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Susceptibility-inducing factor (suppressor) from *Blumeria graminis* f. sp. *hordei* has no effect on the primary infection of the fungus

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ABSTRACT

When fungal germlings, after forming haustoria of *Blumeria graminis* f. sp. *hordei* (*B. graminis*), were removed from the surfaces of barley coleoptiles by cellulose acetate, followed by challenge inoculation with the non-pathogen *Erysiphe pisi*, they infected the nonhost barley coleoptile cells. This phenomenon was not observed on the coleoptile surface when the fungal germlings of *B. graminis* were removed before the formation of haustoria. Also, when the surface was inoculated with the pathogen of barley *B. graminis* as a challenger, after removing the fungal germlings of inducer post haustorial formation, the penetration efficiency of the fungi increased significantly compared with that of the control. Furthermore, when we extracted the crude-susceptibility inducing factor (suppressor) from coleoptiles before and after the formation of haustoria of *B. graminis*, suppressor activity against infection with *E. pisi* was observed only in the extract of barley coleoptiles that included haustoria of *B. graminis* about 18 h or later after inoculation. Surprisingly, however, the extract did not increase the penetration efficiency of *B. graminis* significantly. Thus, we hypothesize that the suppressor extracted from barley coleoptiles in which *B. graminis* had formed haustoria has no effect on increasing the penetration efficiency of the primary infection from the appressorium of *B. graminis* but has an effect on the infection with non-pathogen *E. pisi*.

1. Introduction

When a pathogen infects a host plant, a challenge inoculation with a non-pathogen or an incompatible race of the pathogen infects the plant successfully [4,7–9,12–14]. This phenomenon is called acquired or induced susceptibility [12,14,20]. The secretion of components of pathogens, such as an ethyl acetate-soluble fraction of spore germination of *Pyricularia oryzae* [2,3], a suppressor of *Mycosphaerella pinodes* [15,16] and AK-toxin of *Alternaria kikuchiana* [11], is thought to be one of the factors responsible for such susceptibility.

Studies on induced susceptibility at the cellular level in barley have demonstrated that prior attack of barley coleoptile cells by the pathogen *Erysiphe graminis* (= *Blumeria graminis*) induced susceptibility to the non-pathogen *Erysiphe pisi* [7,8]. That is, the non-pathogen *E. pisi* can form haustoria in barley coleoptile cells previously infected with the pathogen *E. graminis*. This phenomenon suggests that the resistance of barley coleoptile cells to the non-pathogen is blocked by the penetration of the pathogen. We previously presented cytological evidence of (a) susceptibility inducing factor(s) of host resistance from *E. graminis* [6]. We call the susceptibility inducing factor(s) "suppressor" for the sake of convenience here. There is a report that a crude suppressor fraction was extracted from barley coleoptile tissue 18 h after inoculation with *B. graminis* [1]. When the lower surfaces of barley coleoptiles were treated with the extract, haustoria formed in the cells by the non-pathogen *E. pisi*, which usually never forms haustoria by itself in barley coleoptile cells. Suppressor activity was not observed in the extract from the coleoptile tissue without haustoria of *B. graminis*.

Although a suppressor would be responsible for induced susceptibility it could not be secreted for a successful challenge inoculation of the non-pathogen. In other words, the suppressor should be secreted for the infection with the pathogen itself prior to penetration because the suppressor suppresses defense reactions in the host and supports the infection with the

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pathogen itself. Therefore, it is unexpected that the suppressor from *B. graminis* was obtained from the coleoptile tissue that only contained haustoria of the fungi, only after successful penetration of *B. graminis*. Thus, this study was carried out to determine 1) when the susceptibility for the non-pathogen *E. pisi* is induced by *B. graminis* infection, 2) whether the induced susceptibility to the non-pathogen is effective for the pathogen *B. graminis* itself, 3) whether the suppressor fraction from coleoptile cells infected with *B. graminis* is effective for the penetration of cells by non-pathogen *E. pisi*, and 4) whether the suppressor that is involved in the induction of susceptibility to the non-pathogen *E. pisi* is effective for the infection with the pathogen *B. graminis* itself.

Although the conditions for accessibility at the cellular and tissue levels must be distinguished from susceptibility observed at the level of the whole plant as pointed by Ouchi et al. [13,14], we use the term "susceptibility" instead of "accessibility" for the sake of convenience here.

2. Materials and methods

2.1. Fungi and host plant

B. graminis f. sp. *hordei* EM. Marchal, race 1 (the pathogen) was used as an inducer of susceptibility, and *E. pisi* D. C., race 1 (the non-pathogen) was used as the challenge fungus to test the state of susceptibility induced by *B. graminis.*

B. graminis was maintained on barley (*Hordium vulgare* L. cv Kobinkatagi) grown at 20 ± 2 °C and 70% relative humidity under fluorescent lights (ca. 23.6 Wm^{-2}) in a 14-h photo-period in growth chambers. *E. pisi* was maintained on peas (*Pisum sativum* L.) under the same conditions but in separate growth chambers.

