Histopathology of Resistant and Susceptible Rice Plants Inoculated with *Pyricularia oryzae* Cavara

By HIRONORI KOGA

Environment Division, Hokuriku National Agricultural Experiment Station
(Joetsu, Niigata, 943-01 Japan)

Rice blast disease is one of the most important diseases of rice in Japan. Breeding of rice for blast resistance is effective in preventing this disease. In order to breed resistant varieties unerringly, it is important to clarify the interaction between host and pathogenic fungus.

Some anatomical studies on rice leaf blades inoculated with *P. oryzae* were developed by Ikata et al.[2], Kawamura & Ono[3], and Suzuki et al.[13]. The results of these studies done with rice leaf blades were obtained before the studies on the distinction of the races and the identification of the resistance genes were undertaken. Therefore it is necessary to undertake studies anew with the new understanding of the relationship between races and resistance genes. On the other hand, it is often difficult, by observing paraffin sections under a light microscope, to clarify the differences among the individual infected sites concerning whether the penetration was successful or not, and what kind of the reactions occurred in and around the attacked epidermal cells. In addition, the infected site that can be observed by this paraffin sectioning method is confined to a very small area. Moreover, it is not clarified what kind of ultrastructural changes occur in relation to the resistance of rice to *P. oryzae*, although resistance reactions of non-host to this fungus were studied electron-microscopically[1].

In the present paper, comparisons were undertaken between the resistant and susceptible rice varieties to *P. oryzae* with aids of light and electron microscopes to clarify the cell reactions that are most closely related with the manifestation of major genes for resistance. In the light microscopical observation, a whole leaf clearing and staining technique[4] was applied to make up for the deficiencies mentioned above.

Spore germination and appressorial formation of *P. oryzae* on rice leaves

Spore germination and appressorial formation of *P. oryzae*, isolate Ken 60–19 on rice leaves were compared among 4 resistant (Toride 1, Pi No. 4, Fukunishiki and Yashiromochi) and 6 susceptible varieties (Shin 2, Aichi asahi, Ishikari shiroke, Kanto 51, Tsuyuake and Koshihikari)[7]. Spore germination and appressorial formation were observed under the fluorescent microscope after samples were stained with Calcofluor White[7,12]. The percentages of spore germination and appressorial formation of *P. oryzae* showed no statistical differences between a highly resistant variety (Toride 1) and a susceptible one (Koshihikari) in the density of spore suspension from 5×10^2 to 5×10^4 spores/ml[7]. The changes with the passage of time for the percentages of spore germination, and immature and mature appressorial formation on the leaves of Toride 1 were not different from those of Koshihikari (Fig. 1). There were no significant differences in percentages of spore germination and mature

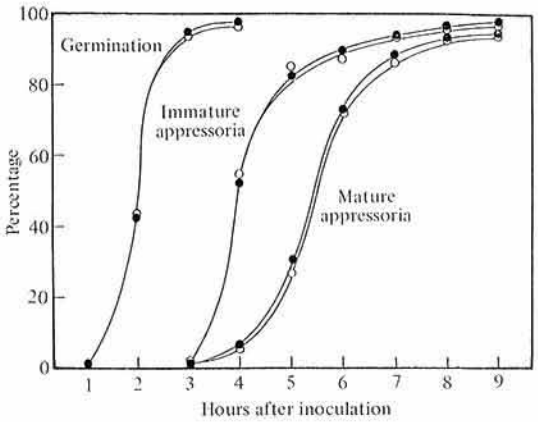

Fig. 1. Changes in the percentages of germination and immature and mature appressorial formation of the spores of rice blast fungus on rice leaves with the passage of time
The susceptible (○: Koshihikari) and the highly resistant variety (●: Toride 1) were inoculated with *P. oryzae*, isolate Ken 60-19.

appressorial formation of *P. oryzae*, isolate Ken 60–19 among 4 resistant and 6 susceptible varieties[7]. From these results, it was suggested that specific resistance of rice varieties tested to *P. oryzae*, isolate Ken 60–19 is not expressed before fungal penetration into rice leaves.

