

イネいもち病菌(Magnaporthe grisea)毒素による宿主および非宿主植物葉細胞のミトコンドリアの微細構造変化

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Ultrastructural Effects of *Magnaporthe grisea* Toxin(s) on Mitochondria of Host and Non-host Plants*

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Abstract

Magnaporthe grisea produces a toxin(s) which is important in the establishment of basic compatibility at the species level between fungus and plant. The effects of the toxin(s) on host (rice and barley) and non-host (finger millet) plants were examined by ultrastructural observation and morphometric measurement. The first ultrastructural changes observed after 1 hr of toxin(s) treatment were a decrease in the number of mitochondrial cristae and in the electron density of the mitochondrial matrix in rice leaf cells. Further, such modifications were observed in both compatible and incompatible inoculated with the fungal strain used for preparation of the toxin(s). However, morphological and ultrastructural changes were not observed in other organelles. Such phenomena were observed in all leaf tissues (epidermis, mesophyll and vascular bundle) of host plants. On the other hand, the effects of toxin(s) were not observed in leaves of the non-host plant. These results suggested that the mitochondrial modifications induced by the toxin(s) were a host-specific phenomenon and possibly, an essential event for *M. grisea* to establish basic compatibility with host plants at the species level.

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Key words : ultrastructure, mitochondria, host, non-host, morphometric measurement, *Magnaporthe grisea* toxin.

INTRODUCTION

The fungus *Magnaporthe grisea* (Hebert) Barr (imperfect stage *Pyricularia oryzae* Cavarra) is known to cause rice blast disease. In this disease, specificity between host and parasite is determined by race-cultivar combinations¹⁴⁾. Further, *M. grisea* is also pathogenic to other gramineae plants such as barley, Italian ryegrass, and corn¹³⁾. Ongoing research has revealed that plant fungal pathogens have to establish two levels of specific compatibility to succeed in colonizing a plant^{6,7)}. The first is compatibility at the fungal species-plant species level (basic compatibility) and the second is compatibility at the race-cultivar level.

Arase *et al.*²⁾ found that *M. grisea* produces a toxin(s) during spore germination which induces susceptibility in the rice cv. Sekiguchi-asahi (a natural mutant rice derived from cv. Asahi). In the presence of this toxin(s), nonpathogenic *Alternaria alternata* infected leaves of rice, barley, Italian ryegrass and other gramineae plants

which are host plants of *M. grisea*. However, *A. alternata* did not infect non-host plants of *M. grisea*^{3,4)}. The specificity of this susceptibility-inducing activity of the toxin(s) corresponded with the host range of *M. grisea*, suggesting that the toxin(s) plays an important role in establishing basic compatibility at the fungal species-plant species level^{3,4)}.

In a previous study of an ultrastructural observation, we reported that the primary site of action of *M. grisea* toxin(s) was in mitochondria in the rice cvs. Sekiguchi-asahi (resistance gene, *Pi-a*) and Asahi (resistance gene, *Pi-a*)¹²⁾. In this study, we observed the ultrastructure of the cellular organelles in host (rice and barley) and non-host (finger millet) leaves treated with *M. grisea* toxin(s) in order to demonstrate the site of action and the specificity of the toxin(s).

MATERIALS AND METHODS

Fungus and plants Strain Naga 69-150 (race 007) of *M. grisea* was used for extracting the toxin(s).

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Rice (cvs. Asahi and Himenomochi) and barley (cv. Kantochusei) were used as host plants and finger millet (cv. Iyazairai) as a non-host plant. Rice cvs. Asahi (resistance gene *Pi-a*) and Himenomochi (resistance gene *Pi-k*) are susceptible and resistant to Naga 69-150 (race 007), respectively. Rice, barley and finger millet seeds were sown in seedling cases (6×10×15 cm, Fujimoto Kagaku Co.). Rice plants were grown for about 30 days, and barley and finger millet plants were grown for 7 days in a greenhouse. The second leaves of rice plants at the 4–5 leaf stage, primary leaves of barley and the second leaves of finger millet plants at the 2 leaf stage were used.

