (Fig. 4b). Germtubes elongated further, yet fluorescence was not induced anymore, suggesting that the conidia might attempt penetration only once from the appressorium-like structures in barley coleoptiles, even if germtubes extended further. The fluorescence was maintained until termination of observation at 24 h after inoculation.

5. *E. graminis hordei* — wheat coleoptile.

Growth patterns of conidia in wheat coleoptiles were very alike to those in barley coleoptiles. When cytoplasmic aggregates were observed below appressoria, circular areas at penetration sites became fluorescent (Fig. 5a, b). However, intensity and area of fluorescence were much less than those in barley coleoptiles. The fluorescence became invisible around 14 h. However, papillae remained intensely fluorescent until termination of observation at 24 h, once they formed.

6. *E. graminis hordei* — rice sheath.

Conidia were capable of forming primary germ tubes and appressoria in rice-sheath cells as similarly as in barley coleoptiles. In a few cases a small, round swelling resembling a haustorium initial arose from an appressorial apex in a rice-sheath cell (Fig. 6a). However, the structure did not grow thereafter. Formation of such a structure evidently shows that conidia certainly attempted penetrations from appressoria, although cytoplasmic aggregates which are supposed to precede haustorial formation could not be observed due to difficulty of seeing cytoplasmic streamings in rice-sheath cells as compared with those in barley and wheat coleoptiles. Fluoresence was never observed around penetration sites (Fig. 6b).

Discussion

The present results indicate that all test fungi are capable of inducing fluorescence at penetration sites of appressoria or appressorium-like structures in living barley coleoptile cells regardless of compatibility and pathogenicity, as similarly as silicon accumulation described earlier[7]. *E. graminis hordei* is able to elicit fluorescence in wheat coleoptiles but its intensity is much less than that in barley coleoptiles.

Host responses to fungal attacks such as cytoplasmic aggregation, papilla and fluorescence, were not seen in rice-sheath cells, even when *E. graminis hordei* evidently succeeded to invade the cells. It is plausibly ascribed to less cytoplasmic activity of rice-sheath cells than that of barley and wheat coleoptile cells. Further studies using other parts of rice should be done before we make a conclusion.

Based on the above results, the followings are pointed out:

i) Occurrence of fluorescence is not a specific response in the *E. graminis* — barley coleoptile system.

ii) The fluorescence is not directly associated with compatibility or pathogenicity, since all test fungi including compatible, incompatible and nonpathogenic fungi to test plants, induced fluorescence likewise in barley coleoptile cells.

iii) Intensity of fluorescence varies with test plants. The fluorescence was visualized more readily in barley than wheat.

Thus, the present observations led us to conclude that occurrence of fluorescence is one of common responses of barley and wheat to fungal attacks as well as cytoplasmic aggregation[1,3,6], halo formation[2,5,11], and papilla induction[1,3,5,6].

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摘　　要

ムギ類うどんこ病菌感染初期の細胞学的研究　(X)

オオムギ・コムギ・イネ上の侵入部位における螢光

山守一男・石崎　寛・久能　均

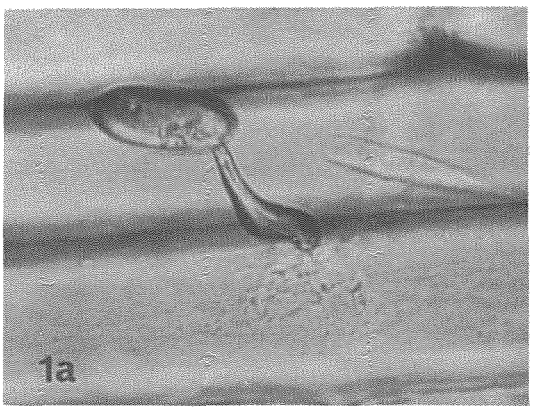
前報で，オオムギうどんこ病菌 (*Erysiphe graminis hordei*) の分生胞子がオオムギ子葉鞘細胞に侵入する際に，侵入点を中心にして円状に緑色螢光が発生することを報告した。この現象が，オオムギと *E. graminis hordei* の系に特異的なものであるかどうかを検討するために，オオムギ・コムギの子葉鞘およびイネ葉鞘に，*E. graminis hordei*, *E. graminis tritici* (コムギうどんこ病菌), *Alternaria kikuchiana* (ナシ黒斑病菌) の分生胞子を接種し，それぞれの菌の付着器下に螢光が発生するか否かを観察した。オオムギ子葉鞘上では，すべての供試菌の付着器または付着器様構造下で螢光が認められた。コムギの子葉鞘上の *E. graminis hordei* の侵入点には弱い螢光が現われた。しかし，イネ葉鞘に同じ菌を接種しても侵入点付近に螢光は現われなかった。以上の結果から，螢光の発生は種々の糸状菌の侵入に対するオオムギ・コムギの子葉鞘の共通的な反応であると結論されるが，螢光の強さは植物によって差があり，オオムギ子葉鞘では強い螢光が発生するようである。

