Characteristic Cellular Responses as Expression of Genes for Resistance to *Erysiphe graminis* f. sp. *hordei* in Barley

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ABSTRACT

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The relationship between fungal development and host cellular responses in barley plants inoculated with *Erysiphe graminis* f. sp. *hordei* was evaluated in cultivars with different single alleles and multiple alleles for resistance at the $JM1_{sn}$ locus and in cultivars possessing two genes for resistance. With increased levels of resistance fewer infection attempts continued beyond the penetration stage. Cessation of fungal growth was most strongly associated with penetration attempts that ended in formation of a papilla and an intensely fluorescent cytoplasmic aggregation. In the multiple allelic lines of $JM1_{sn}$ locus, the cessation of fungal growth followed by the appearance of fluorescence of epidermal cells was highly correlated with the degree of resistance. The results suggested that the genes for resistance could determine a recognition event, controlling the rate of degree of cytoplasmic aggregation followed by the formation of fluorescent collapsed cells in the incompatible interactions.

Additional key words: genetics of resistance, *Hordeum vulgare*, papilla formation.

Ellingboe and co-workers (2,11,14) have shown how host-parasite specificity in powdery mildew of barley controlled by certain genes for incompatibility is expressed. They determined the time after inoculation when various host genes interacted with corresponding parasite genes to prevent the development of *Erysiphe graminis* f. sp. *hordei* during primary infection. Ellingboe (2) also suggested that each gene for incompatibility could present a series of barriers for the parasite and that only some infection units from the parasite population overcome each barrier. Stanbridge et al (12) found similar multiple stages at which fungal development ceased in each of several hosts possessing different genes for resistance. Johnson et al (4) have recently described a binary pathway system that permits statistical comparison of probability for different events within a given host-parasite combination. They also indicated that incompatibility was expressed at several stages in the incompatible combination with the $M1_a$ gene. However, evaluation of characteristic cytological events in relation to the resistance genes has not been fully done.

Results of our work during the last 5 yr has shown that fluorescence microscopy is useful for examining cytological changes in host cells in powdery-mildewed barley leaves and has revealed certain cytological events that are specific for expression of resistance genes during the course of the disease (6–8,10,13). Recently, the authors (5) defined the cytological responses in the early stage of the disease. Namely, small papillae and aggregation of epidermal cytoplasm around the papillae were observed beneath appressorial tips of the fungus 12–13 hr after inoculation in all barley cultivars tested. If papillae increased in size and fluoresced 18 hr after inoculation, the mass of aggregated cytoplasm disappeared and there was no fungal penetration. When successful penetration through papilla was accomplished 15 hr after inoculation in highly resistant and resistant barleys, the aggregated cytoplasm fluoresced and increased in amount and fluorescent intensity until 18 hr. Eventually, all the cytoplasm of the affected epidermal cells became granulated and showed strong fluorescence 48 hr after inoculation. In the present study, the authors use the term "cytoplasmic aggregation" to describe the later case, rather than the aggregation for papillae deposition (1).

The present study was undertaken to evaluate the relationship between the degree of resistance conferred by specific alleles and corresponding cytological events in barley cultivars with defined genes for resistance, including lines with different alleles at the $JM1_{sn}$ locus and cultivars containing two genes for resistance. The host responses studied were papilla formation (Pap), cytoplasmic aggregation (Agg), autofluorescence, and collapse of epidermal cells (CEC), and collapsed autofluorescent mesophyll cells (CMC) as previously described (9,13).

MATERIALS AND METHODS

Thirty cultivars of barley, *Hordeum vulgare* L., and a wild strain of barley, *Hordeum spontaneum nigrum*, were used (Table 1). The resistance genes in the cultivars were defined by Hiura (3), who also showed the infection type and assigned the gene symbols. The growth of plants and inoculation with *E. graminis* f. sp. *hordei*, race I, were conducted as previously reported (6). The density of spores dusted onto the leaves was 100–300 conidia per square centimeter.

