한국식물병리학회지 2(1) : 37-42(1986)
Korean J. Plant Pathol. 2(1) : 37-42(1986)

Biochemical Reactions of Barley Leaves at Intervals After Inoculation with *Erysiphe graminis* f.sp. *hordei*

Dong Up Song, Baik Ho Cho and Ki Chung Kim*

*Department of Agricultural Biology, College of Agriculture, Chonnam National University, Kwangju 500, Korea

보리 흰가루病菌 接種後 보리葉內 經時的 生化學反應

宋 東 業·趙 白 皓·金 基 淸*

*全南大學校 農科大學 農生物學科

ABSTRACT

Papilla and cytoplasmic aggregates clearly formed on the epidermal cells of barley leaves in response to the primary germ-tubes of *Erysiphe graminis* f. sp. *hordei*, but their sizes were much smaller than those in response to the appressoria. Some cells of barley leaves exposed to powdery mildew for 36 - 48h were more deeply stained as compared to the other cells by acid fuchsin. However, the content of malondialdehyde in powdery mildewed leaves, one of the product of lipid peroxidation, did not increase by 96h after inoculation. Positive reactions for callose, protein and phenolics were recognized in the papilla and cytoplasmic aggregates at 6h after inoculation, but cutin, suberin, cellulose and lignin were not noticeable until 72h after inoculation. The total phenol content in methanol extracts increased with increasing time after inoculation. All histochemical reactions were not race-specific in barley-powdery mildew combinations tested.

Key words: E. graminis f.sp. hordei, barley, papillae, cytoplasmic aggregate, phenols.

要 約

0.5% lactophenol acid fuchsin으로 染色해 본 結果 보리흰가루 病菌의 第1次 發芽管에 反應하여 보리表皮細胞에 papillae 및 cytoplasmic aggregate가 形成되었으나 그 크기는 附着器에 反應하여 形成된 것 보다는 훨씬 작았으며 또한 接種後 36-48時間 以後의 表皮細胞內에는 acid fuchsin에 依해서 다른 細胞들에 비해 細胞全體가 좀 더 진하게 染色되는 곳이 군데군데 관찰되었다. 그러나 接種 後 96時間까지 脂質過酸化産物의 일종인 *malondialdehyde*의 含量은 增加하지 않았다. 接種 後 6時間에 形成된 papillae 및 cytoplasmic aggregate 內에 callose, protein, phenol 物質 등은 集積되었으나 接種 後 72時間까지도 cellulose, cutin, suberin, lignin 등은 檢出되지 않았다. 보리-흰가루病 相互組合의 上記 모든 反應은 race 非特異的이었다.

INTRODUCTION

Specificity in barley-powdery mildew combinations has been known to be determined after the penetration of infection hyphae because race specific incompatibility has never been expressed before infection pegs are produced by the appressorium (6, 10). Recently, the fact that barley cell may respond briefly to small primary germ-tube(PGT) of *Erysiphe graminis* f.sp. *hordei* Marchal at the early stage prior to the production of infection pegs, and the development and probable function of the PGT on the growth of *E. graminis* has been investigated (2, 13). Kunoh *et al* (15) found papilla beneath these tubes 4 - 6h after inoculation. They also showed that infection pegs were produced by some PGT as early as 2h after inlculation (16). This is well before the appressorium differentiates and incites a response from the host cell. Early host-pathogen interactions at the PGT stage were also indicated in oat-powdery mildew (11) and in wheat-powdery mildew combination (8).

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 the resistance was race non-specific (3). The significance of PGT of inducer race on the induction of resistance against challenger race was discussed in this paper.

MATERIALS AND METHODS

Plant and Isolate. Barley cultivar Pallas (*Hordeum vulgare* L) and one near-isogenic line of Pallas, 011301, and races, KH1 and KH2 of *E. graminis* f. sp. *hordei* were used. The isolates reacted differentially with the two barleys as follows:

Barley	Resistance gene	Infection type with KH1	KH2
Pallas	none	4	4
011301	M1-a1	0	4

0: incompatible reaction, no visible symtoms.

4: fully compatible reaction.

The 1st leaves fully expanded were used. Maintainance and inoculation of the fungus were the same as described previously (3).