Toxin preparation Strain Naga 69-150 (race 007) of *M. grisea* was cultured on oatmeal agar medium at 26°C for 14–21 days. Synchronization of sporulation and preparation of spore germination fluid (200 ml) of *M. grisea* were carried out by the methods described previously²⁾. Spore germination fluid was extracted with ethyl acetate. The extract was then separated by silica gel column chromatography (Wako gel C-200), by successive elution with chloroform and ethyl acetate. The toxin(s) was successively eluted by the ethyl acetate. Dry weight of the ethyl acetate fraction was measured after evaporation.

Toxin treatment After measurement of the dry weight of the ethyl acetate fraction, the extract was dissolved in 1 ml of acetone, then 2 ml of distilled water were added. This solution was evaporated *in vacuo* to remove the acetone and used as the toxin solution. The susceptibility-inducing activity of the toxin(s) estimated by lesion formation by nonpathogenic *A. alternata* in rice plants was first detectable at a concentration of 0.25 mg/ml, and became significant at the concentration of 1 mg/ml. The final concentration of the toxin solution, therefore, was adjusted to 1 mg/ml by the addition of distilled water. Five μ l of the toxin solution was placed on the wounded site of detached leaves of rice, barley, and finger millet. Distilled water was used as the control. The treated leaves were incubated in moist plastic cases at 26°C for 1, 6 and 12 hr.

Electron microscopy The tissues in the vicinity of toxin- and water-treated sites on the leaves were cut into small pieces (about 0.5×3 mm) with a razor blade. The pieces were prefixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 hr at room temperature. After rinsing with the buffer, specimens were postfixed with 2% buffered osmium tetroxide for 2 hr at room temperature. After rinsing with distilled water overnight, the specimens were dehydrated in ethanol and embedded in Spurr resin. Thin sections from the resin blocks were made using a Porter-Blum MT2-B, ultramicrotome, then stained with uranyl acetate for 1 hr and with lead citrate for 10 min. They were observed with a Hitachi HU-12A transmission electron microscope. Mitochondrial modifications were examined at a magnification of ×30,000. Invaginations of the plasmalemma in the vicinity of plasmodesmata were examined at a magnification of ×10,000 in epidermal, mesophyll and vascular bundle cells of toxin-treated or water-treated leaves. The disappearance of cristae and the loss of matrix were used as ultrastructural markers for modified mitochondria. Invaginations of the plasmalemma in the vicinity of plasmodesmata served as ultrastructural markers for plasmalemma modifications.

Ultrastructural morphometry Vascular bundle cells and mesophyll cells adjacent to the bundle sheath of leaves were examined for ultrastructural morphometry. Electron micrographs were taken randomly at a magnification of ×5,000 or ×15,000 in the vascular bundle and the mesophyll cells adjacent to the bundle sheath of toxin- and water-treated leaves. The negatives were enlarged to a magnification of ×20,000 or ×60,000 for printing for morphometrical analysis. The area ratios of cristae to mitochondria and of starch grains to chloroplasts were calculated by computer analysis with an NIH Image and Adobe Photoshop 2.5J system. The numbers of Golgi vesicles, osmiophilic globules and starch grains were counted on electron micrographs. The lengths of plasmalemma were measured with a Curvimeter. The cellular organelles measured are summarized in Table 1.

Table 1. Cellular components examined, parameters used, expression of values measured, observation sites, and magnification in leaves treated with partially purified toxin(s) or water

Cellular components examined	Parameters	Expression of values measured	Observation sites	Magnification
1. Osmiophilic globules ^{a)}	number	No. of osmiophilic globules/a chloroplast	mesophyll cells adjacent to vascular bundle	×20,000
2. Starch grains ^{a)}	area	starch grains/a chloroplast	mesophyll cells adjacent to vascular bundle	×20,000
3. Starch grains ^{a)}	number	starch grains/a chloroplast	mesophyll cells adjacent to vascular bundle	×20,000
4. Golgi vesicles ^{a)}	number	No. of Golgi vesicles/0–0.5 μ m inner part of cytoplasm from plasmalemma	vascular bundle	×60,000
5. Crista	area	crista/mitochondrion	vascular bundle	×60,000

a) These components were examined in only rice leaves.

