Localization of Induced Resistance and Susceptibility in Barley Leaves Inoculated with the Powdery Mildew Fungus

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ABSTRACT

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Localization of induced susceptibility and resistance was studied with genetically defined barley *(Hordeum vulgare)* cultivars inoculated with races of *Erysiphe graminis hordei* and *E. graminis tritici* by a double inoculation method. Establishment of a rejection reaction required 6 hours, whereas inhibition of secondary hyphae became detectable after a 9-hour interval. Both the rejection and growth inhibition activities elicited by a nonpathogenic race of *E. graminis* were restricted to the site of primary incompatible interaction, suggesting that the primary recognition and subsequent physiological conditioning toward resistance is strictly localized. Barley leaves exposed to a compatible race became susceptible to nonpathogenic *Sphaerotheca fuliginea*. The degree of susceptibility of cells was dependent on their distance from the cells that harbored the primary compatible haustorium. The nonpathogen established infection at a high frequency (75%) in cells that harbored compatible haustoria, but at low frequency in cells located four cell-rows away from the primarily induced cells. Thus, the induced susceptibility also was localized. These results suggest that the primary recognition becomes irreversible once the host cells are physiologically conditioned and that information required for these cellular conditionings is not transferred a long distance, at least not in powdery mildews.

Additional key words: primary recognition, cross-protection.

Preliminary inoculation of plant cells with a compatible pathogen predisposes the invaded cells to a primarily incompatible race and to nonpathogens (11, 13, 14, 15, 16, 17, 18, 19, 20). In previous papers (13, 14), we have shown with powdery mildew of barley that leaves previously inoculated with an incompatible race were induced to become resistant to a compatible race. Because the gene-for-gene relationship is well known in powdery mildew of barley (2, 3, 6, 8, 9, 10), these phenomena pose a critical question about gene functions of the host cell and parasite. On the basis of modification of cellular response to a permissive state, we inferred that the induction of accessibility by a compatible race in the invaded cells must be an active and irreversible process determined by gene interaction (14). The induction in this context is defined to involve both the primary recognition of an invading compatible pathogen and subsequent physiological conditioning toward accessibility.

This communication shows that induced accessibility and resistance are localized in leaves of barley inoculated with a compatible or an incompatible race, and discusses the importance of this phenomenon in the interpretation of the events associated with primary cell-parasite interaction.

MATERIALS AND METHODS

Hosts.—Two cultivars of barley (*Hordeum vulgare* L.) were used: Kobinkatagi (compatible to race 1) and H.E.S. 4 (compatible to race Hh4). One cultivar of wheat [*Triticum aestivum* (L.) ssp. *vulgare* (Vill.) MK 'Norin No. 4'] and a cultivar of melon (*Cucumis melo* L. 'Earl's Favourite') were used for the propagation of their respective powdery mildew fungi. Seeds of barley and wheat were soaked overnight in tap water and germinated on a filter paper bed in a petri dish. Germinated seeds of a uniform size were planted in vermiculite in clay pots (15 cm in diameter) and grown in a phytotron controlled at 20 C for 10-12 days. The melon plants were grown at 25 C in potted soil until 10-12 leaves were fully developed.

Fungi.—*Erysiphe graminis* DC. f. sp. *hordei* Marchal, race 1 and race Hh4; *E. graminis* DC. f. sp. *tritici* Marchal, race t2; and *Sphaerotheca fuliginea* (Schl.) Pollaci (undetermined race) were used. The races of barley and wheat fungi were supplied by U. Hiura and were cultured on their respective compatible cultivars. Conidia were synchronized as follows: leaves of 10-day-old seedlings were inoculated with fresh conidia, incubated for 8 days at 20 C, conidial chains were blown away, and the conidia which then emerged within the subsequent 12 hours were used as inoculum. Conidia of *S. fuliginea* were obtained from colonies on melon leaves that had been infected for 14-20 days.

Induction of resistance and accessibility.—Leaves of 10- to 12-day-old seedlings were inoculated at the middle part of their abaxial surface with a compatible or an incompatible race by employing a soft hair brush to give about 10-20 conidia per one microscopic field at × 150 magnification, incubated in an artificially illuminated growth chamber (Toshiba Plantlux, 2,000 lux) controlled at 20 C for an appropriate period of time. The primary inocula were removed by rubbing the inoculated site with

a wet cotton ball. Noninoculated leaves also were rubbed to serve as controls. The leaves then were challenge-inoculated with a compatible or an incompatible race to measure the degree of induced accessibility or resistance. The second inoculation was made, unless otherwise mentioned, at the site of primary inoculation. Several leaves were retained unchallenged to check the efficiency of the primary inducer race. The average number of spores left over from rubbing was calculated and was taken into account in the estimation of the infection frequency of challenger. The percentage of spores that produced elongating secondary hyphae (elongating secondary hyphae frequency) and the length of secondary hyphae (secondary hyphae length) were measured 48 hours after inoculation and were taken as parameters for the degree of affinity of the pathogen for invaded cells, as was described previously (13, 14).

