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Characteristics of 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 誘導抵抗性의 特性

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ABSTRACT

Some characteristics of the resistance induced by *Erysiphe graminis* f. sp. *hordei* on near-isogenic barley leaves were evaluated. Inoculation of heat-killed incompatible inducer conidia did not protect barley leaves against compatible challenger race when the inducer inoculum was removed prior to inoculation of challenger. However, the colony formation of challenger race was greatly reduced by 87.1 to 91.2% when the heat-killed inducer inoculum was not removed from the leaves. Although incompatible inducer conidia were removed before they penetrate the host cell, colony formation of challenger was markedly decreased without change in its infection type. After penetrating the host cell by inducer, however, a change in infection types occurred on the challenged leaves. Irrespective of compatibility of previously inoculated inducer on middle part of leaves, there was no reduction in colony formation of challenger race both on the adjacent acropetal and basipetal parts of the same leaves free of inducer inoculation. The colonies formed on the basipetal part by challenger race showed normal 4 type, whereas the infection type of colonies formed on the acropetal part was somewhat changed, thereby sporulation being reduced. The possibility of translocation of resistance-inducing factors was discussed.

Key words: induced resistance, powdery mildew, barley.

要 約

近 同質遺傳子 보리系統에서 보리흰가루病에 對한 誘導抵抗性의 몇 가지 特性이 평가되었다. 熱處理하여 살생시킨 非親和性菌의 胞子를 보리 1系統에 前接種한 後 經時的으로 前接種源을 除去한 다음 同一部位에 親和性菌을 後接種하였을 때 抵抗性은 誘導되지 않았다. 그러나 살상된 前接種菌을 除去하지 않고 親和性菌을 後接種하였을 경우는 親和性菌에 依한 菌叢形成이 87.1~91.2%까지 減小하였다. 感染이 이루어지기 前에 前接種했던 非親和性菌을 除去하고 親和性菌을 後接種하였을 경우에는 親和性菌에 依한 菌叢形成의 減少만이 일어났으나 感染이 이루어진 後에 非親和性菌을 除去하고 親和性菌을 後接種한 경우에는 親和性菌

의 菌叢形成感少는 물론, 形成된 菌叢의 病斑型이 모두 변하였고 그 中 몇몇은 菌叢주위에 褐色斑點이 形成되었다. 親和性菌 혹은 非親和性菌을 보리 1次展開葉의 中央에만 前接種한 後 다시 잎의 全面에 經時的으로 親和性菌을 後接種하였을 경우 中央部位隣接上段과 隣接下段에서 後接種源에 依해 形成된 菌叢의 數는 대조구와 차이가 없었다. 그러나 隣接下段에서는 形成된 親和性菌叢의 病斑型이 변하지 않았으나 隣接上段에서는 菌叢의 病斑型이 약간 변화하였고 또 褐變되었고 이들 菌叢들에 依해 形成된 胞子의 數는 대조구보다 顯著히 感少하였다.

INTRODUCTION

Kuć and co-workers(8) who has intensively studied on induced resistance determined a difference between two functionally different forms of induced resistance, systemic and local. In the system of barley and powdery mildew, induced systemic resistance has recently been demonstrated by Hwang and Heitefuss(6) who found that inoculation of the lower leaves with a compatible or an incompatible race of *Erysiphe graminis* f. sp. *hordei* partially protected the upper leaves against a compatible race. Induced local resistance against a compatible race of *E. graminis* f. sp. *hordei* was also demonstrated in barley by prior inoculation with an incompatible race of the fungus(11,12).

In our previous work, localized induced resistance in barley leaves could be detected within an hour following challenge inoculation with a virulent or an avirulent inducer race of *E. graminis* f. sp. *hordei* and it was proportional to the amount of inducer inoculum applied, and the resistances elicited by virulent or avirulent inducer race were nonspecific (1).

This paper reports a change in infection type of colonies formed by challenger race of *E. graminis* f. sp. *hordei* and a translocation of the resistance induced by powdery mildew fungus in barley leaves. Characteristics of the resistance made by host-inducer interaction is discussed.

MATERIALS AND METHODS

Barley and mildew isolates: Two near-isogenic lines of barley (*Hordeum vulgare* L.), No. 011301 and No. 112405, and two races of *Erysiphe graminis* f. sp. *hordei*, A6 and H21, were used. The barley lines possess different resistance genes which control resistance against mildew fungus, and react contrary to each other with the races A6 and H21:

Near-isogenic barley lines	Resistance gene	Infection type A6	Infection type H21
011301	M1-a1	4	0
112405	M1-a13 + M1(Ru3)	0	4

0: incompatible reaction,
4: highly compatible reaction.

