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Localized Induced Resistance to *Erysiphe graminis* f. sp. *hordei* in Near-Isogenic Barley Lines.

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近 同質遺傳子 보리系統에서 보리흰가루病에 對한 局部的 誘導抵抗性

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ABSTRACT

Localized resistance against a virulent race of *Erysiphe graminis* f. sp. *hordei* by prior inoculation of a virulent or an avirulent race of the same fungus was induced on the near-isogenic barley leaves. Induced resistance could be detected within one hour following challenge inoculation with a virulent or an avirulent inducer race, but the resistance increased greatly as the interval between the two inoculations was increased, showing the highest level by 6-9 hours of exposure to the inducer race. The level of the induced resistance was proportional to the amount of inducer inoculum applied. The resistances elicited by virulent or avirulent inducer races were similar with respect to the level of resistance and the time needed for its induction.

Key words: induced resistance, powdery mildew, barley.

要 約

近 同質遺傳子를 가진 2 보리系統을 使用, 2 系統에 親和性 혹은 非親和性 反應을 나타내는 보리흰가루病菌 1菌系를 前接種하여 抵抗性을 誘導하고 接種部位로부터 前接種源을 經時的으로 除去한 다음 同一部位에 보리 2 系統에 各各 親和的인 2 菌系(1 菌系는 前接種源과 同一)를 後接種하여 본 結果 前接種源에 親和性 및 非親和性 反應을 나타내는 2 系統 모두 前接種源과 한시간 以內의 짧은 接觸에 依해서도 抵抗性이 誘導되었고 그 抵抗性의 程度는 接觸時間이 길어짐에 따라 增加하였으며 6～9 時間에 最大値에 達하였다. 前接種源에 親和的, 非親和的인 系統間의 誘導抵抗性의 程度와 抵抗性이 誘導되는데 必要한 時間은 差異가 없었다. 그러나 양 組合 모두 誘導抵抗性의 程度는 使用된 前接種源의 量에 比例하여 增加하였다.

INTRODUCTION

It seems to be a well known phenomenon that host plants are protected against virulent pathogens

by prior inoculation with avirulent pathogens. Probably because of technical difficulties making the experiment, however, localized induced resistance to virulent pathogens have never been demonstrated by prior inoculation with the same virulent pathogens. Ouchi et al. (10,11) found that inoculation of barley leaves with avirulent races of *Erysiphe graminis* f. sp. *hordei* elicited resistance against subsequent infection by virulent races. The resistance was strictly localized and the establishment of resistance by the avirulent race required 6 hours. Only avirulent races were shown to act as inducers.

The objectives of this investigation are to examine if previous inoculation of virulent races of *E. graminis* f. sp. *hordei* may locally protect barley leaves from subsequent infection by the same virulent races in near-isogenic barley lines and to determine the different levels of induced resistances elicited by virulent and avirulent races and the time needed for resistance induction.

MATERIALS AND METHODS

Barley and mildew isolates: Two near-isogenic lines of barley (*Hordeum vulgare* L.), No. 011301 and No. 112405, were obtained from Miss Munk at Department of Plant Pathology, The Royal Veterinary & Agricultural University, Denmark. The former line possesses the resistance gene Ml-a1, and the latter possesses two resistance genes, Ml-a13 and Ml-(Ru3). Each of the three genes controlled high resistance against *E. graminis* f. sp. *hordei*. The barley seedlings were grown in plastic pots (8 x 8 x 8cm) with nutrient soil. The environmental conditions for cultivation of plants were ca. 20°C and illumination of 8000 lux (400w fluorescent lamp) for 14h of light/day.

The races of *E. graminis* f. sp. *hordei* used were A6 and H21. Reactions of the two barley lines to the races were:

Near isogenic barley line	Resistance gene	Infection type A6	H21
011301	Ml-a1	4	0
112405	Ml-a13+Ml(Ru3)	0	4

0: incompatible reaction, 4: highly compatible reaction

The isolates were maintained on the susceptible cultivar Pallas in a separate green house under the same environmental conditions as described above.

Inoculation: When the 1st or 2nd leaves of barley plant were fully expanded, 8 leaves of the same age from each pot were horizontally fixed with the adaxial side up on a polyacrylamide plate by using rubber bands. The inducer conidia formed on susceptible plants 8 days after inoculation were uniformly inoculated into the fixed leaves by the aid of a 50 cm high rectangular inoculation tower. The tower was kept in position for 30 minutes until all spores had settled. The application of inoculation tower ensured that only a particular area of the horizontally fixed leaves was inoculated with the inducer conidia. Two spore concentrations of ca. 20 and 400 conidia/mm^2 leaf area were used for inducer inoculation.

The inducer inoculum was removed by gently rubbing the inoculated leaves in an upward direction with wet cotton at intervals after inoculation. The challenger races were then inoculated by shaking infected leaves 2m above the plants in an inoculation chamber. The challenger conidia were obtained from susceptible infected plants 6 days after inoculation. The use of young conidia prevented the formation of spore lumps on the plants.