Table 2. Comparative frequency of modified mitochondria in rice, barley and finger millet leaves treated with partially purified toxin(s) or water^{a)}

Plant (cultivar)	Treatment	Treatment time (hr)	Epidermis			Mesophyll			Vascular bundle		
			No. of MIT ^{b)}	No. of modified MIT	%	No. of MIT	No. of modified MIT	%	No. of MIT	No. of modified MIT	%
Rice (cv. Asahi)	Water	1	102	6	5.9	138	1	0.7	204	15	7.4
		6	157	12	7.6	345	7	2.0	261	25	9.6
		12	97	8	8.3	324	8	2.5	208	11	5.3
	Toxin	1	87	6	6.9	139	17	12.2	159	41	25.8
		6	66	10	15.2	183	24	13.1	141	35	24.8
		12	85	21	24.7	137	18	13.1	232	58	25.0
Rice (cv. Himenomochi)	Water	1	118	12	10.2	227	12	5.3	251	31	12.4
		6	205	22	10.7	284	11	3.9	202	12	5.9
		12	166	10	6.0	250	4	1.6	235	32	13.6
	Toxin	1	146	32	21.9	253	33	13.0	160	37	23.1
		6	123	25	20.3	403	48	11.9	199	43	21.6
		12	123	30	24.4	267	32	12.0	177	57	32.2
Barley (cv. Kantochusei)	Water	1	114	11	9.7	137	13	9.5	296	28	9.5
		6	114	7	6.1	216	23	10.6	265	24	9.1
		12	97	7	7.2	272	11	4.0	330	49	14.8
	Toxin	1	73	10	13.7	141	20	14.2	237	23	9.7
		6	111	30	27.0	145	32	22.1	179	44	24.6
		12	69	13	18.8	177	35	19.8	305	83	27.2
Finger millet (cv. Iyazairai)	Water	1	69	3	4.3	266	5	1.9	270	9	3.3
		6	53	5	9.4	227	11	4.8	211	12	5.7
		12	93	3	3.2	326	5	1.5	221	5	2.3
	Toxin	1	78	6	7.7	387	13	3.4	277	18	6.5
		6	131	15	11.5	290	18	6.2	332	24	7.2
		12	92	4	4.3	311	5	1.6	235	9	3.8

a) Mitochondria were examined at a magnification of $\times 30,000$ with transmission electron microscopy.

b) The abbreviation MIT denotes mitochondria.

RESULTS

Ultrastructural changes in leaves of toxin-treated host and non-host plants

M. grisea toxin(s) was applied to rice, barley and finger millet leaves, and mitochondrial modifications and invaginations of plasmalemma in the vicinity of plasmodesmata in epidermal, mesophyll and vascular bundle cells were investigated. The cristae and electron density of the matrix in water-treated leaves of rice cvs. Asahi and Himenomochi did not differ from controls (Plate I-1 and 3). Modification of mitochondria was observed in all three experimental sites (epidermal, mesophyll and vascular bundle cells) of the leaves of rice cvs. Asahi and Himenomochi treated with the toxin(s) for 1 hr (Table 2). In modified mitochondria, both the number of the cristae and the electron density of matrix had decreased (Plate I-2 and 4).

In barley host leaves, mitochondrial modifications in epidermal, mesophyll and vascular bundle cells were also observed after 1 hr of the toxin treatment. However, the modifications in barley leaves became more

pronounced after a 6 hr toxin treatment (Table 2). The mitochondrial modifications in toxin-treated barley leaves were similar to that observed in toxin-treated rice leaves (Plate II-1 and 2). On the other hand, ultrastructural changes were not observed in other organelles such as the plasmalemma and chloroplasts.

In leaves of non-host finger millet, the frequency of modified mitochondria in toxin-treated and water-treated leaves did not differ (Table 2, Plate II-3 and 4).

The frequency of plasmalemma invaginations in the vicinity of plasmodesmata did not differ between toxin-treated and water-treated leaves of rice, barley and finger millet (Table 3).

Ultrastructural morphometric analysis

In rice cvs. Asahi and Himenomochi, the area ratio of cristae to mitochondria in vascular bundle cells of leaves treated with the toxin(s) for 1, 6 and 12 hr was significantly lower than that in water-treated leaves. The area-ratio of cristae to mitochondria did not change with the length of toxin treatment (Fig. 1).