The 1st fully expanded leaves of barley plant were used. Experimental conditions and general inoculation procedure followed the previous methods(1) unless stated otherwise.

Heat-treatment of conidia: Heat-killed inducer conidia were obtained by placing the diseased plants in pots with 7 day-old conidia in the controlled humid incubator at ca. 38°C for 24 hours. The conidia never germinated on barley leaves in this experiment.

Inoculation on different leaf parts: The adaxial surface of the 1st leaves on a polyacrylamide plate were vertically compartmented 3 parts by using rubber bands. Inducer of a compatible or an incompatible race was inoculated on the middle part, 3cm in length, using an inoculation tower, after covering all the other adjacent acropetal and basipetal parts (each 2cm in length) with polyvinyl bag. The bag was removed 24 hours after inducer inoculation. Challenger race was then inoculated on the whole adaxial surface of the same leaves 24, 48 and 72 hours after exposure to inducer races.

Measurement of conidia: The number of mildew colonies on the acropetal or basipetal part of leaves were counted 9 days after the challenge inoculation. The parts of leaves were then carefully

cut with scissors, and put into the small test tubes filled with 10ml of Fluorochemical-45. The tubes were shaken thoroughly, and the mildew conidia in suspension were counted using a haemocytometer.

Accessement of induced resistance: Induced resistance was quantitatively estimated by counting mildew colonies per 20cm^2 of leaf area, or by counting mildew spores per 300 colonies produced on the same leaf area 9 days after the challenge inoculation. The plants inoculated only with the compatible challenger race were used as controls. The infection types of challenger races were read on 0 to 4 scale 9 days after inoculation (10).

RESULTS

Heat-killed incompatible conidia as a inducer: Compatible challenger race produced colonies of normal infection type 4 on susceptible barley leaves which had been previously inoculated with heat-killed incompatible inducer race when the inducer inoculum was removed prior to inoculation of challenger (Table 1). There was no reduction in colony formation of challenger race inoculated subsequently 24, 48 and 72 hours after inducer inoculation. However, colony formation of challenger race was reduced by 87.1 to 91.2% when heat-killed inducer inoculum was not removed from the leaves prior to inoculation of challenger, but the colonies formed did not change in their infection types. Similar results were obtained when heat-killed compatible conidia were previously inoculated on the same leaves of barley isoline and challenge inoculated with the compatible races.

Changes in infection type of challenger race: When incompatible inducer inoculum was removed 1, 6, 24 and 36 hours after inoculation and then immediately challenged with a compatible race, the number of colonies formed by challenger race were significantly reduced, thereby resulting in ca. 80% reduction by 24 to 36 hours of exposure to the inducer races (Table 2). When inducer inoculum was removed within 6 hours after inoculation, the colonies of challenger race formed on the predisposed barley leaves showed normal infection type 4. When the inducer inoculum was removed 24 and 36 hours after inoculation, however, infection types of all the colonies formed by challenger race were changed from originally 4 to 1, 2 or 3 type, some of them showing necrosis around them. When inducer inoculum remained during challenge inoculation, few colonies were formed by challenger race despite of challenge inoculation within an hour after inducer inoculation. A few colonies formed were changed in their infection types, accompaning with some necrosis around them.

Table 1. Number of colonies formed by the compatible challenger race A6 of *Erysiphe graminis* f. sp. *hordei* on the barley isoline 011301 previously inoculated with heat killed incompatible inducer race 1121[a]

Time interval[b] (h)	Inducer removed		Inducer not removed	
	No. of colonies / 20 cm^2 leaf area	Infection type	No. of colonies / 20 cm^2 leaf area	Infection type
Control	341 ± 15 (100.0)	4	341 ± 15 (100.0)	4
24	343 ± 15 (100.0)	4	30 ± 27 (8.8)	4
48	339 ± 12 (99.4)	4	44 ± 21 (12.9)	4
72	340 ± 12 (99.7)	4	37 ± 33 (10.9)	4

[a] Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves. The inducer and challenger were inoculated on the adaxial surface of the first developed leaves. Inoculum density of heat killed inducer race was ca. 400 conidia/mm^2 and that of challenger race ca. 17 conidia/cm^2.