Estimation of localization.—Localization of induced resistance was estimated by dividing the leaf surface into three areas, induced (middle) area and proximal and distal areas (each 10 mm in length), each 5 mm from the induced area. Accessibility induction was estimated on a cell-to-cell basis by designating the cell invaded by the inducer race as D_0, and the cell transversely adjacent to it as D_1, the one next to it as D_2, and so forth. Spore density was controlled by use of a soft hair brush. Four leaves were stroked in succession with a brush impregnated with conidia, and the third and fourth stroked leaves were used for challenge inoculation. These leaves ordinarily had less than 10 conidia per microscopic field at × 150 magnification. The final microscopic examination was restricted to those fields with less than three conidia per field.

RESULTS

Time course of preinfectional behavior of compatible or incompatible races and cellular response.—Barley leaves of cultivar H.E.S. 4 were inoculated with the compatible race of *E. graminis hordei* and race t2 of *E. graminis tritici*. Germination, appressorium formation, and halo formation at the inoculated site were observed. Results are shown in Fig. 1 for the incompatible host-parasite combination. The compatible and incompatible races had comparable percentages of conidial germination and appressorium formation; these responses reached a plateau at about 15 hours. Halo formation, detected by cotton-blue staining, was observed as early as 7 hours after inoculation with the nonpathogenic race; maximum halo development occurred by 18 hours postinoculation. Halo formation in the compatible interaction seemed to be initiated slightly later than in the incompatible combination. In the compatible combination, primordial haustoria became observable 9-10 hours after inoculation, and 50% of the appressoria were accompanied by the primordial haustoria within 15 hours postinoculation.

Time required for establishment of a resistant reaction.—Leaves of barley cultivar H.E.S. 4 were inoculated with race t2, incubated at 20 C for certain periods of time, and then challenged at the same site with

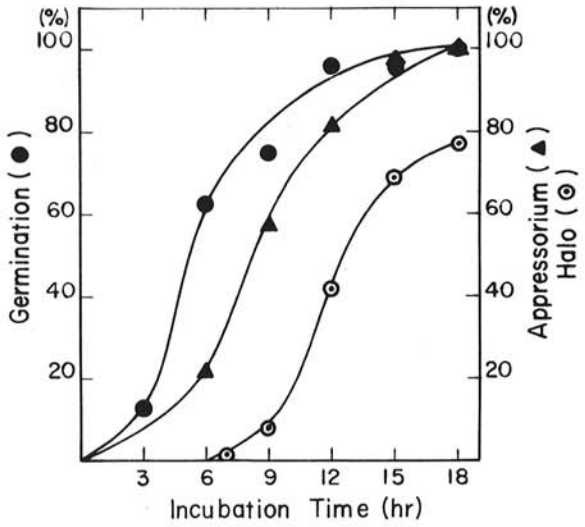

Fig. 1. Germination and appressorium formation by *Erysiphe graminis* f. sp. *tritici* (race t2) on barley leaves (cultivar H.E.S. 4), and halo formation in epidermal cells. Germination is calculated (percentage) at 18 hours as 100. Others are observed values.