Inoculated primary leaves were collected 2 and 6 days after inoculation. The leaves were immediately boiled at 80 C in alcoholic lactophenol for 2 min and then decolorized completely in fresh solution. This fixation was suitable for observation of fluorescent papilla, CEC, and CMC, without any cellular destruction, under a fluorescence microscope. Fluorescence found in attacked host cells was shown to be autofluorescence due to fungal infection, but not artificial treatment (9). To reveal fungal development, the cleared specimens were stained in a diluted solution of alcoholic lactophenol containing 0.05% aniline blue for several minutes. Fluorescent papilla, CEC, and CMC stained blue.

Three inoculated leaves of each cultivar were selected, and a 2-cm-long leaf segment was cut 1 cm from the tip of each. About 500 infection sites were observed on the adaxial face of each section. The cellular responses found at each infection site were classified into Pap, Agg, CEC, and CMC, and the frequency of

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fungal population, which ceased growth at the stages of Pap, Agg, CEC, and CMC. The following indicators of fungal development were also measured: the percentage of mature haustoria formed, the greatest colony diameter 2 and 6 days after inoculation, and the number of conidiophores per colony. Correlations between each cellular response and fungal development were analyzed statistically within 30 barley cultivars and within multiple allelic lines of the Ml_{sn} locus.

RESULTS

Fungal development and cellular responses associated with cessation of fungal growth in 30 cultivars inoculated with race I were summarized in Table 1. The cultivars are arranged in the order of colony size at 6 days after inoculation. A detailed investigation of fungal development in each cultivar was conducted to determine what parameters other than infection type would be appropriate indicators of susceptibility. The number of conidiophores per colony was highly correlated with colony size 6 days after inoculation ($r = 0.83$). Also, a high correlation was found between colony size 2 and 6 days after inoculation ($r = 0.76$) and between colony size at 2 days and the percentage of infection sites with haustoria 2 days after inoculation ($r = 0.78$). Therefore, both colony size and the rate of haustorial formation 2 days after inoculation in each cultivar were used as parameters of susceptibility in the following comparisons. High correlation ($r = -0.869$) between the rates of haustorial formation and fungal population, which ceased growth at the penetration stages involving Pap, Agg, and CEC was shown in Fig. 1. When the cellular responses, which were associated with cessation of fungal growth, were examined in Fig. 2, the highest correlation was with intense cytoplasmic aggregation ($r = -0.862$). When compared with the average length of colonies in each cultivar, similar correlation coefficients were also observed (Fig. 2B). These results clearly indicated that with an increase in the level of resistance there was an increase in the number of encounters that were stopped at the penetration stages. Another cytological event in incompatible interactions was CMC. The correlation between the occurrence of CMC at the infection sites and the degree of susceptibility was shown in Fig. 3. The correlation of CMC with either haustorial formation or colony size was not significant in the 30 barley cultivars ($r = 0.129$ and 0.231, respectively). CMC was generally observed in cultivars in which haustorial formation occurred at 30–50% of infection sites.

TABLE 1. Resistance genes, infection types, cellular responses, and development of powdery mildew in barleys possessing identified genes for resistance to *Erysiphe graminis* f. sp. *hordei*, race I