[b] Hours between the inducer and challenge inoculation. At the time intervals indicated, heat-killed inducer inoculum was removed by using wet cotton or not removed prior to inoculation of the challenger.

Table 2. Number of colonies formed by the compatible challenger race A6 of *Erysiphe graminis* f. sp. *hordei* on the barley isoline 011301 previously inoculated with incompatible inducer race H21[a]

Time interval[b] (h)	Inducer removed		Inducer not removed	
	No. of colonies / 20 cm² leaf area	Infection type	No. of colonies / 20 cm² leaf area	Infection type
Control	330 ± 12 (100.0)	4	330 ± 12 (100.0)	4
1	261 ± 13 (79.1)	4	5 ± 9 (1.5)	1-3
6	81 ± 34 (24.5)	4	6 ± 5 (1.8)	1-3
24	66 ± 21 (20.0)	1-3	13 ± 14 (3.9)	1-3
36	64 ± 32 (19.4)	1-3	8 ± 4 (2.4)	1-3

[a] Values are means ± standard deviations of three replicates. Each replicate consist of 8 leaves. The inducer and challenger were inoculated on the adaxial surface of the first expanded leaves. Inoculum density of inducer race (H21) was ca. 400 conidia/mm², and that of challenger race (A6) ca. 17 conidia/cm².

[b] Hours between the inducer and challenge inoculation. At the time intervals indicated, inducer inoculum was removed by using wet cotton or not removed prior to inoculation of the challenger.

Table 3. Number of colonies and spores formed by the compatible challenger race H21 of *Erysiphe graminis* f. sp. *hordei* on the adjacent acropetal and basipetal parts (free of inducer inoculation) of leaf surfaces of barley isoline 112405 previously inoculated with the incompatible inducer race A6[a]

Time interval[b] (h)	Acropetal part			Basipetal part		
	No. of colonies/ 20cm² leaf area	No. of spores/300 colonies (x10⁵)	Infection Type	No. of colonies/ 20cm² leaf area	No. of spores/300 colonies (x10⁵)	Infection Type
Control	333 ± 23 (100.0)	32.0 ± 3.1 (100.0)	4	351 ± 12 (100.0)	33.5 ± 2.4 (100.0)	4
24	317 ± 27 (95.2)	23.2 ± 6.4 (72.5)	4	351 ± 17 (100.0)	33.9 ± 4.8 (100.0)	4
48	327 ± 16 (98.2)	23.0 ± 6.2 (71.9)	2-4	345 ± 13 (98.3)	33.4 ± 3.5 (99.7)	4
72	325 ± 32 (97.6)	23.5 ± 7.4 (73.4)	2-4	347 ± 14 (98.9)	33.3 ± 3.8 (99.4)	4

[a] Values are means ± standard deviations of three replicates. Each replicate consist of 16 leaves. Inducer was inoculated on the middle part (3cm in length) of the adaxial surface of the first expanded leaves and challenger was then inoculated on the whole adaxial surface of the same leaves. Inoculum density of inducer was ca. 400 conidia/mm², and that of challenger ca. 17 conidia/cm².

[b] Hours between the inducer and challenger inoculations.

Table 4. Number of colonies and spores formed by the compatible challenger race A6 of *Erysiphe graminis* f. sp. *hordei* on the adjacent acropetal and basipetal parts (free of inducer inoculation) of leaf surfaces of barley isoline 011301 previously inoculated with the compatible inducer race A6[a]

Time interval[b] (h)	Acropetal part			Basipetal part		
	No. of colonies/ 20cm² leaf area	No. of spores/300 colonies (x10⁵)	Infection Type	No. of colonies/ 20cm² leaf area	No. of spores/300 colonies (x10⁵)	Infection Type
Control	310 ± 17 (100.0)	30.3 ± 2.2 (100.0)	4	321 ± 9.0 (100.0)	32.5 ± 3.3 (100.0)	4
24	315 ± 28 (100.0)	21.6 ± 6.7 (71.3)	2-4	329 ± 12.0 (100.0)	31.8 ± 3.2 (97.8)	4
48	306 ± 30 (98.7)	21.0 ± 6.4 (69.3)	2-4	315 ± 19.0 (98.1)	31.4 ± 2.6 (96.6)	4
72	317 ± 21 (100.0)	20.9 ± 6.8 (69.0)	2-4	313 ± 15.0 (97.5)	31.7 ± 3.1 (97.5)	4

[a] Values are means ± standard deviations of three replicates. Each replicate consist of 16 leaves. Inducer was inoculated on the middle part (3cm in length) of the adaxial surface of the first expanded leaves and challenger was then inoculated on the whole adaxial surface of the same leaves. Inoculum density of inducer was ca. 400 conidia/mm², and that of challenger ca. 17 conidia/cm².