Accessment of induced resistance: Induced resistance was quantitatively estimated by counting mildew colonies per 20cm^2 of leaf area 7 days after the challenge inoculation. Plants inoculated only with the compatible challenger race were used as controls. Changes in the infection types of challenger races were read on 0 to 4 scales 9 days after inoculation (9).

RESULTS

Removal of mildew conidia from inoculated leaves: The aim of this work was to determine whether or not prior inoculation of a virulent race of *E. graminis* f. sp. *hordei* can protect barley against subsequent infection by the same race. To achieve this goal it was absolutely necessary to develop a technique by which the inducer inoculum could be removed from the preinoculated leaves before inoculation with challengers.

The efficiency by which conidia of the compatible race H21 could be removed from the adaxial leaf surface of barley isoline 112405 was shown from Table 1. Only very few mildew colonies were produced when conidia were removed within 9 to 12 hours after inoculation. At an inoculum density of 20 conidia/mm^2, 4 to 7 colonies formed on the 20cm^2 of leaf area. In contrast, the number of mildew colonies on the control leaves from which conidia inoculated had not been removed were uncountable. Since the infection pegs of the fungus penetrate the epidermal tissues, it seems difficult to remove the conidia infected 12 hours after inoculation. As a result, 156 and 242 mildew colonies were produced on 20cm^2 of leaf area 18 hours after inoculation at the inoculum densities of 20 and 400 conidia/mm^2, respectively (Table 1). Similar results were also obtained in the time course studies with the compatible interaction between race A6 and barley isoline 011301.

Table 1. Numbers of colonies formed after removal of the preinoculated compatible conidia (H21) of *Erysiphe graminis* f. sp. *hordei* on leaves of the barley isoline 112405

Hours after inoculation[a]	No. of colonies/20cm^2 leaf area after inoculation of:	
	20 conidia/mm^2	400 conidia/mm^2
Control	Uncountable	Uncountable
1	5 ± 5[b]	10 ± 4
3	5 ± 3	9 ± 3
6	6 ± 2	9 ± 8
9	4 ± 1	10 ± 3
12	7 ± 4	27 ± 8
15	58 ± 27	150 ± 47
18	156 ± 33	242 ± 31

a The time when the compatible conidia inoculated were removed by gently rubbing the leaf surfaces with wet cotton.

b Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves.

It was examined whether the physical removal of the inducer race with wet cotton could alter the susceptibility of barley plants to powdery mildew. The rubbing of barley leaves with wet cotton did not affect the number of mildew colonies compared to the untreated control (Table 2).

Table 2. The effect of physical rubbing of barley leaves with wet cotton on susceptibility to *Erysiphe graminis* f. sp. *hordei*

Hours before inoculation[a]	No. of colonies/20cm^2 leaf area[b]	
	011301/A6[c]	112405/H21
Control	305 ± 15	338 ± 11
0	311 ± 20	325 ± 19
48	297 ± 23	335 ± 26
96	307 ± 8	328 ± 29

a After rubbing the adaxial leaf surfaces with wet cotton at the indicated time intervals, the compatible conidia were inoculated on the same leaf surfaces.

b Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves.

c Barley line/mildew isolate.

Exposure time required for induction of resistance to inducer races: To determine the time needed for induced resistance, leaves of the two barley isolines were inoculated at time intervals with an incompatible or a compatible inducer race of *E. graminis* f. sp. *hordei*. After removal of the inducer, the compatible challenger race was inoculated at the same sites of barley leaves. Both incompatible and compatible inducer races caused a reduction in number of mildew colonies produced by subsequent compatible races (Table 3 and 4). The reduction occurred in less than one hour of the time, being greater with increasing the time for which the leaves were exposed to the inducer race

Table 3. Induction of resistance in leaves of barley isoline 112405 by pre-exposure to the incompatible inducer race A6 of *Erysiphe graminis* f. sp. *hordei*[a]

Exposure time to inducer(h)	No. of colonies/20cm² leaf area	
	1st leaf	2nd leaf
Control	348±22(100.0)	359±25(100.0)
1	299±38(85.9)	291±39(81.1)
2	278±46(79.9)	-
3	-	176±43(49.0)
4	122±47(35.1)	-
6	87±37(25.0)	104±11(29.0)
9	91±21(26.1)	101±19(28.1)

a Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves. The inducer and challenger were inoculated on the adaxial leaf surface. Inducer inoculum was removed immediately prior to challenge inoculation. Inoculum density of inducer race (A6) was ca. 400 conidia/mm² and that of challenger race (H21) ca. 20 conidia/cm².