Fungal penetration into rice leaves and host cell responses

Early infection process of *P. oryzae*, isolates Ken 60–19, HR 77–189 and Ina 72 in leaves of susceptible, resistant, and highly resistant varieties was observed under the light microscope with an aid of the whole-leaf clearing and staining technique[4] (Plate 1). The penetration peg pierced through host cell wall and developed invaded hyphae. There were a few percentages of appressoria with invaded hyphae 36 hr after inoculation and the percentages increased between 48 hr and 72 hr after inoculation (Fig. 2)[5,6,12]. The percentages of successful penetration on leaves of Toride 1 (highly resistant variety), and Fukunishiki (resistant), were less than on Koshihikari (susceptible) 48 hr after inoculation[5,6,12]. Growth of invaded hyphae on leaves of Toride 1 and Fukunishiki were less than on Koshihikari 48 hr after inoculation (Fig.

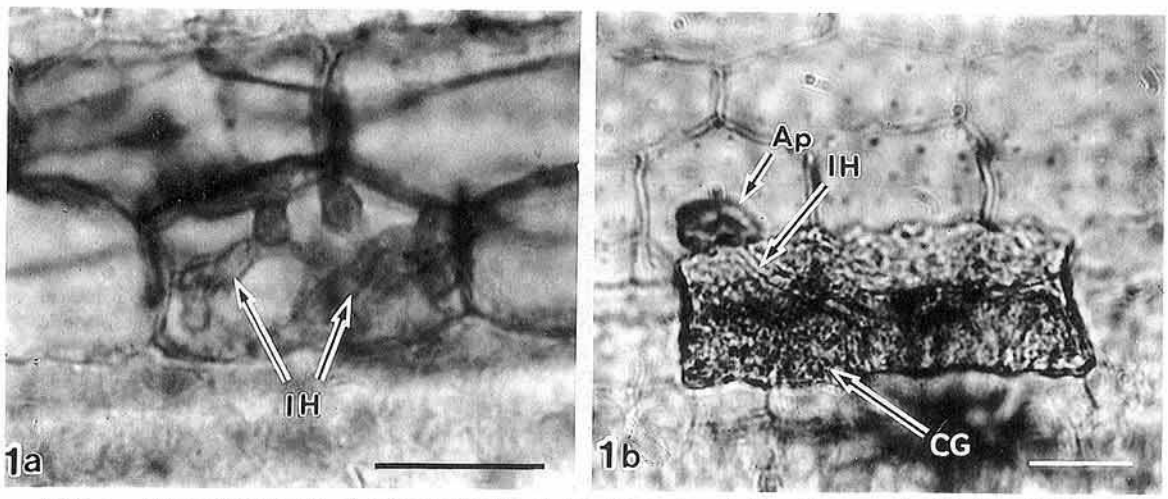

Plate 1. Microphotographs of epidermal cells of rice leaves invaded by *P. oryzae*, isolate Ken 60-19
a: Invaded hyphae that are branched are observed, but the epidermal cellular contents are not undergoing granulation (Koshihikari, 72 hr after inoculation).
b: A short and slender invaded hypha which is not branched is observed and granulation of the epidermal cellular contents is going on (Toride 1, 144 hr after inoculation).
Ap: Appressorium, CG: Granulation of the epidermal cellular contents, IH: Invaded hyphae.
The scale in each picture represents 20 μm.

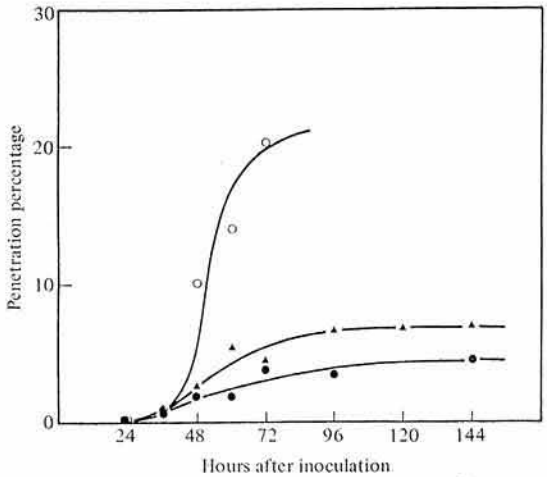

Fig. 2. Comparison of the percentages of penetration among the susceptible (○: Koshihikari), the resistant (●: Fukunishiki) and the highly resistant (▲: Toride 1) rice leaves inoculated with *P. oryzae*, isolate Ken 60-19

