Defense Reaction of Barley Cultivars to Non-Pathogenic Fungi

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Abstract

Defense reaction of barley cultivars to three nonpathogenic fungi were studied microscopically to clarify whether there are differences among cultivars and the nature of nonhost resistance was discussed by comparing with that of race-specific resistance. In all barley cultivars and non-pathogenic fungi combinations, it is shown that the penetration failure by non-pathogenic fungi was almost associated with papilla formation and cell wall alteration which fluoresced under fluorescence microscopy, of leaf epidermal cells. It is therefore suggested that papilla formation and cell wall alteration associated with defense reaction is major components of nonhost resistance in barley. In addition these components of nonhost resistance are most likely related to the induced resistance caused by previous inoculation with hypo- and hyper-virulent pathogens.

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Key words : nonhost resistance, barley, pathogen non-pathogenic to barley, papilla formation, autofluorescence.

Introduction

Nonhost resistance was defined by Heath¹⁾ as the resistance shown by plant species not considered to be hosts for the pathogen. Attention has been focused on it because it is not only highly effective to the majority of microorganisms but also greatly durable²⁾. For example, Sherwood and his co-worker^{9,12,13)} using reed canarygrass and fungi non-pathogenic to it, indicated that papilla or appositional cell wall thickness and its lignification might play important roles in resistance to fungal penetration. Later, they hypothesized that all species of Gramineae characteristically respond to fungal penetration attempts by appositional wall formation¹⁰⁾. Ride and Pearce⁷⁾ showed that failure of *Botrytis cinerea* to penetrate the intact surface of primary wheat leaves was closely associated with the production of lignified papilla and alteration of upper epidermal and adjacent lateral walls, and discussed their possible roles in resistance.

On nonhost resistance in barley, however, a few observations are reported and most of them have been studied using only one cultivar^{10,14)}. As pointed out by Heath²⁾ and Sherwood and Vance¹⁰⁾, it is important and interesting to clarify whether or not there are differences in responses among lines and cultivars within a host species.

The purpose of this experiment is to clarify microscopically whether or not defence reaction of barley to some nonpathogenic fungi differs among cultivars and to discuss

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the nature of nonhost resistance.

Materials and Methods

Plants and fungi. Five barley cultivars (*Hordeum vulgare* L.), Goseshikoku (GS), Kobinkatagi (KB), Hanna (HA), Russian No. 12 (R12) and Turkey 290 (TK) were used in the present study. These plants were grown in vermiculite supplied with 0.02% Hyponex solution every other day in a growth chamber maintained at 20 ± 1 C with 12h photoperiod.

Alternaria alternata (Fries) Keissler, *Erysiphe polygoni* DC. and *Colletotrichum lagenarium* (Pass.) Ellis et Halsted were used as non-pathogenic inocula to barley. *A. alternata* was isolated from a sunflower plant by Dr. Tsuda, Pesticide Research Institute, Faculty of Agriculture, Kyoto University and maintained on potato sucrose agar (PSA) medium. *E. polygoni* (KU 86-1) was isolated from common buckwheat in the experimental field of Kyoto University and maintained on its host. *C. lagenarium* (104-T, Stock culture in Laboratory of Plant Pathology, Kyoto University) was maintained on PSA medium.

Preparation of inocula and inoculation. Conidia of *A. alternata* grown on PSA medium at 25 C for 7 to 8 days were collected with a soft hair brush, washed three times with distilled water by centrifugation, and then conidia were suspended in 0.05% Tween 20 solution to give a final concentration of 5×10^3 conidia/ml. Fresh conidia of *E. polygoni* formed on buckwheat leaves 15 days after inoculation were used as inocula. Conidia of *C. lagenarium* were obtained by the same methods as those for *A. alternata* and adjusted to a final concentration of 3×10^3 conidia/ml.