Cultivars	Resistance genes	Infection types	Cellular responses (%) Day 2			Fungal development Day 2		Fungal development Day 6	
			Pap[a]	Agg[b]	CEC[c]	Colony size (μm)	Haustorial formation (%)	Colony size (μm)	Conidiophores per pustule (no.)
Goseshikoku	···	4	7	2	9	373	82	2,500	>200
Hanna C.I. 906	···	4	15	4	2	281	81	2,400	>100
A. 222 (Almora)	Ml^{al}_{sn}	1–2	11	11	8	239	70	2,100	40
Arlington Awnless C.I. 702	Ml_k	1	19	20	31	242	30	1,800	35
Kairyobozu-mugi	Ml_{kb}	1	27	38	31	152	4	1,760	15
Psaknon C.I. 6305	Ml_p	0–1	31	37	10	296	22	1,500	12
Nakaizumi-zairai	Ml_{nz}	0–1	16	41	3	214	40	1,420	18
Russian No. 81	Ml_{r81}	0–1	40	49	1	292	10	1,380	11
Hosomugi C	Ml_{nz}	1	27	38	5	304	30	1,380	19
Kwan C.I. 1016	Ml_k	1–2	35	27	8	327	30	1,220	28
Colsess C.A. 772	Ml_{r12}	1	32	19	4	338	45	1,200	18
Russian No. 12	Ml_{r12}	1	46	21	3	258	30	1,200	14
H.E.S. 4	Ml^{h4}_{sn}	0′	33	15	20	229	32	1,100	4
Kleinwanzleben	Ml_{r74}	0′	37	14	4	229	45	230	0
Heil's Hanna 3 C.I. 682	Ml^{h4}_{sn}	0′	28	25	4	219	43	220	0
Russian No. 74	Ml_{r74}	0′	24	36	16	180	24	190	0
Goldfoil C.I. 928	Ml_g	0′	27	56	4	122	13	180	0
Nigrate C.I. 244	Ml^{n}_{sn}	0	43	40	25	100	2	100	0
Weichenstephaner I	Ml_g	0	33	60	6	0	0.7	0	0
J. 5	Ml^{mc}_{sn}	0	20	51	28	0	0.6	0	0
Monte Cristo C.I. 1107	Ml^{mc}_{sn}	0	23	52	23	0	2	0	0
Gopal C.I. 1107	Ml_{sn}	0	19	47	33	0	1.1	0	0
Russian No. 68	Ml_{sn}	0	21	38	37	0	3.7	0	0
Engledow India C.I. 7555	Ml_{sn}	0	25	62	12	0	1.1	0	0
H. spont. nigrum	Ml_{sn}	0	18	47	35	0	0.5	0	0
Russian No. 66	Ml^{h4}_{sn} + Ml^{d}_{sn}	0′	34	21	21	292	24	510	0
Hosomugi	Ml^{h4}_{sn} + Ml_{nz}	0′	24	29	23	269	24	500	0
Six-rowed Chevalier	Ml^{h4}_{sn} + Ml_{r12}	0′	33	27	13	268	27	300	0
Dajokan	Ml^{h4}_{sn} + Ml_{r12}	0′	30	21	21	288	28	300	0
Golden Melon	Ml^{h4}_{sn} + Ml_{kb}	0′	29	29	11	157	31	200	0

[a] Papilla without cytoplasmic aggregation.
[b] Papilla with intense cytoplasmic aggregation and epidermal fluorescence.
[c] Collapsed epidermal cells.
[d] Resistant to race IX.

In cultivars with multiple alleles for resistance at the $JM1_{sn}$ locus, the relationship between colony size and haustorial formation after 2 days was higher than that obtained from the 30 barley cultivars with a single allele (Table 2). Again, the early cessation of fungal growth was most highly associated with the occurrence of intense cytoplasmic aggregation. The incidence of papilla formation was not significantly correlated with the cessation of fungal growth.

The results shown in Table 3 indicated a gene-dosage effect on powdery mildew development. The cultivars possessing two independent resistance genes were more resistant than those with one or the other gene. The colony size was much reduced, and no production of conidia was observed in the former cultivars. Haustorial formation in cultivars with two genes was less than that in others. These gene-dosage effects also reflected on cellular responses of host cells. The degree of fungal penetration failure followed by intense cytoplasmic aggregation increased in the cultivars possessing two genes. However, the number of CMC in each infection site did not change with the change in gene-dosage.