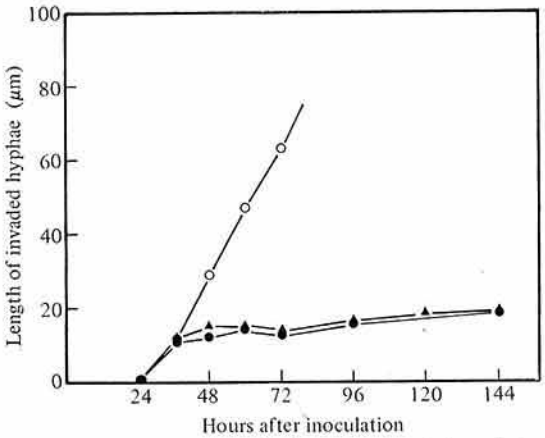

Fig. 3. Comparison of the growth of invaded hyphae of *P. oryzae*, isolate, Ken 60-19 inside the rice leaves among the susceptible (○: Koshihikari), the resistant (●: Fukunishiki) and the highly resistant variety (▲: Toride 1)

3). In the highly resistant combination (Ken 60-19/Toride 1), and the resistant combination (Ken 60-19/Fukunishiki), invaded hyphae ceased growing within the primarily invaded epidermal cell except brown spot lessions.

The host-parasite encounter sites were grouped into 6 types (Fig. 4, N, A-E), according to fungal development and host responses. The rates of no-reaction at penetration sites in the highly resistant and the resistant combinations were almost the same as those of susceptible ones (Fig. 5). Therefore, it seems that unsuccessful penetration without any host reactions was independent of the major gene for resistance to *P. oryzae*. The granulation (types A and B) of cellular contents of epidermal cells which was suggested to be dead, appeared 36 hr after inoculation and the occurrence rates increased until 72 hr after inoculation, when most of encounter sites accompanied the cytoplasmic granulation except no-reaction type (type N) (Figs. 5 and 6). The epidermal cells with cytoplasmic granules were often pale-browned 144 hr after inoculation (Plate

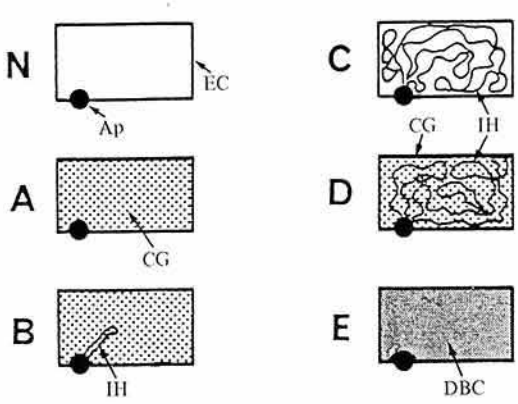

Fig. 4. Classification of infection types according to the penetration of rice blast fungus, fungal growth and the reactions of the rice cells

N: Unsuccessful penetration without any host reaction, A: Unsuccessful penetration with host cytoplasmic granulation in an epidermal cell, B: A poorly-developed invaded hyphae with host cytoplasmic granulation in epidermal cell, C: Well-developed invaded hyphae without host cytoplasmic granulation in an epidermal cell, E: Deep browning of the infected epidermal and parencymatous cells. Ap: Appressorium, CG: Granulation of the epidermal cellular contents, DBC: Epidermal cell with deep browning, EC: Epidermal cell, IH: Invaded hyphae.