Explanation of plate

Figs. 1a, b. A conidium of *Erysiphe graminis hordei* inducing a cytoplasmic aggregate beneath the appressorium (a) and a wide, circular, fluorescent area around the penetration site (b) in barely coleoptile 11.5 h after inoculation. The intense fluorescence at the appressorium apex represents the response of a papilla (b). ×690.

Figs. 2a, b. A conidium of *Erysiphe graminis tritici* inducing a cytoplasmic aggregate (a) and intense fluorescence (b) in a wheat coleoptile 12 h after inoculation. ×700.

Figs. 3a, b. An *E. pisi* conidium having an appressorium of swelling apex which has induced a cytoplasmic aggregate (a) and fluorescence (b) in a barely coleoptile 10 h after inoculation. ×540.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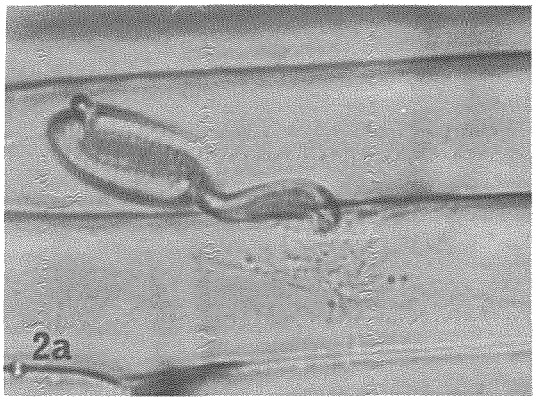
1b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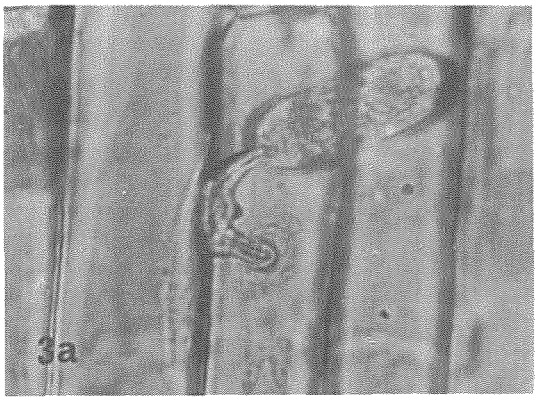
2b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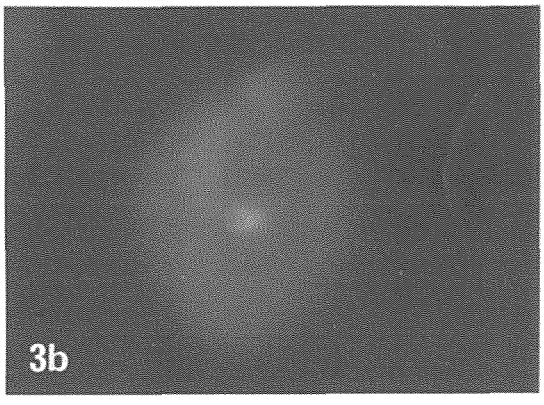
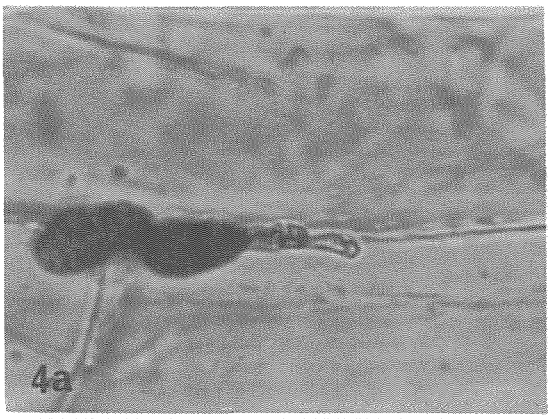

3b

Figs. 4a, b. A germinating conidium of *Alternaria kikuchiana* in a barley coleoptile 8 h after inoculation (a). An intense fluorescence is visible around the germ tube (b). ×690.

