島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 17 : 141–146, 1983

Light Microscopical Studies on Host and Non-host Response of Rice Plants against Several *Pyricularia* spp.*

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各種いもち病菌に対するイネの宿主・非宿主反応の顕微鏡観察

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Introduction

In many studies on the process of race-specific resistance and the resistance of non-host plants to plant pathogenic fungi, studies with light microscope has revealed the timing of the cessation of fungal growth during resistant reactions and the relationship between fungal development and host cell death.[1-6] To investigate the specific processes involved in these types of resistance may provide not only information about the way in which host-parasite specificity is determined but also some promise for more precise and longlasting control of plant pathogens in the future. Unfortunately, however, similar evidence is remarkably scarce for several *Pyricularia* spp. infection.[7-10] Therefore, the object of the present study is to search for significant and key responses of the rice plants against pathogens or non-pathogens in their early infection.

Materials and Methods

Plants. Two cultivars of rice plants, Reishiko (*Pi-k*) and Aichi-asahi (*Pi-a*), were employed for this work, and were grown as described previously.[8]

Fungi. Pyricularia oryzae (isolates Hoku 1 and Naga 87), *P. grisea* and *Pyricularia* spp. from Italian ryegrass (Italian-isolate), ragi (ragi-isolate), hachiku (hachiku-isolate) and madake (madake-isolate) were used. *Pyricularia* spp. are non-pathogenic on rice plants. Conidia of all *Pyricularia* isolates were prepared as described previously.[9]

Microscopical observations. Leaf-sheath inoculation method devised by Takahashi[11] was used. When inner surface of detached sheaths was inoculated with diluted conidial suspension (ca. 5-10 conidia under×150 microscopic field) of *P. oryzae*, responses of invaded cells were examined under microscope after 18-42 hr-inoculation at 28 C. Infected tissues were soaked in 0.8 M KNO_3 solution prior to the microscopic examination in order to test for the plasmolytic ability of the invaded cells and their neighbouring cells. On the other hand,

* This study was supported in part by a Crant-in-Aid from Ministry of Education, Science and Culture, Japan (No. 56760034), and portion of an M. S. thesis submitted to Tottori University by the senior author.
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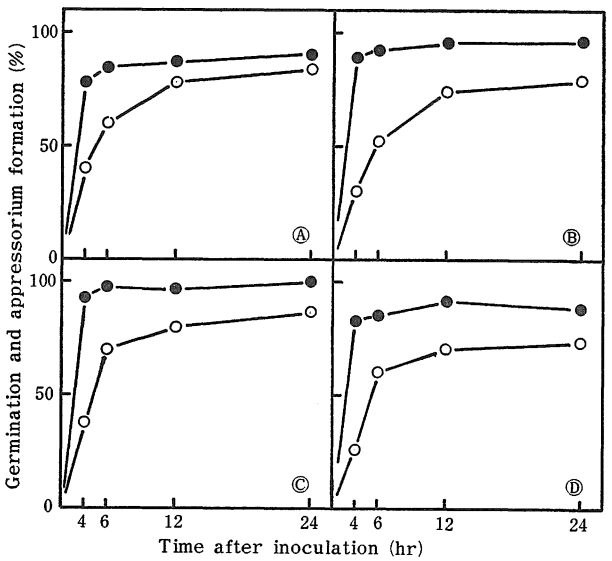

Fig. 1. Germination and appressorium formation of *P. oryzae* on the leaves.
—●— : Germination(%).
—○— : Appressorium formation(%).
A : cv. Reishiko-isolate Hoku 1 (Incompatible).
B : cv. Reishiko-isolate Naga 87 (Compatible).
C : cv. Aichi-asahi-isolate Hoku 1 (Compatible).
D : cv. Aichi-asahi-isolate Naga 87 (Incompatible).