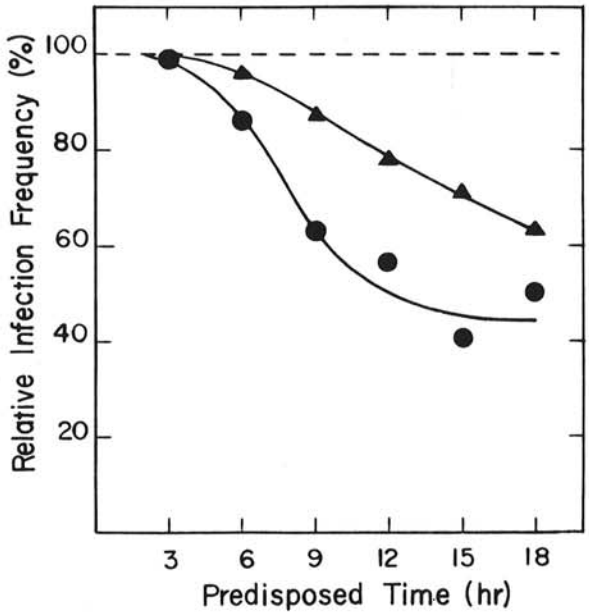

Fig. 2. Establishment of infection by *Erysiphe graminis hordei* (race Hh4) on leaves of barley (cultivar H.E.S. 4), which were predisposed with *E. graminis tritici* (race t2) for various times. Elongating secondary hyphae frequency (●) and secondary hypha length (▲) are shown as a percentage of the values for inoculations on nonpredisposed leaves.

the compatible race Hh4. Affinity indices of compatible race in leaves with induced resistance are shown in Fig. 2. A statistically significant decrease in frequency of elongating secondary hyphae was observed on leaves that had been double-inoculated at a 6-hour interval, whereas secondary hyphae length decreased when inoculated with a 9-hour interval. No detectable necrosis or chlorosis was observed in the leaves with induced resistance within the experimental period.

Location of induced resistance.—Leaves of cultivars Kobinkatagi and H.E.S. 4, previously inoculated with race t2 were challenged with their respective compatible, race 1 and race Hh4, to estimate the degree of localization of induced resistance. Results (Fig. 3) show that the induced resistance was localized at the site where the preliminary inoculation was made with the nonpathogenic race. This type of resistance therefore was not translocated to areas 5 mm from the induced area, at least not in the early phase of the infection.

Location of induced accessibility.—Leaves of barley cultivar H.E.S. 4 were inoculated with race Hh4 at a density less than 10 conidia per microscopic field, incubated at 20 C for 48 hours, and then challenged with *S. fuliginea* to determine whether or not the establishment of infection by the nonpathogen depends on the distance from the cell harboring the primary compatible haustorium. Results (Fig. 4) showed that the nonpathogen colonized the cells harboring the compatible haustorium with a very high frequency. The cells transversely adjacent to the haustorium-harboring cells also were accessible to the nonpathogen, even though the frequency of colonization decreased rather sharply. Cells in the second and third rows had little or no colonization by the nonpathogen. Other experiments suggest that this translocation of accessibility from cell to cell is dependent on time from primary inoculation to challenge inoculation.

DISCUSSION

Morphological and ultrastructural studies on the early phase of powdery mildew infection indicate that no difference can be detected between the compatible and incompatible interactions until the penetration hyphae of the parasite reach the plasmalemma of the host cell (2, 3, 7). The events associated with specificity occur after penetration; apparently, specificity is determined by interaction of the genic components of the host and the parasites (2, 3, 6, 8, 9, 10).

Our results extend previous findings on the primary host-parasite interaction in powdery mildews. Host cells first placed in contact with a nonpathogenic (or incompatible) race became inaccessible to the primarily compatible race. The induced resistance was evident as a reduction in ability of the pathogen to infect, and as inhibition of hyphal growth. The frequency of infection by the compatible race was reduced significantly when the leaves were inoculated with a nonpathogenic race 6 hours prior to inoculation with a compatible race; retardation of hyphal growth was not evident until 9 hours after inoculation. These observations, together

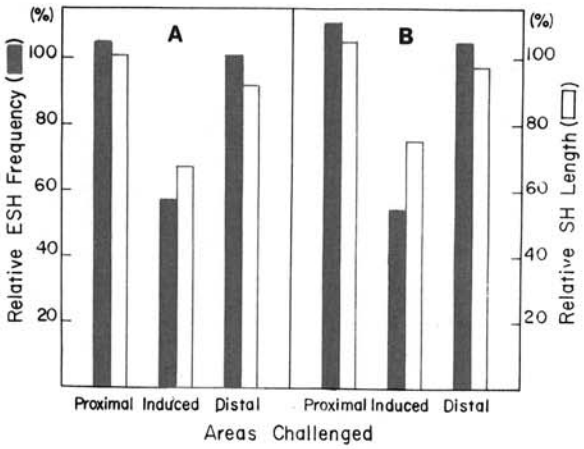

Fig. 3. Effect of induced resistance on establishment of infection by a compatible race on barley leaves. Leaves were first inoculated with *Erysiphe graminis tritici* (race t2), then were challenged with the compatible race. Indices of respective noninduced control were expressed as 100. Tested with cultivar Kobinkatagi-*E. graminis tritici* race t2 – *E. graminis hordei* race 1(A), and cultivar H.E.S. 4 – *E. graminis tritici* race t2 – *E. graminis hordei* race Hh4 (B). Proximal and distal areas were separated respectively 5 mm from the induced middle part of leaves (10 mm long).