2.2. Specimen preparation

Barley was grown from seeds at 20 ± 2 °C and 70% relative humidity under fluorescent lights (ca. 23.6 Wm^{-2}) in a 14-h photo-period in growth chambers. Coleoptiles were excised from seedlings nine days after sowing, and single-cell epidermal layers of partially dissected coleoptiles were prepared as described previously [17].

2.3. Inoculation, removal of germlings and challenge inoculation

Coleoptiles were inoculated with at least one conidium or two conidia of *B. graminis* per coleoptile cell and then floated on 1 mM $CaCl_2$ and incubated for the required period. After incubation, conidia and germ tubes of the inducer *B. graminis* were removed at various times after onset of incubation by painting the coleoptile surface with cellulose acetate dissolved in acetone and then peeling away the cellulose acetate strip after allowing the acetone to evaporate (within 1 min) as reported by Lyngkjaer and Carver [10]. As controls, uninoculated coleoptile surfaces were treated with cellulose acetate. Immediately after removing the inducer germlings the treated coleoptiles and controls were inoculated with challenge inoculum, *B. graminis* or *E. pisi*. The challenge-inoculum density was adjusted to give 40–50 conidia per coleoptile. The coleoptiles were floated on 1 mM $CaCl_2$ and incubated at 20 °C for 24 h in a dark paper box.

Experiments were repeated at least three times, and data are expressed as means with standard deviations. Significance of differences was determined using Student's *t*-test.

2.4. Extraction of the suppressor

The crude suppressor fraction was extracted as described previously [1]. About 40–50 coleoptiles at various times after inoculation with *B. graminis* were homogenized in liquid nitrogen. The homogenate was suspended in a mixture of 1 ml of deionized water and 2 ml of 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 at 1000 × g for 10 min. 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. The dry weight of the extract was determined, and the extract was kept at −20 °C until use.

3. Results and discussion

3.1. Timing of induced susceptibility by B. graminis infection

The fungal germlings of *B. graminis* were removed by treatment with cellulose acetate at each time after inoculation as shown in Fig. 1: at 7.5–8.5 h after inoculation (maturation of appressoria), at 10–11 h after inoculation (beginning of penetration), at 14 h after inoculation (beginning of haustoria formation) and at 18 h after inoculation (developing haustoria). Immediately after removal of the inducer germlings the treated coleoptiles and controls were inoculated with a challenge inoculum of *B. graminis* or *E. pisi*.

When induced susceptibility was assessed by using the non-pathogen *E. pisi* as a challenger, susceptibility was not observed in the coleoptiles in which the germlings had been removed before haustoria formation (Fig. 1). Induced susceptibility was observed only when germlings of the inducer had been removed 18 h after inoculation, after haustoria of *B. graminis* had formed in the coleoptile. In contrast, when the induced susceptibility was assessed by

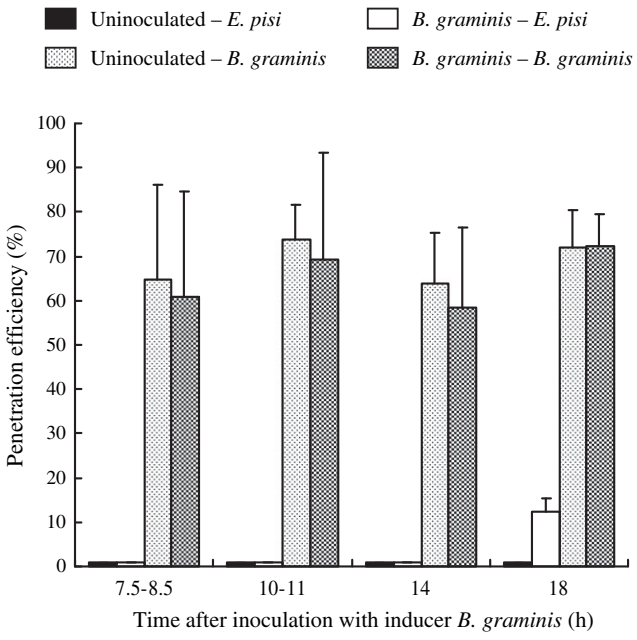

Fig. 1. Evaluation of susceptibility to the non-pathogen *Erysiphe pisi* or to the pathogen *Blumeria graminis* induced by prior attack by *B. graminis.* The fungal germlings of *B. graminis* were removed by treatment with cellulose acetate at various times after inoculation: at 7.5–8.5 h after inoculation (maturation of appressoria), at 10–11 h after inoculation (beginning of penetration), at 14 h after inoculation (beginning of haustoria formation), and at 18 h after inoculation (developing haustoria). Immediately after removal of the inducer germling, the treated coleoptiles and controls were inoculated with a challenge inoculum of *B. graminis* or *E. pisi*.