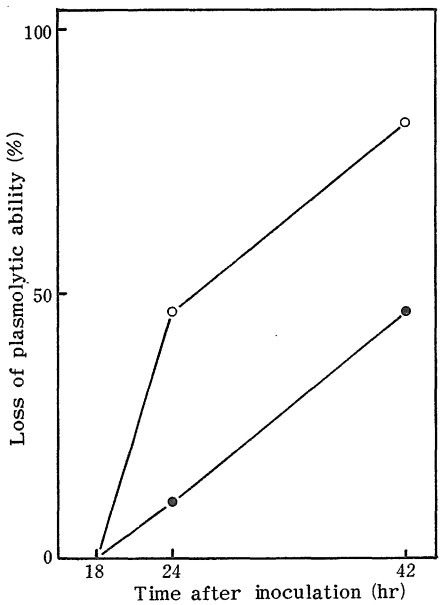

Fig.2. Time course in death of host cells after inoculation. Incompatible cv. Reishiko (—○—) and compatible cv. Aichi-asahi (—●—) were inoculated with conidia of *P. oryzae* (isolate Hoku 1), and were kept at 28 C.

when non-pathogenic *Pyricularia* spp. were inoculated, plant responses and fungal growth were observed 40-44 hr after inoculation. Index of fungal growth in rice cells was determined by Takahashi's method.[11,12]

Results

Pre-infectional behaviors of the compatible and incompatible race of P. oryzae on rice leaves

Rice leaves of the cultivars Reishiko and Aichi-asahi were inoculated with race 007 (isolate Hoku 1) and race 131 (isolate Naga 87) of *P. oryzae*. Germination and appressorium formation at the inoculated site were observed. Results are shown in Fig. 1. These races had comparable percentages of conidial germination and appressorium formation, regardless of cultivar-race combination ; rates of these behaviors reached a plateau at 12 hr.

Relationship between infection behavior of Pyricularia oryzae isolates and response of rice cultivars

When leaf-sheaths of rice plants were inoculated with race 007 (isolate Hoku 1), the penetration pegs were formed 18 hr after inoculation and began to penetrate the cell wall of both the incompatible cultivar (Reishiko) and the compatible cultivar (Aichi-asahi). Until this period, there were no remarkable differences in infection behaviors of the pathogen into the leaf-sheaths. The primary hyphae beneath each appressoria showed same value, 0.5 in the growth index. At this stage, all the invaded or their neighbouring host cells were keeping the ability to plasmolyse when they were soaked in 0.8 M KNO_3 solution. As first response of host cells 18 hr after inoculation, however, plasma membrane became indis-

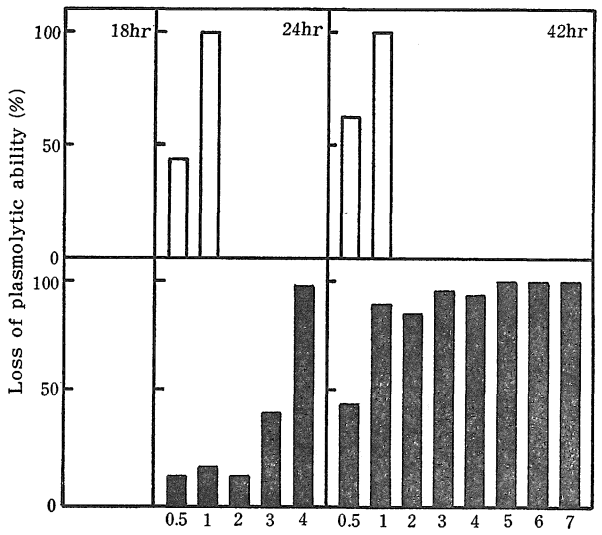

Fig.3. Relationship between death of host cells and the index of fungal growth in the host cells during infection stages after inoculation. Incompatible cv. Reishiko (□) and compatible cv. Aichi-asahi (■) were inoculated with conidia of *P. oryzae* (isolate Hoku 1), and were kept at 28 C. Index of fungal growth in the host cells was determined by Takahashi's method. [11]

logical responses of such dead cells were fine granulation of host cytoplasm accompanying uncolor or slightly colored change. In the incompatible host, indistinctness of outline of plasma membrane was also observed at the cells adjoining the invaded cells.

After 42 hr, infection hyphae were restricted within most of the primarily invaded cells in the incompatible host, and, as shown in Figs. 3 and 4, the index of fungal growth was almost the same value as that at 24 hr after inoculation. In the compatible host, number of cells having 0.5 value in the developmental index rapidly decreased, and conversely, number of cells with 2.0-7.0 in the developmental index increased. Most of the invaded cells or their neighbouring cells lost their plasmolytic ability. At this period, such pathological changes as browning, decoloration and coagulation occurred in many invaded cells. However, fungal tinctive only in the incompatible host cells beneath appressoria, but not in the compatible host.