Histochemical examination. Histochemical tests were made on the stripped abaxial epidermis(SAE) 6 - 72h after inoculation. Papilla and cytoplasmic aggregates were stained with 0.5% lactophenol acid fuchsin. Specific metabolites in the appositions were examined as follows: Tollens silver solution for callose (14), I_2KI-lithium chloride for cellulose (18), sudan IV for cutin & suberin (9), phloroglucinol-HCl for lignin (9), $FeCl_3$-potassium ferricyanide for phenolics (19), and bromphenol blue for proteins (18).

Spectrophotometric analysis of the compounds. Six to ninety six hours after inoculation, the inoculated leaves were rubbed with wet cotton to remove the ectoepidermic portion of the parasite, and cut into the segments.

Product of lipid peroxidation. The level of lipid peroxidation in the tissue was measured in terms of malondialdehyde (MDA) content determined by the thiobarbituric acid (TBA) reaction with the modified method of Dhindsa *et al* (4). A leaf sample (0.5g fr. wt.) was homogenized in 20 ml 0.1% trichloroacetic acid (TCA), and stirred at 20°C for 5h. The homogenate was centrifuged at 10,000g for 20 min.. Four ml of 20% TCA containing 0.5% TBA were added to 1ml aliquot of the supernatant. The mixture was heated at 95°C for 30 min. and then quickly cooled in tap water. After centrifuging at 10,000g for 10 min. The absorbance of the supernatant was read at 532 nm wavelength.

Total phenols. A leaf sample (0.5g fr. wt.) was kept in 50 ml of 70% methanol at 80°C for 20 min. (three times). The 150 ml of extract was then filtered and evaporated to dryness in *vacuo* at 40°C. The residue was dissolved in 15 ml distilled water at 40°C and centrifuged at 10,000g for 10 min.. One ml Folin-ciocalteu (1N HCl) solution was added to 1 ml aliquot of the supernatant, and were well mixed. After 3 min., 1 ml of 1N Na_2CO_3 solution is added and the tubes are thoroughly shaken. After 1 hour, the absorbance were determined at 725nm using as a blank water and reagent only.

RESULTS

Papillae and cytoplasmic aggregates. Papilla and

cytoplasmic aggregates clearly formed on the epidermal cells of barley leaves in response to the primary germ tubes of *E. graminis* f. sp. *hordei*, but their sizes were much smaller than those in response to the appressoria (Plate 1). Some cells of barley leaves exposed to powdery mildew for 36 - 48 h were more deeply stained as compared to the other cells by acid fuchsin (Plate 2). Thus, it was examined if the deep staining of the cells was due to the hypersensitive reaction of barley leaves. The level of lipid peroxidation was measured assuming cell death in the inoculated leaves. However, the content of malondialdehyde in powdery mildewed leaves, one of the product of lipid peroxidation, did not increase by 96h after inoculation. No sign of cell membrane deterioration was detected.

Histochemical analysis were carried out to examine the chemical constituents of papillae and cytoplasmic aggregate in response to primary germ tubes in barley leaves. Positive reactions for callose (Plate 3), protein (plate 4) and phenolics (Plate 5) were recognized in papilla and cytoplasmic aggregates at 6h after inoculation, but cutin, suberin, cellulose and lignin were not noticeable until 72h after inoculation (Table 1).

All reactions discribed above were not race-specific in barley-powdery mildew combinations.

Phenolic compounds. The total phenol content in methanol extracts from healthy and infected leaves at intervals after inoculation were compared. Phenolic compounds increased with increasing time after inoculation (Fig. 1). Regardless of compatible or incompatible, approximately 0.14 increase of optical density relative to control was recorded 24h after inoculation.

DISCUSSION

Localized resistance to a virulent race of *E. graminis*

Table 1. Comaprisons of different histochemical tests for some metabolites in the papillae and cytoplasmic aggregate produced by barley - powdery mildew interactions

Reagent	Substance tested	Time(hr) after inoculation								
		Pallas-KH1[a]			011301-KH1			011301-KH2		
		6	24	72	6	24	72	6	24	72
Tollens silver sol.	Callose	+[b]	+	+	+	+	+	+	+	+
I_2 KI-LiCl	Cellulose	−	−	−	−	−	−	−	−	−
Sudan IV	Cutin, Suberin	−	−	−	−	−	−	−	−	−
Phloroglucinol-HCl	Lignin	−	−	−	−	−	−	−	−	−
Bromphenol blue	Protein	+	+	+	+	+	+	+	+	+
$FeCl_3$-$K_3Fe(CN)_6$	Phenolics	+	+	+	+	+	+	+	+	+

[a] Barley-powdery mildew interactions.