Ten leaves of 7-day-old barley were fixed with rubber bands horizontally on a plastic sheet (19×18 cm) and 30 ml of conidial suspension of either *A. alternata* or *C. lagenarium* was sprayed onto the adaxial surface of the leaves. Inoculated plants were placed in a clear plastic case with water to give a high relative humidity throughout the experiment and incubated in a growth chamber described above. Inoculation with conidia of *E. polygoni* was conducted by shaking diseased plants over 30 cm height and incubated as mentioned above.

Preparation of specimens and observation. Inoculated leaves were harvested at appropriate times. Fixation, staining and observation were conducted by the methods described by Tosa and Shishiyama¹¹⁾.

For *A. alternata*, 20 conidia (60 to 100 attempted penetration sites), and for *E. polygoni* and *C. lagenarium*, more than 500 conidia on three leaves were observed.

Results

Symptoms on barley cultivars to three non-pathogenic fungi were observed 5 and 7 days after inoculation. No visible symptom was observed on all cultivars.

Results of microscopic observation on *A. alternata* were summarized in Table 1. About 90% of conidia germinated 6h after inoculation on all cultivars. By 48h after in-

oculation 61 to 98 appressoria were formed per 20 conidia which had formed 3.1 to 4.9 appressoria in average. More than 90% of attempted penetration from these appressoria failed with papilla which fluoresced under fluorescence microscopy. (Fig. 1 A, B). Only 1 penetration was observed in HA and R12 36h after inoculation.

About 90% of conidia of *E. polygoni* germinated 6h after inoculation and more than

Table 1. Development of *Alternaria alternata* on leaf epidermal cells on barley

Cultivar	Time after inoculation (h)	Germination percentage (%)	Number of sites per 20 conidia showing		
			Appressorial formation	Penetration failure with papilla	Successful penetration
Goseshikoku	24	94.3	12 (0.6) ^{a)}	8 (66.7) ^{b)}	0 (0.0) ^{b)}
	36	93.4	55 (2.8)	51 (92.7)	0 (0.0)
	48	93.3	70 (3.5)	66 (94.6)	0 (0.0)
Russian No. 12	24	94.3	18 (0.9)	18 (100.0)	0 (0.0)
	36	94.3	45 (2.3)	39 (86.7)	1 (2.2)
	48	93.4	98 (4.9)	97 (98.9)	0 (0.0)
Turkey 290	24	96.6	15 (0.8)	14 (93.3)	0 (0.0)
	36	95.3	39 (2.0)	38 (97.4)	0 (0.0)
	48	96.2	64 (3.2)	62 (96.9)	0 (0.0)
Hanna	24	96.1	48 (2.4)	43 (89.6)	0 (0.0)
	36	97.1	55 (2.8)	53 (96.4)	1 (1.8)
	48	88.9	61 (3.1)	60 (98.4)	0 (0.0)

a) Average number of appressoria per conidium. b) Percentage.

Table 2. Development of *Erysiphe polygoni* on leaf epidermal cells of barley

Cultivar	Time after inoculation (h)	Germination percentage (%)	Appressorial formation (%)	Percentage of attempted penetration sites showing		
				Failure with papilla	Failure with H R ^{a)}	Successful penetration
Goseshikoku	24	95.8	98.6	78.7	4.7	0.0
	36	97.1	98.6	92.8	1.9	0.0
	48	95.8	98.4	95.8	1.3	0.0
Kobinkatagi	24	89.2	97.5	58.3	1.8	0.0
	36	92.3	96.2	74.0	2.6	0.0
	48	91.7	97.1	76.1	4.8	0.0
Turkey 290	24	97.0	96.9	46.9	21.4	0.0
	36	94.4	99.8	72.5	17.8	0.0
	48	93.4	99.4	75.6	17.2	0.0
Hanna	24	96.7	98.4	71.3	0.9	0.0
	36	96.0	99.2	90.0	1.1	0.0
	48	95.0	97.5	90.3	0.0	0.0
Russian No.12	24	89.2	97.6	80.7	5.9	0.0
	36	— ^{b)}	—	—	—	—
	48	93.9	97.7	76.5	8.5	0.5

a) Hypersensitive reaction of epidermal cell. b) Not observed.