After 24 hr, index of fungal growth was 0.5-1.0 in the incompatible cells, and was 0.5-4.0 in the compatible cells. About 50 percent of the invaded cells of the incompatible host lost their plasmolytic ability (Fig. 2). On the other hand, only 10 percent of those of the compatible host lost their plasmolytic ability. Results in Fig. 3 showed that death-rate of the host cells was proportional to the index of fungal growth in the infected cells. Morpho-

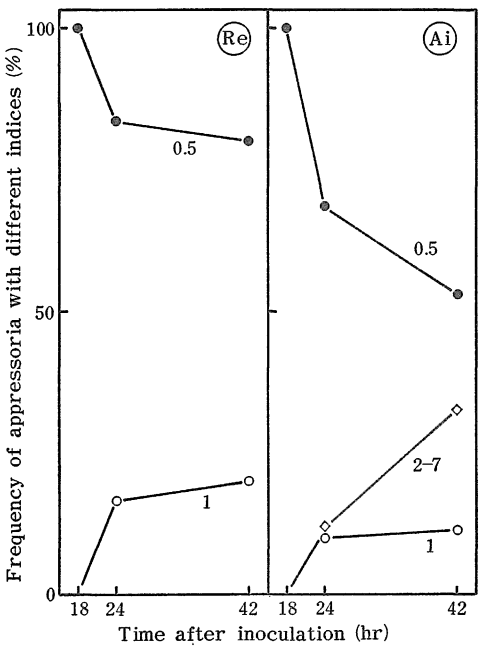

Fig.4. Time course of the fungal growth in the host cells of differnt rice cultivars after inoculation. Incompatible cv. Reishiko (Re) and compatible cv. Aichi-asahi (Ai) were inoculated with conidia of *P. oryzae* (isolate Hoku 1), and were kept at 28 C. Index of fungal growth in the host cells was determined by Takahashi's method. [11]
—●— : Fungal growth index 0.5.
—○— : Fungal growth index 1.
—◇— : Fungal growth index 2–7.

growth continued to develop further even in such damaged host cells. Indistinctness of outline of plasma membrane tended to appear at the invaded or their neighbouring cells in the compatible host rather than in the incompatible host, and, in such cells, plasma streaming or Browian movement was observed clearly.

Table 1. Penetration and fungal growth of *Pyricularia* spp. in the sheath cells of rice plants

Cultivar	*P. oryzae*[a]	*P. grisea*	*Pyricularia* sp. from			
			Italian ryegrass	ragi	madake	hachiku
Reishiko	23[b](0.7)[c]	38(1.0)	19(0.8)	6(1.0)	11(0.5)	14(0.5)
Aichi-asahi	34(6.0)	—[d]	24(0.5)	11(0.5)	—	4(1.0)

Detached sheaths were inoculated with conidia of *Pyricularia*, and penetration and fungal growth were observed 40—44 hr after inoculation.
a) Isolate Hoku 1 (race 007). b) Penetration (%). c) Average index of fungal growth.
d) Not observed.

Non-host interaction of rice plant cells with non-pathogenic Pyricularia spp.

Poor penetration and fungal growth occurred at the all infection sites in rice sheath cells inoculated with non-pathogenic isolates of *Pyricularia* spp., and there was little difference in their penetration rates. After 40-44 hr, average penetration ranged 6 % (ragi-isolate) to 38 % (*P. grisea*). Further the average and highest index values of fungal growth from appressoria of all non-pathogenic *Pyricularia* spp. showed less than 0.8 and 2, respectively. All intercellular hyphae were located in the primarily infected cells (Table 1). In all combinations, during such non-host responses of rice plant, different cellular changes appeared in rice sheath cells. As reported previously[8], most of the changes in non-host interaction were very similar to those induced in the host interaction with the incompatible races of *P. oryzae*.

Discussion

Detailed microscopical observations showed that conidial germination and fungal development on the leaf surface are not determinants of varietal resistance, and that the growth of invading hyphae in the host cells was closely related to morphological changes of the host cells. The same changes of the infected host cells were previously observed by Takahashi[13] and Ohata *et al.*[14] Generally, in case of the compatible combination, the death of the invaded cells occurred only at the latter period of infection (42 hr after inoculation) when the pathogen had considerably colonized the suscept. In contrast to this, in case of the incompatible combination, the invaded host cells quickly died, became granular and turned brown soon (24 hr after inoculation) after the penetration of infection hyphae. From these results, it was suggested that, although *P. oryzae* is not an obligate parasite, there may be a kind of symbiotic phase in the early stages of compatible interaction, or that the hypersensitive cell death of host plays an important role in blast resistance.