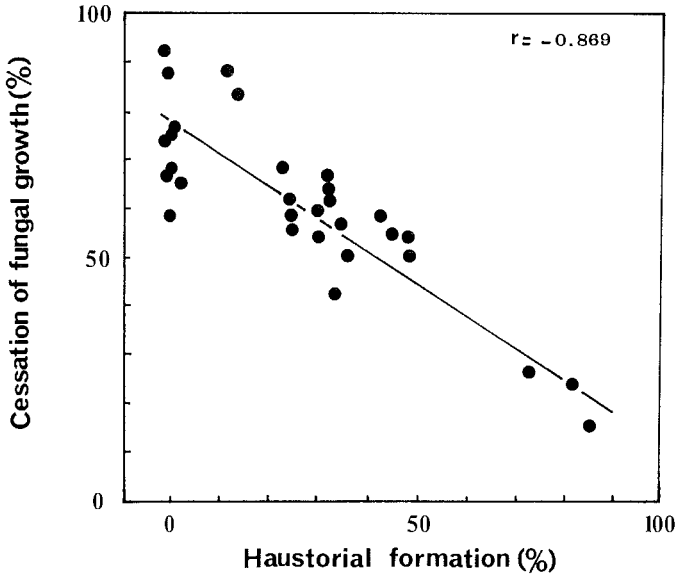

Fig. 1. Correlation between percentage of conidia producing haustoria and ceasing growth at the penetration stage in barley cultivars posssessing identified genes for resistance to *Erysiphe graminis* f. sp. *hordei*, race 1 (2 days after inoculation). The dots in figure indicate data from the 30 barley cultivars shown in Table 1. Correlation coefficient was calculated at $P = 0.05$.

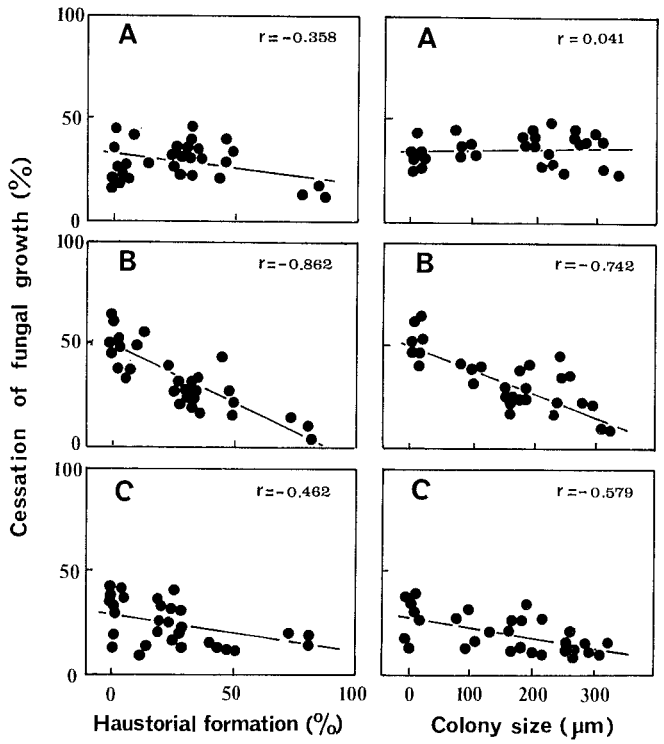

Fig. 2. Correlation between degrees of haustorial formation or colony size and cessation of fungal growth at various infection stages, such as **A,** papilla formation, **B,** cytoplasmic aggregation, and **C,** collapse, of attacked epidermal cells in barley cultivars possessing identified genes for resistance to *Erysiphe graminis* f. sp. *hordei*, race I (2 days after inoculation). The dots in each figure indicate data from the 30 barley cultivars listed in Table 1. Correlation coefficient was calculated at $P = 0.05$.

DISCUSSION

In the present study, the most characteristic cellular event relating to cultivar-specific resistance in powdery mildew of barley was shown to be the intense cytoplasmic aggregation. The aggregation was followed by fluorescence and the collapse of epidermal cells (5). It is significant that the degree of resistance found in various multiple allelic lines of $JM1_{sn}$ gene was highly correlated with occurrence of cytoplasmic aggregation after penetration. The cytoplasmic aggregation described in the present study was distinguished from the aggregation for papilla deposition (1): the aggregation in this study expanded and eventually resulted in epidermal cell collapse.