[b] +: Positive reaction (detected), −: Negative reaction (not detected).

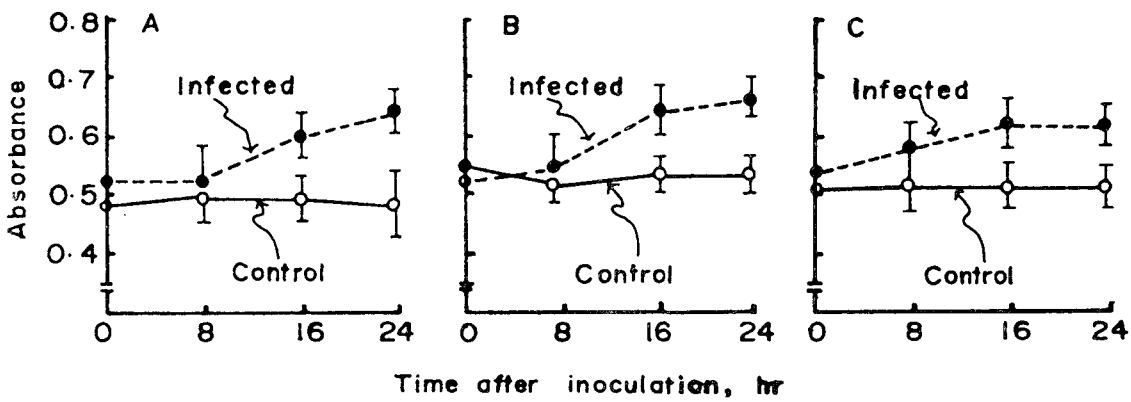

Fig. 1. Time course increase of the phenolic compounds in barley leaves infected with *Erysiphe graminis* f. sp. *hordei*. Inoculum density was ca. 200 conidia/mm^2. A: Barley cv. Pallas, race KH1(compatible), B: Barley isoline 011301, race KH1(incompatible), C: Barley isoline 011301, race KH2(compatible). Vertical bars indicate standard deviations.

Explanation of plates

Micrographs of histological reactions in epidermal strips of barley infected with *Erysiphe graminis* f. sp. *hordei*.