Table 4. Induction of resistance in leaves of barley isoline 011301 by pre-exposure to the compatible inducer race A6 of *Erysiphe graminis* f. sp. *hordei*[a]

Exposure time to inducer(h)	No. of colonies/20cm² leaf area	
	1st leaf	2nd leaf
Control	397±23(100)	415±22(100)
1	330±42(83.1)	344±30(82.9)
2	306±42(77.1)	-
3	-	187±52(45.1)
4	161±56(40.6)	-
6	109±21(27.5)	125±37(30.1)
9	121±34(30.5)	104±35(25.1)

a Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves. The inducer and challenger were inoculated on the adaxial leaf surface. Inducer inoculum was removed immediately prior to challenge inoculation. Inoculum density of inducer race (A6) was ca. 400 conidia/mm² and that of challenger race (A6) ca. 20 conidia/cm².

prior to the challenge inoculation, regardless of previous infection of incompatible or compatible races. The phenomenon was characterized only as a reduction in number of mildew colonies.

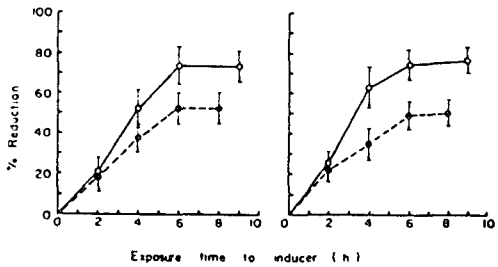

Fig. 1. Effects of conidia concentration of and exposure time to inducer races on induction of resistance in barley leaves to subsequent infection by challenger races of *Erysiphe graminis* f. sp. *hordei*. Ordinates indicate % reduction in number of colonies of challenger. Inducer inoculum was removed immediately before challenge inoculation. Vertical bars represent the standard deviation. A: barley isoline 011301, inducer race H21 (incompatible), challenger race A6 (compatible). B: barley isoline 112405, inducer race H21 (compatible), challenger race H21 (compatible). Inoculum density of inducer: ca. 400 conidia/mm² leaf area (o--o), ca. 20 conidia/mm² leaf area (●···●).

There was no change in infection types by 6-9 hours of exposure to inducer races. No differences were found in the results obtained in the 1st and the 2nd leaves.

The effect of inoculum density of the inducer race on induction of resistance: The production of mildew colonies of challenger races was gradually reduced with increasing inoculum density of the inducer races (Fig. 1). No differences were in the results inoculated with incompatible and compatible inducer race. Figure 1 shows that inoculation with 20 conidia of the compatible inducer race per mm² leaf area caused 50% reduction in number of mildew colonies produced by subsequent challenge inoculation with the same race. When 400 conidia per mm² leaf area was applied, 75% of reduction resulted in number of mildew colonies. A time interval of 6-9 hours between the first and second inoculation showed the highest level of protection against powdery mildew.

DISCUSSION

Ouchi et al.(10,11) found that inoculation of barley leaves with an avirulent race of *E. graminis* f. sp. *hordei* protected against virulent races subsequently applied to the same leaves. Recently, Hwang and Heitefuss(5) found that inoculation of the lower leaves of barley plants with a virulent or an avirulent race of mildew fungus systemically protected the upper leaves against infection by virulent races. However, local protection against *E. graminis* f. sp. *hordei* incited by previous inoculation with a virulent race has not been known.

A prerequisite for using virulent races of *E. graminis* as inducers is that the inducer inoculum must be effectively removed from the leaf surfaces prior to the application of challenger races. Our results demonstrated that the use of wet cotton may be very effective in removing inducer inoculum from the leaves, provided it is done before the inducer become established in the host. Under our experimental conditions the virulent inducer races produce few colonies when inoculum was removed within 9 hours after inoculation. Since physical agent may give rise to accumulation of phytoalexin (1), it is essential to note that the physical effect of rubbing the leaves with wet cotton did not alter the susceptibility of barley leaves to powdery mildew.(Table 2). The destructed conidial debris of inducer race which may be remained on leaf surfaces after rubbing with wet cotton doesn't seem to induce resistance of barley plants to powdery mildew (unpublished our data).

Resistance was induced within one hour following inoculation with a virulent or an avirulent inducer race, being more intensive as the interval between the two inoculations was increased by 6 hours. This early host response suggest that induction of resistance may be initiated prior to appressorial penetration which occur at the earliest 6-8 hours after inoculation(3,6). However, recent studies of the early stages of barley powerdy mildew interactions have demonstrated that primary germ tubes which are produced during the initial stage of conidial germination may penetrate the host cell wall and incite a host response as early as 1-2 hours after inoculation(2,8). This occurres before the appressoria differentiate and establish interactions with the host. An early host-pathogen interaction at the pre-appressorial stage of *E. graminis* was also found in wheat(4) and in oat(7). These results agreed with our findings that one hour of host exposure to an inducer race is enough to establish resistance against a subsequent challenger race, suggesting that at least the early stage of induced resistance in barley against *E. graminis* f. sp. *hordei* may result from interactions between the non-appressorial, primary germ tube and host cell.

The fact that the level of induced resistance was proportional to the amount of inducer inoculum may explain an increase in the predisposed cells by inducer since the induced resistance seemed to be localized to site of inducer inoculation(10).

The resistances elicited by compatible and incompatible inducer races were similar with respect to the level of resistance and the time needed for induction of resistance, indicating that the resistance is non-specific in its effect.

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