95% of germinated conidia formed appressoria by 24h on all cultivars. Even 48h after inoculation, successful penetration was low or absent. However, unlike *A. alternata*, percentage of penetration failure with fluorescent papilla (Fig. 1 C, D) ranged from 75 to 95% and it seemed to differ among the cultivars used. In TK, about 20% of attempted penetrations failed to develop haustorial initial with aggregation or granulation of cytoplasm (hypersensitive reaction) of epidermal cells (Table 2).

Results with *C. lagenarium* were shown in Table 3. About 85% of conidia germinated 90% of them formed pigmented appressoria. However penetration failure with papilla which also fluoresced was low and its percentage was 13.4 to 45.5 and 19.9 to 46.4%, 48 and 72h after inoculation, respectively. By 5 days after inoculation more than 55% of conidia with normal appressoria failed to penetrate with papilla (Fig. 1 E, F). When appressoria were wiped off with a wet cotton ball to observe the site beneath them, however, a large number of papillae were observed beneath the appressoria even 48h

Table 3. Development of *Colletotrichum lagenarium* on leaf epidermal cells of barley

Cultivar	Time after inoculation (h)	Number of conidia with normal appressoria	Number of attempted penetration sites showing	
			Failure with papilla	Successful penetration
Goseshikoku	48	621	83 (13.4) ^{a)}	0 (0.0) ^{a)}
	72	658	131 (19.9)	0 (0.0)
	120	682	380 (55.7)	0 (0.0)
Kobinkatagi	48	606	81 (13.4)	0 (0.0)
	72	634	141 (22.2)	0 (0.0)
	120	850	485 (57.1)	0 (0.0)
Turkey 290	48	598	154 (25.8)	0 (0.0)
	72	594	169 (28.5)	0 (0.0)
	120	734	403 (54.9)	0 (0.0)
Hanna	48	809	368 (45.5)	0 (0.0)
	72	939	436 (46.4)	0 (0.0)
	120	610	413 (67.7)	0 (0.0)
Russian No. 12	48	529	146 (27.6)	0 (0.0)
	72	552	177 (32.1)	0 (0.0)
	120	552	376 (68.1)	0 (0.0)

a) Percentage.

Table 4. Papilla formation in barley epidermal cells in response to attempted penetration by *Colletotrichum lagenarium*

Cultivar	Time after inoculation (h)	Number of appressoria	Number of papilla	Percentage of papilla
Goseshikoku	48	1,484	1,104 ^{a)}	74.4
	72	1,402	1,014	72.3
Kobinkatagi	48	1,621	1,094	67.5
	72	1,574	1,128	71.7

a) Papilla was observed after removing appressoria with wet cotton ball.

after inoculation. The estimated number showed almost the same value in both 48 and 72h after inoculation (Table 4, Fig. 2).

Fig. 1. Light (A, C and E) and fluorescence (B, D and F) microscopic photographs showing papilla formation in response to attempted penetration of three non-pathogenic fungi. A; Papilla formed at attempted penetration site of *A. alternata*. B; Same as A. Note that lateral wall adjacent to penetration site fluoresced, showing alteration of its site. C; Papilla formed at attempted penetration site of *E. polygoni*. Penetration peg was observed at the centre of papilla. D; Same as C. E; Papilla formed at attempted penetration site of *C. lagenarium*. F; Same as E. Lateral wall also fluoresced as those observed with *A. alternata*. AP; Appressorium, C; Conidium, N; Nucleus, P; Papilla, PP; Penetration peg, WA; Wall alteration. Each bar represents 10 μ m.