TABLE 2. Development of powdery mildew and cellular responses in barley plants whose resistance genes for *Erysiphe graminis* f. sp. *hordei*, race I, are multiple allelic on one locus[a]

Cultivars	Resistance genes	Colony size (μm)	Haustorium formation (%)	Pap[b] (%)	Agg[b] (%)	CEC[c] (%)
H. spontaneum nigrum	$JM1_{sn}$	0	0.5	18	47	35
Monte Cristo C.I. 1017	$JM1_{sn}^{mc}$	0	2.0	23	52	23
Nigrate C.I. 2444	$JM1_{sn}^{n}$	100	2.0	43	40	25
H.E.S. 4	$JM1_{sn}^{h4}$	229	32.0	33	15	20
A. 222	$JM1_{sn}^{a1}$	239	70.0	11	11	8
Goseshikoku	···	373	82.0	7	2	9
Coefficients of correlation (r) with haustorium formation		0.92	—	−0.68	0.94	−0.94

[a]The results were obtained 2 days after inoculation.
[b]Refer to Table 1.
[c]Calculated at $P = 0.05$.

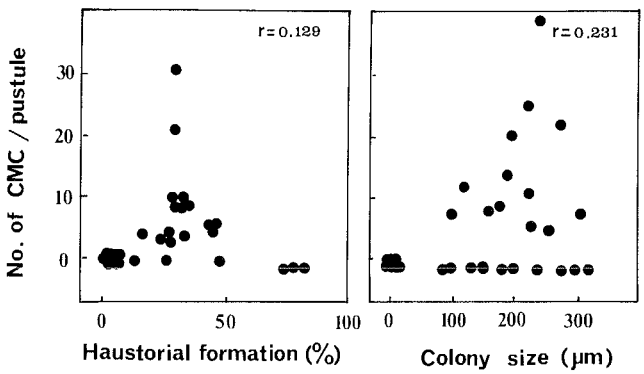

Fig. 3. Correlation between the occurrence of collapsed mesophyl cells (CMC) and the degrees of haustorial formation or colony size in barley cultivars possessing identified genes for resistance to *Erysiphe graminis* f. sp. *hordei*, race I (2 days after inoculation). The dots in each figure indicate data from the 30 barley cultivars shown in Table 1. Correlation coefficient was calculated at $P = 0.05$.

TABLE 3. Effects of gene-dosage on resistance of barley plants to *Erysiphe graminis* f. sp. *hordei*, race I

Cultivars	Resistance genes	Colony size (μm)	Conidiophores per pustule (no.)	CMC[a] (no.)	Haustorium formation (%)	Agg[b] (%)
H.E.S. 4	$JM1^{h4}_{sn}$	1,100[c]	4[c]	12[c]	32[d]	35[d]
Nakaizumi-zairai	$JM1_{nz}$	1,420	18	6	40	44
Hosomugi	$JM1^{h4}_{sn} + JM1_{nz}$	500	0	10	24	52
H.E.S. 4	$JM1^{h4}_{sn}$	1,100	4	12	32	35
Russian No. 12	$JM1_{r12}$	1,200	14	19	30	24
Colsess	$JM1_{r12}$	1,200	18	11	45	23
Six-rowed Chevalier	$JM1^{h4}_{sn} + JM1_{r12}$	200	0	18	27	40
Dajokan	$JM1^{h4}_{sn} + JM1_{r12}$	300	0	14	28	42

[a]Collapsed mesophyll cells per pustule.
[b]Refer to Table 1.
[c]6 days after inoculation.
[d]2 days after inoculation.

Ellingboe (2), Stanbridge et al (12), and Johnson et al (4) proposed the concept that independent genes for incompatibility are expressed at various stages during the development of the mildew infection. Our observations support this hypothesis. However, the present data from multiple allelic lines seem to show that the strength of these resistance genes is reflected quantitatively by the difference in the rate of cessation of fungal growth followed by cytoplasmic aggregation and fluorescence of epidermal cells. From the evidence, the authors suppose that the strength of resistance genes is the degree of host recognition leading to different rates of such cytoplasmic aggregation in incompatible combination.