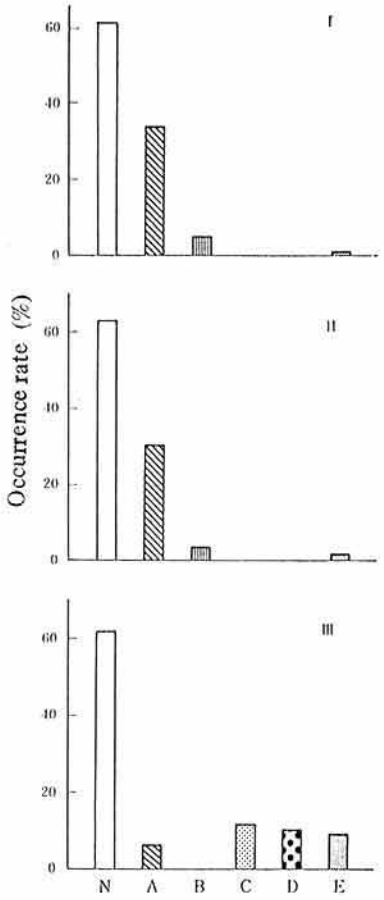

Fig. 5. Occurrence rates of the various infection types in the highly resistant (I: Toride 1), the resistant (II: Fukunishiki) and the susceptible varieties (III: Koshihikari) under attack by *P. oryzae*, isolate Ken 60-19, 72 hr after inoculation
Refer to Fig. 4. for infection types.

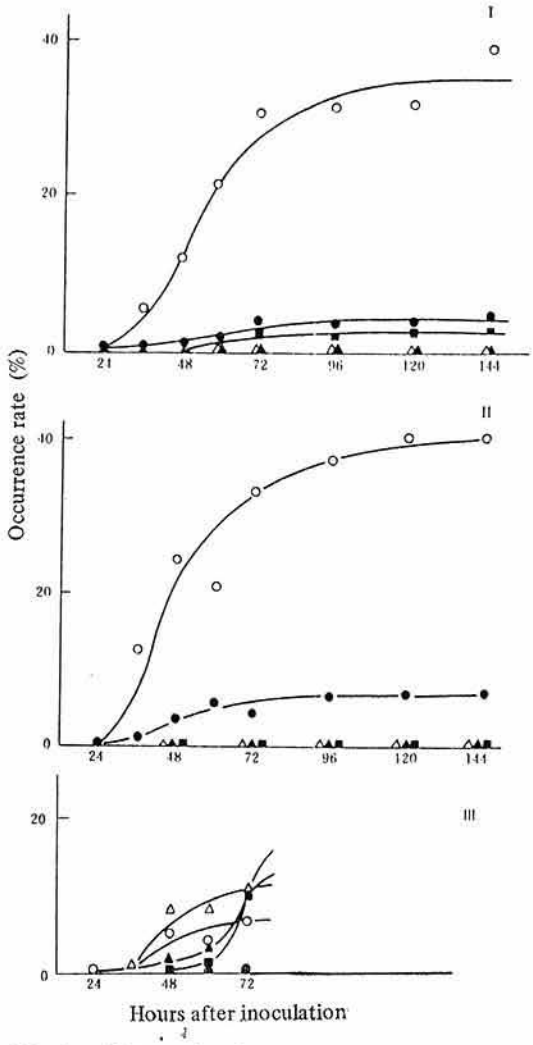

Fig. 6. Changes in the occurrence rates of the various infection types (○: Type A, ●: Type B, △: Type C, ▲: Type D, ■: Type E) in the resistant (I: Fukunishiki), the highly resistant (II: Toride 1) and the susceptible varieties (III: Koshihikari) under attack by *P. oryzae*, isolate Ken 60-19
Refer to Fig. 4 for infection types.