[b] Hours between the inducer and challenge inoculations.

Translocation of the induced resistance: Although inducer conidia, incompatible (Table 3) or compattible (Table 4), were previously inoculated on the middle part of leaves, there was no reduction in colony formation of challenger race both on the acropetal and basipetal part of the same leaves until 72 hours after exposure to inducer race, and the colonies formed on the basipetal part showed normal infection type 4. However, almost all the colonies formed by challenger race on the acropetal part were changed in their infection type from originally 4 to 2 or mostly 3 type within 24 hours for which middle part of the leaves was exposed to the inducer race thereby resulting in reduction in the number of mildew spores of challenger race (Table 3 & 4). The color of mildew colonies on the acropetal part became slightly brown. The level of resistance induced on the acropetal parts was similar in the compatible and incompatible barley leaves to inducer race.

DISCUSSION

Kuć(8) described that mechanical injury, and injury by dry ice, chemicals or fungal and plant extract do not cause protection in cucurbits. Inoculation of heat-killed inducer conidia did not protect barley leaves against compatible challenger race of *E. graminis* f. sp. *hordei* (Table 1). However, colony formation of challenger race was greatly reduced by 87.1 to 91.2% when the heat-killed inducer inoculum was not removed from the leaves prior to inoculation of challenger. An inoculum density of heat-killed inducer race, ca. 400 conidia/mm^2 leaf area, may be enough to be a barrier of which challenger conidia can not contact with leaf surface.

When the incompatible inducer conidia were removed before they penetrate the host cell within 6 hours after inoculation, the induced resistance was expressed only as a colony reduction of challenger race. The colonies of challenger race on the predisposed leaves showed infection type 4 like the original one. On the other hand, when the inducer inoculum was removed after they had penetrated the host cell (24 or 36 hours after inoculation), a higher level of resistance might be induced on the predisposed leaves since no colonies of infection type 4 occurred. But 1, 2 or 3 type colonies were observed on the same leaves, and total number of colonies in their infection types were similar to the number of colonies formed on the predisposed leaves from which the inducer inoculum was removed 6 hours after inoculation. This fact suggest that induction of resistance expressed as a reduction in colony formation of challenger race may be determined prior to penetration of inducer race on the leaves, and the enhanced host response following penetration of inducer may affect colony formation of challenger races. However, the possible intervention of an antagonistic effect can not be excluded since all the parasitic units of inducer races may not be removed from the leaves after their infection pegs penetrate the host cells. Also a question may arise whether or not the destructed conidial debris of inducer which might be remained on leaf surfaces after rubbing with wet cotton could affect the induced resistance of barley plants to powdery mildew fungus. However, there was no reduction in colony formation of the challenger race on the barley leaves on which heat-killed inducer conidia had previously been inoculated and from which the heat-killed conidia were removed by rubbing with wet cotton (Table 1). In the case of removal of inducer prior to the inoculation of challenger, only one hour interval between inducer and challenge inoculation was enough to completely protect barley leaves against challenger race.

Our results suggest that induced resistance may be strictly localized since no reduction in colony formation occurred on the adjacent acropetal and basipetal parts of the leaves free of inducer inoculation. However, acropetal part was clearly affected by previously inoculation with inducer races on middle part of the same leaves (Table 3 & 4). Some resistance-inducing factors may be translocated to the acropetal rather than to the basipetal part of the leaves. Translocation of resistance-inducing, principles, to the acropetal rather than to the basipetal

direction in plant rust disease(13), from stem to leaves in tobacco inoculated with *Peronospora tabacina*(2), from the bulb tissues to the entire orchid tuber infected with *Rhizoctonia repens*(3), and from one half to the opposite half of the same leaf in the wild fire disease of tobacco (9) has been reported. Recently Kuć and co-workers (5) also demonstrated that the signal for induced resistance in cucurbits is graft-transmissible from root stock to scion(7), that the effect of induced resistance is stronger for a foliar pathogen above the inducer leaf than below it and that girdling the petiole of the inducer leaf prevents protection above or below the inducer leaf. Nevertheless, roots can be protected by infecting leaves(4). However, resistance-inducing factors for protection on the acropetal part may be not specific since the level of induced resistance were similar in both compatible and incompatible barley leaves to inducer races.

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