Fig. 2. Papilla formation in barley epidermal cells in response to attempted penetration by *C. lagenarium*. A ; light microscopic photograph of inoculated barley epidermis. No reaction was observed. B ; Fluorescent microscopic photograph of the same site. C ; Fluorescent microscopic photograph showing papillae detected after removal of appressoria. Note that a large number of papillae were formed. AP and P ; Refer to Fig. 1. Each bar represents 10 μ m.

Discussion

As defense reactions of plants against non-pathogenic fungi, lignified papilla^{7,9,12,13}, callosity¹⁴, appositional wall formation¹⁰ and lignification^{3,4,6} at the penetration site have been reported in several plant species and their possible roles in defense reaction has been discussed. However, these results were obtained, in many cases, with only one plant cultivar.

In the present study it was shown using 5 barley cultivars that penetration failure by non-pathogens was almost associated with papilla formation and cell wall alteration, which fluoresced under fluorescence microscopy, although there are some differences among cultivars and fungi used. Therefore, papilla formation and cell wall alteration at the attempted penetration site may be major components of nonhost resistance at least in barley. However, it is unclear why the percentages of papilla formation were less than the others in TK and KB at 24h after inoculation when *E. polygoni* was used.

In *E. polygoni*, 10 to 25% of attempted penetration failed to develop haustorial initials with hypersensitive reaction of epidermal cell. This reaction may serve as a barrier after papilla formation because at these sites the fungus penetrated through a small papilla.

It has been suggested in race-specific interaction of barley cultivars and *E. graminis* f. sp. *hordei* that *JMlsn^t* and *JMr₁₂*, major genes for resistance, identified in TK and R12, respectively, control hypersensitive reactions of epidermal and mesophyll cells and that *JMlh*, identified in HA, controls mesophyll reaction at least¹¹⁾. No major gene, however, has so far been identified in GS and KB.

The results in the present study indicated that defense of barley to non-pathogenic fungi is due to the inhibition of penetration by papilla formation and that it might be distinguished from race-specific resistance which is mainly due to hypersensitive reactions of epidermal and mesophyll cells which fluoresced under fluorescence microscopy^{8,11)}. However, it is unclear whether or not there is substantial or essential difference between race-specific and nonhost resistance because there are some cases in which papilla formation plays important role to inhibit fungal penetration in incompatible combination of barley and *E. graminis* f. sp. *hordei* and the hypersensitive reaction of epidermal cell are also observed in nonhost barley plants. Therefore, comparative studies on recognition mechanisms in molecular level and on initiation time of defense reactions should be made in relation to specificity of race-cultivar and species-species levels.

In double inoculation system using barley and its powdery mildew fungi, Sahashi and Shishiyama⁸⁾ suggested that the increase of papilla formation is a major factor of induced resistance and discussed that the resistance induced might differ from race-specific resistance mediated by a single major gene. These findings together with the present study provide an important clue to understanding the nature of resistance induced by previous inoculation with hyper- and hypo-virulent pathogen.

Further studies should be conducted to clarify what the nature of nonhost and race-specific resistance is in many plant species.

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和 文 摘 要

佐橋憲生・獅山慈孝：非病原性糸状菌に対するオオムギ品種の防御反応

オオムギ 5 品種の第一葉に 3 種の非病原性糸状菌, *Alternaria alternata*, *Erysiphe polygoni* および *Colletotrichum lagenarium* を接種し, それらオオムギ品種の防御反応を光学および蛍光顕微鏡を用いて観察した。その結果, 供試した品種および糸状菌間では, パピラによる侵入阻止率に差が認められた。しかし, ほとんどの侵入阻止部位では, パピラ形成や蛍光顕微鏡下で自発蛍光として現われる細胞壁の変化が観察された。以上の結果から, パピラ形成によって起こる侵入阻止反応は, オオムギが非病原性糸状菌に対して示す抵抗反応の一つとして重要であることが示唆された。