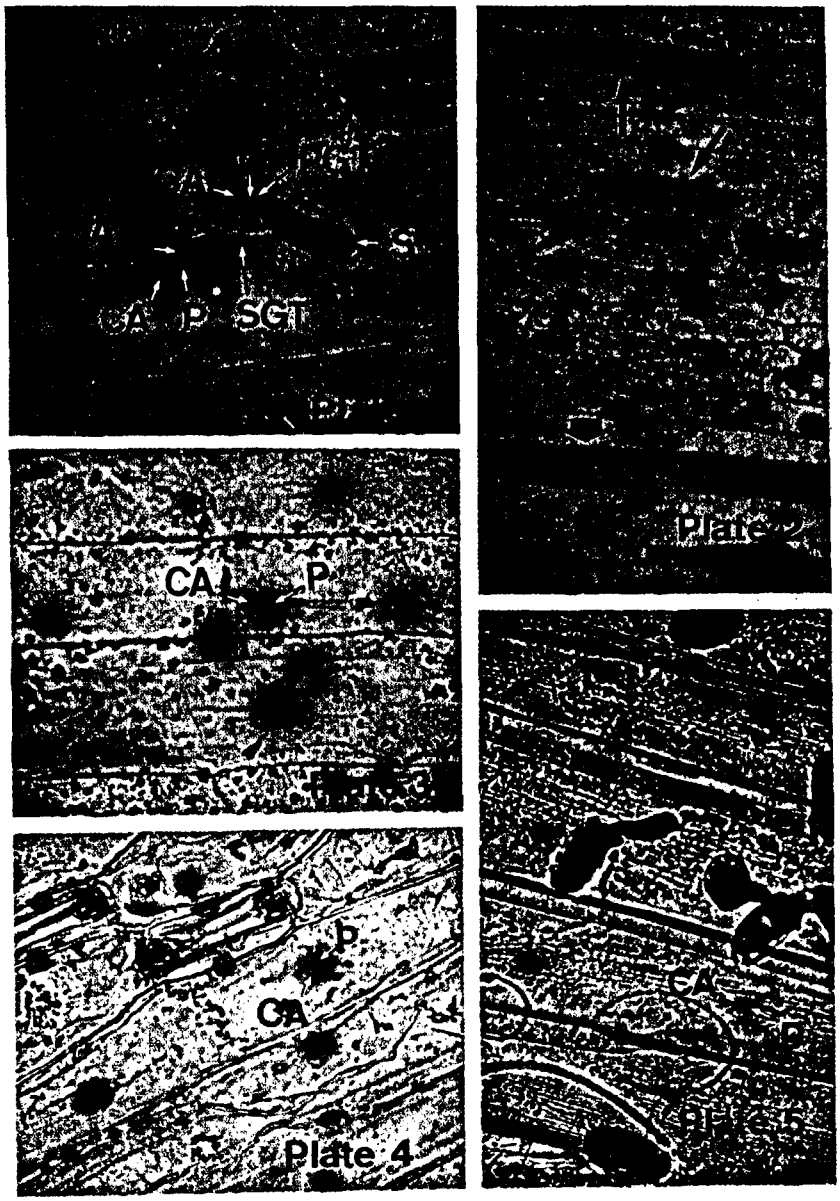

Plate 1. Cell wall appositions stained by 0.5% lactophenol acid-fuchsin in the epidermal strip of barley cv. Pallas 12 h after inoculation with the race KH1. (x 400)

Plate 2. Cells deeply stained by 0.5% lactophenol acid-fuchsin in the epidermal strip of barley isoline 011301 48h after inoculation with the race KH2. (x 100)

Plate 3. Positive reaction for callose in the epidermal strip of barley isoline 011301 6h after inoculation with the race KH1, stained by Tollens silver solution. (x 400)

Plate 4. Positive reaction for protein in the epidermal strip of barley isoline 011301 6h after inoculation with the race KH1 by bromphenol blue. (x 400)

Plate 5. Positive reaction for phenolics in the epidermal strip of barley cv. Pallas 6h after inoculation with the race KH1, stained by $FeCl_3$-$K_3Fe(CN)_6$. (x 400)

Abbreviations used in micrographs. A: appressorium, CA: cytoplasmic aggregate, P: papillae, S: spore, PGT: primary germ tube, SGT: secondary germ tube.

hordei by prior inoculation of a virulent or an avirulent race of the same fungus was induced on barley leaves (3). The resistance greatly increased as the interval between inducer and challenge inoculation increased, showing the highest level by 6h exposure to the inducer races. This time is well before the appressorium differenciated. Since the inducer races were removed before challenge inoculation, only the 6h exposure of barley leaves to the inducer races may be the cause of the induced resistance. Thus, barleypowdery interactions at 6h after inoculation were histochemically investigated.

Papilla and cytoplasmic aggregates clearly formed on the epidermal cells of barley leaves in response to the primary germ tube of *E. graminis hordei* (Plate 1). The primary germ tube has been known to be developed from mildew conidia 0.5–6h after inoculation (15). The significance of papilla formation and cytoplasmic aggregation in resistance mechanism has been discussed (1).

Histochemical approaches to papilla deposition led to postulate the existance of some metabolites in the papilla incited by appressoria of barley powdery mildew (1, 5, 12, 16), but the papilla deposition incited by primary germ tube haven't been studied before. Callose, protein and phenolics were recognized in the papilla and cytoplasmic aggregates at 6h after inoculation in this experiment. This fact suggest that primary germ tubes may elicit resistance reaction in the inoculated barley leaves. Moreover, phenolic compounds increased with increasing time after inoculation (Fig. 1). The increase is probably due to the activation of synthesis of the compounds. This result is well in agreement with Frić's (7).

Induced resistance elicited by compatible and incompatible inducer races were similar with respect to the level of resistance and the time needed for induction of resistance (3). All reactions in this experiment were also race non-specific in barley-powdery mildew interactions combinated.