1-b)[6]. Occurrence rates of cytoplasmic granulation at penetration sites (types A and B) in the highly resistant and the resistant combinations were distinctly higher than in the susceptible one (Fig. 5). The host-parasite encounter sites where fungal penetration or hyphal growth ceased within the primary attacked cells except reaction type N were accompanied with granulation of epidermal cellular contents (types A and B). The similar results were also gained in the highly resistant combinations (Ken 60-19/Pi No. 4, HR 77-189/Tsuyuake and HR 77-189/Kanto 51) and the resistant combination (Ken 60-19/Yashiromochi). Therefore, it was sug-

gested that the granulation of the epidermal cellular contents closely correlates with the expression of the major genes for resistance of Toride 1, Pi No. 4, Tsuyuake, Kanto 51, Fukunishiki and Yashiromochi.

The occurrence rates of the brown-spot lesions in primary leaves inoculated with *P. oryzae* were less than 1% to appressoria observed in the combinations of Ken 60–19/Toride 1, a few percent in Ken 60–19/Fukunishiki, and 5–15% in Ken 60–19/Koshihikari (Fig. 5). It suggests that the occurrence rates of the brown-spot lesions were not coincident with the strength of resistance of rice leaves to *P. oryzae*. In the brown-spot lesions in primary leaves inoculated with *P.* electron-microscopically found in the necrotic parencymatous cells, but transformed at various grades. Some hyphae contained swollen mitochondria, extremely-developed vacuoles and very small amounts of lipid granules, and the other hyphae seemed to be deceased with the granulation of their contents[8].

Ultrastructural aspects of granulation of epidermal cellular contents under the attack of *P. oryzae*

Electron microscopical studies were carried out to observe cytoplasmic granulation which was detected under a light microscope in the epidermal cells of rice leaves responding resistingly to the incompatible race of *P. oryzae*. The first obvious changes in the infected epidermal cells of the highly resistant variety (Toride 1) inoculated with *P. oryzae*, Ken 60–19 were invagination of plasma membrane and appearance of lomasome-like structures 24 hr after inoculation. Many invaginations in plasma membranes occurred 48 hr after inoculation (Plate 2). The invaginated structures of plasma membrane became globu-

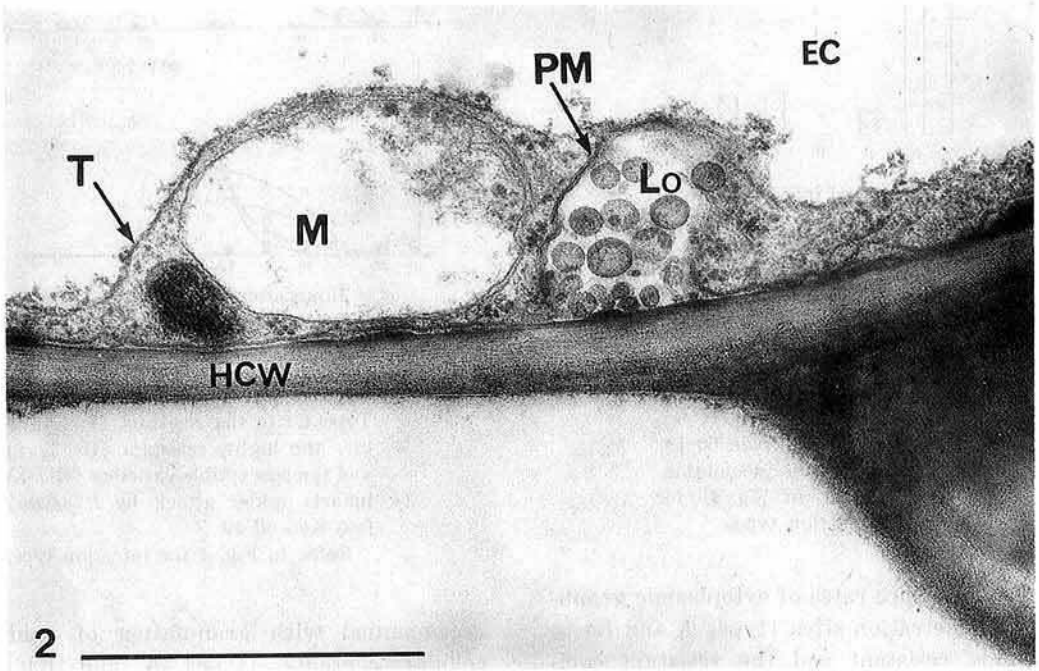

Plate 2. Ultrastructure of a rice leaf of Toride 1 (highly resistant variety) inoculated with *P. oryzae*, isolate Ken 60-19, 48 hr after inoculation
A swollen mitochondrion (M) and lomasome-like structure (Lo) observed in an epidermal cell.
HCW: Host cell wall, PM: Plasma membrane, T: Tonoplast.
The scale represents 1 μm.