Figs. 5a, b. A conidium of *E. graminis hordei* inducing a cytoplasmic aggregate (a) and fluorescence (b) in a wheat coleoptile 12 h after inoculation. Intensity of fluorescence is less than that in Fig. 1b. ×540.

Figs. 6a, b. A germinating conidium of *E. graminis hordei* inducing a small structure resembling a haustorium initial (arrow) (a) but no fluorescence has been induced, 15 h after inoculation. ×540.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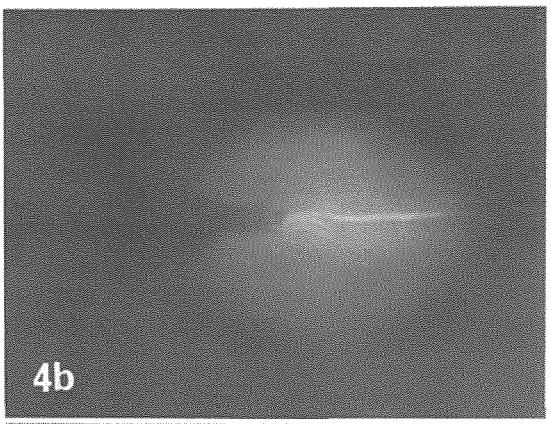
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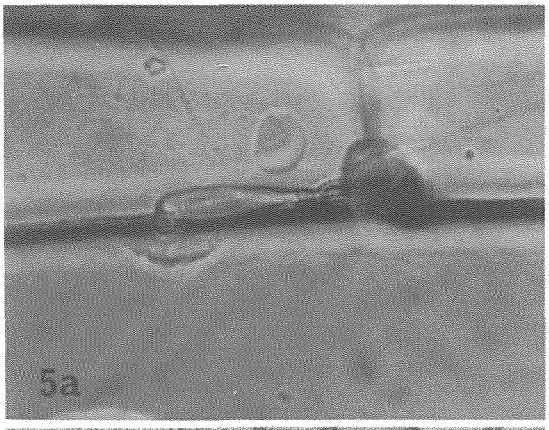
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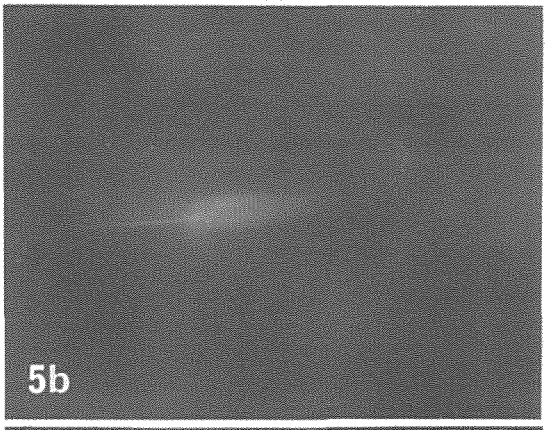
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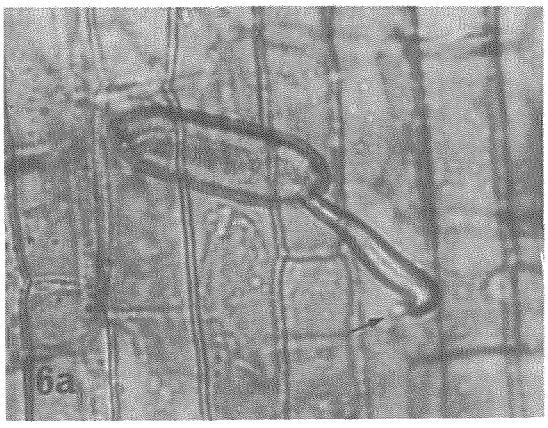
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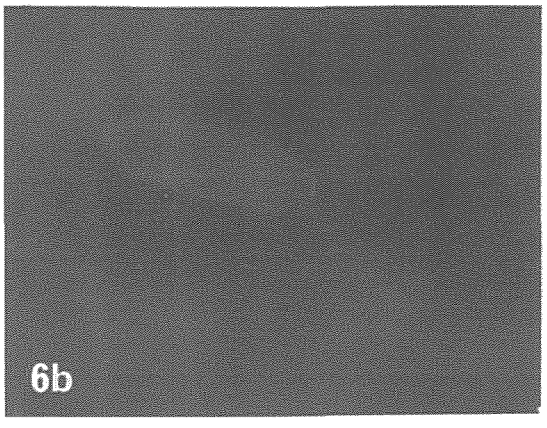
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