REFERENCES

1. AIST, J. R. (1976). Papilla and related would plugs of plant cells. *Ann. Rev. Phytopathol.* 14: 145-163.
2. CARVER, T. L. W. & BESHNELL, W. R. (1983). The probable role of primary germ tubes in water uptake before infection by *Erysiphe graminis*. *Physiol. Plant Pathol.* 23: 229-240.
3. CHO, B. H. & SMEDEGAARD-PETERSEN, V. (1985). Localized induced resistance to *Erysiphe graminis* f. sp. *hordei* in near isogenic barley lines. *Korean J. Plant Pathol.* 1: 22-27.
4. DHINDSA, R. S., PLUMB-DHINDSA, P. & THORPE, T. A. (1981). Leaf senscence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Experimental Botany* 32: 93-101.
5. EDWARD, H. H. (1970). A basic staining material associated with the penetration process in resistant and susceptible powdery mildewed barley. *New Phytol.* 69: 299-301.
6. ELLINGBOE, A. H. (1972). Genetics and physiology of primary infection by *Erysiphe graminis*. *Phytopathology* 62: 401-406.
7. FRIĆ, F. (1969). Phenolische Stoffe und Oxydasen vom Standpunkt der Resistenz von Gerste gegen Mehltau (*Erysiphe graminis* f. sp. *hordei* Marchal). *Biologia* (*Bratislava*) 24 (1): 54-69.
8. GREEN, N. E.,HADWIGER, L. A. & GRAHAM, S. O. (1975). Phenylalanine ammonia lyase, tyrosine ammonia lyase and lignin in wheat inoculated with *Erysiphe graminis* f. sp. *hordei*. *Phytopathology* 65: 1071-1074.
9. JOHANSEN, D. A. (1940). *Plant microtechnique*. McGraw-Hill, New York. 523p.
10. JOHNSON, L. E. B., BUSHNELL, W. R. & ZEYEN, R. J. (1979). Binary pathway analysis of primary infection and host response in populations of powdery mildew fungi. *Can. J. Botany* 57: 497-511.
11. KIDGER, A. L. & CARVER, T. L. W. (1981). Autofluorescence in oats infected by powdery mildew. *Tran. Br. Mycol. Soc.* 76: 405-409.
12. KITA, N., TOYODA, H. & SHISHIYAMA, J. (1980). Histochemical reactions of papilla and cytoplasmic aggregate in epidermal cells of barley leaves infected by *Erysiphe graminis hordei*. *Ann. Phytopath. Soc. Japan* 46: 263-265.
13. KUNOH, H. (1982). Primary germ tubes of

Erysiphe graminis conidia. In *Plant Infection, The Physiological and Biochemical Basis*. Ed. by Y. ASADA, W. R. BUSHNELL, S. OUCHI & C. P. VENCE, pp. 45-59. Japan Scientific Societies Press, Tokyo.

14. KUNOH, H. & AKAI, S. (1969). Histochemical observations of the halo on the epidermal cell wall of barley leaves attacked by *Erysiphe graminis hordei*. *Mycopathol. Mycol. Appl.* 37: 113-118.
15. KUNOH, H., ISHIZAKI, H. & NAKAYA, K. (1977). Cytological studies of the early stages of powdery mildew in barley and wheat leaves. II. Significance of the primary germ tube of *Erysiphe graminis* on barley leaves. *Physiol. Plant Pathol.* 10: 191-199.
16. KUNOH, H., TSUZUKI, T. & ISHAZAKI, H. (1978). Cytological studies of the early stages of powdery mildew in barley and wheat. IV. Direct ingress from superficial primary germ tubes and appressoria of *Erysiphe graminis* f. sp. *hordei* on barley leaves. *Physiol. Plant Pathol.* 13: 327-333.
17. MAYAMA, S. & SHISHIYAMA, J. (1978). Localized accumulation of fluorescent and u.v. absorbing compounds at pentration sites in barley leaves infected with *Erysiphe graminis hordei*. *Physiol. Plant Pathol.* 13: 347-354.
18. OKAMOTO, K., UEDA, M., MADEA, R., MIZUTANI, A. & SUGIYAMA, T. (1976). *Microscopic histochemistry*. Igaku Shoin Ltd., Tokyo.
19. SHERWOOD, R. T. & VANCE, C. P. (1976). Histochemistry of papilla formed in reed canary grass leaves in response to noninfecting pathogenic fungi. *Phytopathology* 66: 503-510.
20. WOOLACOTT, B. & ARCHER, S. A. (1984). The influence of the primary germ tube on infection of barley by *Erysiphe graminis* f. sp. *hordei*. *Plant Pathol.* 33: 225-231.