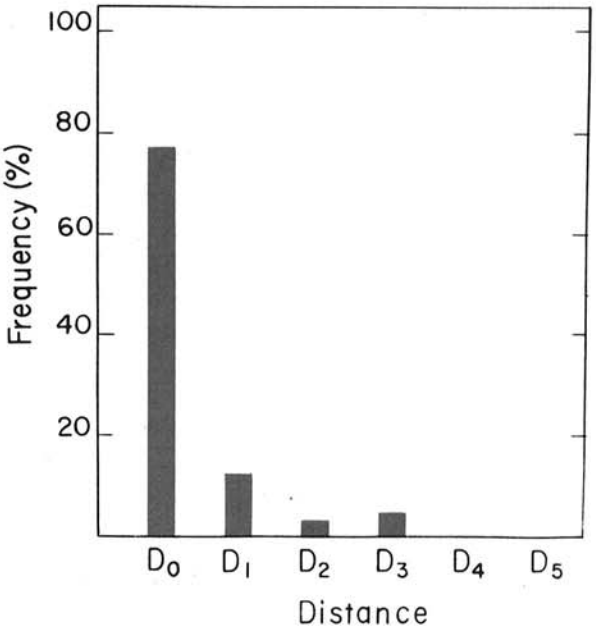

Fig. 4. Effect of preinoculation with *Erysiphe graminis hordei* (race Hh4) (a compatible fungus) on infection by *Sphaerotheca fuliginea* in leaves of barley (cultivar H.E.S. 4). Induction period was 48 hours. D_0 represents cell that harbored the haustorium of the compatible race (Hh4), D_1, cells transversely adjacent to it, D_2 the one next to it, and so forth.

with previous results (13, 14), support the hypothesis of secondary resistance (8). The data also indicate that the rejection reaction is established much earlier than the cellular conditioning for accessibility, which required 15-18 hours (14). The difference in time required for induction of resistance and induction of accessibility should be considered in relation to the time course of the infection process. Assuming that no inhibitory mechanism operates until the penetration hyphae come in contact with the host cell plasmalemma (10 hours), the above results indicate that cellular conditioning toward inaccessibility will be established within 16 hours, whereas conditioning for accessibility requires 25-28 hours. These times are too long to explain the actual infection process, because by these times, the incompatible race should have been rejected and the compatible race should have formed haustoria bearing few projections (Fig. 1). Therefore, the recognition and cellular conditioning must have occurred much earlier. In a previous paper, we have shown that phytoalexin activity can be detected as early as 8 hours after inoculation with an incompatible race (12). Since phytoalexins are elicited by and produced after the recognition of an incompatible race, the recognition and subsequent physiological conditionings should have been accomplished prior to 8 hours of incubation. Thus, the results indicate that rejection and accessibility became detectable at a later time. The same rationale applies to the interpretation of the incubation time (12 hours) required for suppression of the resistance response and to induction of the hypersensitive response in potato tissue by compatible or incompatible races of *Phytophthora infestans* (18). The rationale applies also to the time (8-12 hours) required for induction of hypersensitivity in oat leaves by the crown rust fungus (15).

The present results also support the previous hypothesis that recognition of invading fungus by host cells becomes irreversible once the cells are physiologically conditioned (14). The cells induced to become inaccessible recognize compatible races as incompatible and those induced to become accessible fail to recognize incompatible races or some nonpathogens as a foreign entity, permitting their entrance and parasitic association. This irreversibility of recognition will be elaborated further elsewhere (Ouchi et al., *unpublished*).

Another important conclusion from our data is that induction of resistance and accessibility is not translocated very far in the tissues. Even though the information on cellular response is transferred eventually to a few nearby cells, the induced inaccessibility or accessibility is highly localized at the induced site. This does not, however, imply the nonparticipation of surrounding cells or tissue in these processes. The results suggest that an active principle involved in the initiation and regulation of the host gene system toward rejection or accessibility may not be a low-molecular-weight compound. Érsek (5) demonstrated that the defense reaction induced in wheat leaves by the barley powdery mildew fungus also was localized. However, resistance information in bean anthracnose appears to become systemic (1, 4).

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