Johnson et al (4) have described a binary pathway system that permits statistical comparison of probability for different events within a given host-parasite combination and for specific events in different host-parasite combinations, and they found the importance of papilla formation as well as host cell collapse in resistance conditioned by the $M1_a$ gene. Koga et al (7) also reported the role of papilla formation in certain incompatible combinations. Both papers suggest that papilla formation may depend on type and age of epidermal cell. However, the present study indicated that the frequency of early cessation of fungal growth followed by only papilla formation did not correlate with the degree of resistance in the 30 barley cultivars and multiple allelic lines of $JM1_{sn}$.

LITERATURE CITED

1. Bushnell, W. R., and Bergquist, S. E. 1975. Aggregation of host cytoplasm and the formation of papillae and haustoria in powdery mildew of barley. Phytopathology 65:310-318.
2. Ellingboe, A. H. 1972. Genetics and physiology of primary infection by *Erysiphe graminis*. Phytopathology 62:401-406.
3. Hiura, U. 1960. Studies on the disease-resistance in barley. IV. Genetics of the resistance to powdery mildew. Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ., Jpn. 11:235-300.
4. Johnson, L. E. B., Bushnell, W. R., and Zeyen, R. J. 1979. Binary pathway for analysis of primary infection and host response in population of powdery mildew fungi. Can. J. Bot. 57:497-511.
5. Kita, N., Toyoda, H., and Shishiyama, J. 1981. Chronological analysis of cytological responses in powdery-mildewed barley leaves. Can. J. Bot. 59:1761-1768.
6. Koga, H., Mayama, S., and Shishiyama, J. 1978. Microscopic specification of compatible and incompatible interactions in barley leaves inoculated with *Erysiphe graminis hordei*. Ann. Phytopathol. Soc. Jpn. 44:111-119.
7. Koga, H., Mayama, S., and Shishiyama, J. 1980. Correlation between the deposition of fluorescent compounds in papillae and resistance in barley against *Erysiphe graminis hordei*. Can. J. Bot. 58:536-541.
8. Mayama, S., and Shishiyama, J. 1976. Histological observation of cellular responses of barley leaves to powdery mildew infection by UV-fluorescence microscopy. Ann. Phytopathol. Soc. Jpn. 42:591-596.
9. Mayama, S., and Shishiyama, J. 1976. Detection of cellular collapse in albino barley leaves inoculated with *Erysiphe graminis hordei* by UV-fluorescence microscopy. Ann. Phytopathol. Soc. Jpn. 42:618-620.
10. Mayama, S., and Shishiyama, J. 1978. Localized accumulation of fluorescent and u.v.-absorbing compounds at penetration sites in barley leaves infected with *Erysiphe graminis hordei*. Physiol. Plant Pathol. 13:347-354.
11. McCoy, M. S., and Ellingboe, A. H. 1966. Major genes for resistance and the formation of secondary hyphae by *Erysiphe graminis* f. sp. *hordei*. Phytopathology 56:683-686.
12. Stanbridge, B., Gay, J. L., and Wood, R. K. S. 1971. Gross and fine structural changes in *Erysiphe graminis* and barley before and during infection. Pages 367–379 in: T. F. Preece and C. H. Dickinson, eds. Ecology of Leaf Surface Micro-Organisms. Academic Press, New York.
13. Toyoda, H., Mayama, S., and Shishiyama, J. 1977. Fluorescent microscopic studies on the hypersensitive necrosis in powdery-mildewed barley leaves. Phytopathol. Z. 92:125-131.
14. Yang, S. L., Moseman, J. G., and Ellingboe, A. H. 1972. The formation of elongating secondary hyphae of *Erysiphe graminis* and the segregation of *M1* genes in barley. Phytopathology 62:1219-1223.