Table 1. Occurrence of plasma membrane invagination, lomasome-like structures and degenerated mitochondria in resistant and susceptible rice leaves infected with *Pyricularia oryzae* Cav. race 037 (Ken 60-19), 48 hr after inoculation

Combination (Variety/Race)	No. of invaginated plasma membranes per cell	No. of lomasome-like structures per cell	Degenerated mitochondrion (%)
Non-inoculated (Toride 1)	0.21[a)]	0.19	0.00
Incompatible (Toride 1/Race 037)	7.05	1.40	77.80
Non-inoculated (Koshihikari)	0.27	0.24	0.00
Compatible (Koshihikari/Race 037)	0.85	0.42	6.90

a): The data represent the mean values of about 50 epidermal cells.

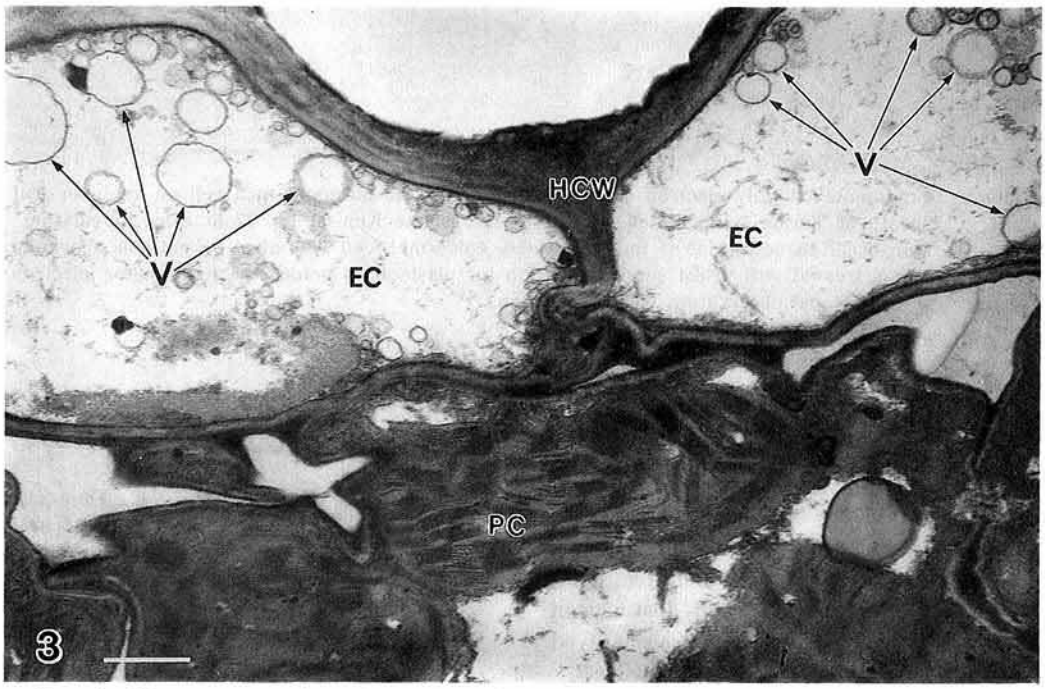

Plate 3. Ultrastructure of a rice leaf of Toride 1 inoculated with *P. oryzae*, Ken 60-19, 72 hr after inoculation
Vesicular structures (V) in various sizes observed in the epidermal cells.
PC: Parencymatous cell.
The scale represents 1 μm.

lar and formed lomasome-like structures. Some globular membrane structures seemed to be detached from the plasma membrane. The outer membranes of lomasome-like structures often disappeared and the vesicles closed in the structures were dispersed into the cytoplasm[9].