Recently, Arase *et al.*[8] demonstrated that rice tissue of each cultivar possesses the cells which differ in degree of the restrictive ability of fungal growth at the early infection with an incompatible race of *P. oryzae*. It seems that differences in the degree of blast resistance

among the rice cultivars largely depend on the rate of hypersensitive cell death, as Doke[15] reported in *Phytophthora infestans*-potato cell system.

The authors newly observed an initial event in the host-parasite interaction of rice blast. It was an indistinctness phenomenon of host plasma membrane under microscope, which occurred beneath or closely near the infction site in the incompatible host (from 18 hr after inoculation) faster than in the compatible host (from 24 hr after inoculation). We reported that an increse of electrolyte leakage from the incompatible host was observed faster than in the compatible host until 12-22 hr after conidial inoculation.[16] From these results, it was suggested that membrane-damage of the incompatible host may occur at early infection.

The results of non-host responses presented here support the conclusion of a recent study[8] that fungal development in infected rice cells was strongly inhibited in all combinations, and that there is difference in resistance induced in a rice cultivar between host and non-host interactions. According to Heath,[1] resistance was expressed at clearly defined stages of fungal development, and the stages of parasitism were controlled by a series of two-way switching-points. On the other hand, Mansfield *et al.*[3] reported that the development of resistant reactions of broad bean and tulip to non-pathogenic *Botrytis* species was associated with less rapid penetration and the death of far fewer plant cells during the early stages of infection. It is necessary that we should compare with role of hypersensitive reaction in host and non-host interactions.

Summary

Host and non-host responses of rice plants against *Pyricularia oryzae* and other *Pyricularia* spp. were observed microscopically. Conidia of *P. oryzae* germinated equally well, and formed appressoria on the leaves of incompatible and compatible host. The appressoria formed the penetration pegs and began to penetrate the host cell wall about 18 hr after inoculation. Until this stage, invaded cells of both the incompatible cv. Reishiko and the compatible cv. Aichi-asahi were alive. However, in the cv. Reishiko, the indistinctness of plasma membrane under light microscope was observed in first invaded cells and appeared to be an initial response of host cells to the pathogens. After 24 hr, most of the invaded cells of the incompatible cultivar indicate slightly colored change, and already lost their plasmolytic ability. Infection hyphae were restricted within the host cells which hypersensitively died, and were inhibited to grow. On the other hand, in the compatible cv. Aichi-asahi, most of invaded cells remained alive for much longer period and the hyphal growth was observed to continue. After 42 hr, in the incompatible combination, the host changes and fungal behavior were nearly the same as those at 24 hr after inoculation. In the compatible combination, although such phenomena as browning, deplasmolysis of host cells and the indistinctness of plasma membrane were observed, fungal growth in infected host cells was not inhibited. On the other hand, in the case of non-host interaction with non-pathogenic blast fungi, rice sheath cells of the cv. Reishiko died hypersensitively and enclosed infection hyphae in the first penetrated cells.

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摘　　要

イネいもち病菌や他の *Pyricularia* 属菌に対するイネ体の宿主・非宿主反応を光学顕微鏡で観察した．イネいもち病菌の分生胞子は非親和性，親和性に関係なく通常2～3時間以内に発芽し，約4時間で付着器を形成した．付着器は接種後約18時間で侵入糸を形成し，宿主細胞壁を貫入し始めた．この時期には，非親和性の荔支江，親和性の愛知旭の両品種とも被侵入細胞は生きていた．しかし，荔支江では原形質膜の薄膜化が付着器下の宿主細胞で観察された．接種後24時間目の荔支江では，被侵入細胞の多くはわずかに褐変するとともに原形質分離能もすでに失活していた．侵入菌糸は過敏感死した宿主細胞内に封入され，その伸展は阻害されていた．これに対して親和性の愛知旭では，侵入菌糸がある程度伸展しても被侵入細胞の多くはなお生き続けていた．接種後42時間目の観察では，荔支江の場合，侵入菌糸の伸展と宿主細胞の変質は24時間目のそれとほぼ同じ状態であった．接種後42時間目の愛知旭においても被侵入宿主細胞の褐変化，原形質分離能の失活，原形質膜の薄膜化などの現象が認められた．しかし，侵入菌糸の宿主内伸展は停止していなかった．一方，非病原性のいもち病菌を用いた非宿主相互間では，荔支江の葉鞘細胞は過敏感細胞死を起こし，侵入菌糸を第一侵入細胞に封じ込めていた．