Degenerated mitochondria and swollen endoplasmic reticula were observed 48 hr after inoculation (Table 1)[9]. At 72 hr after inoculation, a lot of vesicles of various sizes (0.1–1.1 μm) were observed in the infected epidermal cells in the highly resistant variety

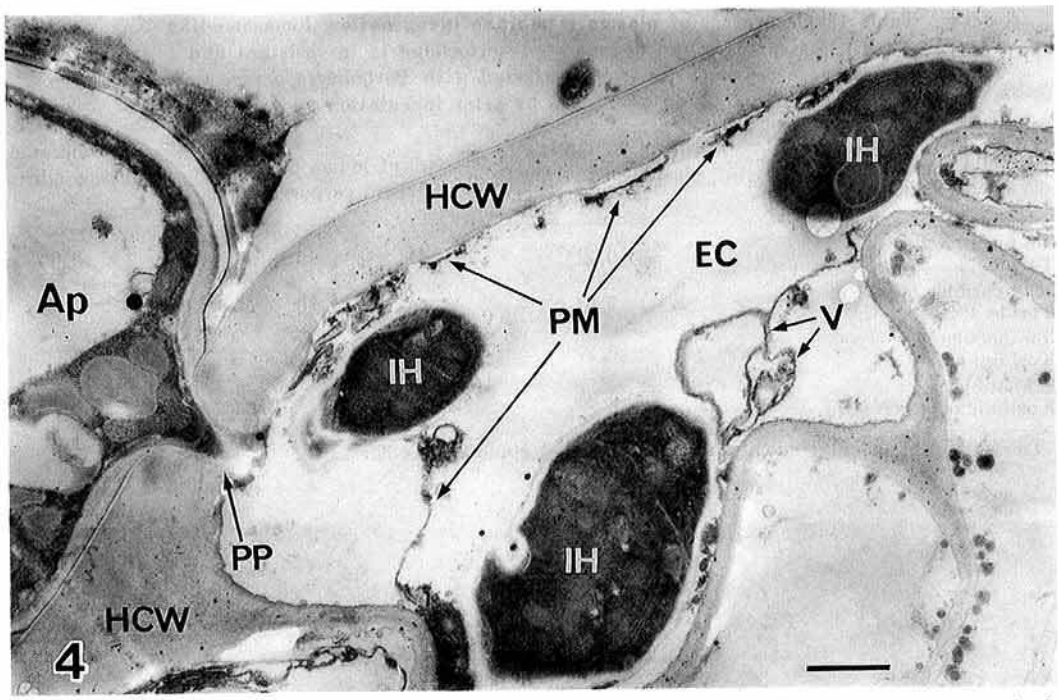

Plate 4. Fragmented, highly electron dense plasma membrane observed in a cell on an inner leaf sheath of Toride 1 inoculated with *P. oryzae*, isolate Ken 60-19, 27 hr after inoculation
In order to determine if the penetrated epidermal cell was alive or not, the specimen was treated with 0.8M sucrose solution for plasmolysis before the fixation for electron microscopic observation was undertaken.
PP: Penetration peg.
The scale represents 1 μm.

(Plate 3)[9]. In the leaf sheath of Ken 60-19/Toride 1 combination, invagination of plasma membrane and lomasome-like structures in the attacked epidermal cells were more frequent than in those in the susceptible one (Ken 60-19/Koshihikari), then fragmentation of plasma membranes and destruction of the unit membrane structure were observed in the infected epidermal cells (Plate 4)[10].

In the susceptible combination (Ken 60-19/Koshihikari), such ultrastructural changes as observed in the highly resistant one were rarely observed at the attacked epidermal cells of both leaf and leaf sheath. Moreover, the cell wall of the invaded hyphae was distinctly in contact with the cytoplasm of the epidermal cells, suggesting that the invaded hyphae penetrated through the plasma membrane of the epidermal cells[11].

References

1) Hashioka, Y. & Ando, N.: Fine structure of the rice blast. XIII. Ultrastructure of *Pyricularia oryzae*—nonhost interfaces. *Res. Bull. Fac. Agr. Gifu Univ.*, 38, 39-47 (1975).
2) Ikata, S., Matsuura, Y. & Taguchi, S.: Experimental results on control of rice blast disease. 1. Resistance of rice cultivars to blast disease. *Nōji Kairyo Shiryo*, 20, 1-140 (1931) [In Japanese].
3) Kawamura, E. & Ono, K.: Resistance of foreign rice varieties to rice blast disease. *J. Nat. Agr. Exp. Sta.*, 4, 13-22 (1948) [In Japanese with English summary].
4) Koga, H. & Kobayashi, T.: A whole leaf clearing and staining technique to observe the invaded hyphae of blast fungus and host responses in rice leaves. *Ann. Phytopathol. Soc. Jpn.*, 46, 679-681 (1980).
5) Koga, H. & Kobayashi, T.: Comparison of the early infection process of *Pyricularia oryzae* Cav. in rice leaves of compatible and

incompatible combinations. *Ann. Phytopathol. Soc. Jpn.*, 48, 506–513 (1982) [In Japanese with English summary].

6) Koga, H. & Kobayashi, T.: Cytological reactions in leaves of the rice variety, Toride 1 to the incompatible race of *Pyricularia oryzae* Cav. *Proc. Assoc. Plant Prot. Hokuriku*, **30**, 12–18 (1982) [In Japanese with English summary].
7) Koga, H.: Comparison of spore germination and appressorial formation of *Pyricularia oryzae* Cav. on rice leaves in compatible and incompatible combinations. *Proc. Assoc. Plant Prot. Hokuriku*, **31**, 7–12 (1983) [In Japanese with English summary].
8) Koga, H., Kobayashi, T. & Horino, O.: Electron microscopical observation of rice leaves infected with *Pyricularia oryzae* Cav. in compatible and incompatible combinations. I. Fine structure of invaded hyphae in host cells. *Ann. Phytopathol. Soc. Jpn.*, 48, 281–289 (1982) [In Japanese with English summary].
9) Koga, H. & Horino, O.: Electron microscopical observation of rice leaves infected with *Pyricularia oryzae* Cav. in compatible and incompatible combinations. II. Resistance expression and cytoplasmic granulation in the epidermal cells. *Ann. Phytopathol. Soc. Jpn.*, 49, 322–330 (1983) [In Japanese with English summary].
10) Koga, H. & Horino, O.: Electron microscopical observation of rice leaves infected with *Pyricularia oryzae* Cav. in compatible and incompatible combinations. III. Resistance expression and loss of capability for plasmolysis in inner epidermal cells of leaf-sheath. *Ann. Phytopathol. Soc. Jpn.*, **50**, 353–360 (1984) [In Japanese with English summary].
11) Koga, H. & Horino, O.: Electron microscopical observation of rice leaves infected with *Pyricularia oryzae* Cav. in compatible and incompatible combinations. IV. The interface between invading hyphae and host cytoplasm in epidermal cells of leaf sheath. *Ann. Phytopathol. Soc. Jpn.*, **50**, 375–378 [In Japanese with English summary].
12) Koga, H., Horino, O. & Kobayashi, T.: Ultrastructural studies on the resistance mechanisms of rice plants infected with *Pyricularia oryzae* Cav. *Bull. Hokuriku Nat. Agr. Exp. Sta.*, 28, 1–56 (1986) [In Japanese with English summary].
13) Suzuki, N., Doi, Y. & Toyota, S.: Histochemical studies on lesions of rice blast caused by *Pyricularia oryzae*. II. On the substance in the cell-membrane of rice reacting red in color with diazo reagent. *Ann. Phytopathol. Soc. Jpn.*, **17**, 97–101 (1953) [In Japanese with English summary].

(Received for publication, Oct. 31, 1988)