Although, starch accumulation and an increase of osmiophilic globules in chloroplasts is well known to occur after fungal infection, toxin treatment and

Table 3. Comparative frequency of invaginations of plasmalemma in the vicinity of plasmodesmata in rice, barley and finger millet leaves treated with partially purified toxin(s) or water^{a)}

Plant (cultivar)	Treatment	Treatment time (hr)	Epidermis			Mesophyll			Vascular bundle		
			No. of Pd ^{b)}	No. of Pd with invaginations	%	No. of Pd	No. of Pd with invaginations	%	No. of Pd	No. of Pd with invaginations	%
Rice (cv. Asahi)	Water	1	0	0	0.0	30	0	0.0	84	2	2.4
		6	28	0	0.0	97	2	2.1	84	0	0.0
		12	27	0	0.0	132	2	1.5	67	1	1.5
	Toxin	1	5	0	0.0	70	0	0.0	41	0	0.0
		6	12	1	8.3	47	0	0.0	64	0	0.0
		12	21	0	0.0	38	1	2.6	53	1	1.9
Rice (cv. Himénomochi)	Water	1	26	0	0.0	124	1	0.8	52	1	1.9
		6	43	0	0.0	145	2	1.4	42	0	0.0
		12	20	0	0.0	111	2	1.8	74	1	1.4
	Toxin	1	16	0	0.0	134	0	0.0	53	0	0.0
		6	19	1	5.3	84	1	1.2	42	3	7.1
		12	31	0	0.0	121	1	0.8	53	3	5.7
Barley (cv. Kantochusei)	Water	1	13	0	0.0	86	3	3.5	120	7	5.8
		6	30	0	0.0	137	0	0.0	170	0	0.0
		12	30	1	3.3	110	0	0.0	73	1	1.4
	Toxin	1	20	0	0.0	117	1	1.7	117	2	5.1
		6	29	0	0.0	126	0	0.0	143	0	0.0
		12	36	0	0.0	103	0	0.0	126	0	0.0
Finger millet (cv. Iyazairai)	Water	1	13	0	0.0	177	2	1.1	118	2	1.7
		6	21	0	0.0	138	1	0.7	85	3	3.5
		12	22	0	0.0	141	0	0.0	79	1	1.3
	Toxin	1	23	0	0.0	177	0	0.0	80	0	0.0
		6	25	0	0.0	209	2	1.0	91	1	1.1
		12	21	0	0.0	106	0	0.0	80	1	1.3

a) Plasmodesmata were examined at a magnification of $\times 10,000$ with transmission electron microscopy.

b) The abbreviation Pd denotes plasmodesmata.

senescence¹¹⁾, we did not observe significant differences in the area ratio of starch grains to chloroplasts or the number of osmiophilic globules and starch grains per chloroplast, nor did we observe a change in the frequency as Golgi vesicles reported by Park *et al.*¹⁰⁾ for host cells treated with AK-toxin (*A. alternata* Japanese pear pathotype) (Tables 4 and 5).

In barley leaves, the area-ratio of cristae to mitochondria in vascular bundle cells of leaves treated with the toxin(s) decreased significantly for 1, 6 and 12 hr as compared to that of water-treated leaves. In finger millet leaves, however, the area-ratio of cristae to mitochondria did not change after the toxin treatment (Fig. 1).

DISCUSSION

In a previous report¹²⁾, we showed that the toxin(s) initially induced modifications of mitochondria in rice leaf cells 1 hr after treatment. Such modifications were observed regardless of tissue type in this study. Further, mitochondrial modifications by the toxin(s) were induced regardless of compatibility of rice cultivars to the

tested fungal strain. Other organelles, including plasmalemma, chloroplasts and Golgi vesicles were not affected. These results suggest that the primary site of action of the toxin(s) in rice leaf cells is mitochondria, and that its mode of action is not cultivar-specific.

In a previous report³⁾, we demonstrated that *M. grisea* toxin(s) has a susceptibility-inducing activity which was involved in establishing basic compatibility at the fungal species-plant species level. The ultrastructural results in this paper support this previous data. The modification in mitochondria was observed in leaf tissues of barley which is a host of *M. grisea*, but not in those of non-host finger millet. These results showed that mitochondrial modifications induced by the toxin(s) are a host-specific phenomenon, corresponding to the specificity of the susceptibility-inducing activity of the toxin(s). This again suggests that mitochondrial modifications induced by the toxin(s) is an essential event for *M. grisea* to establish basic compatibility with host plants.

In this study, the effect of *M. grisea* toxin(s) was specific only to host mitochondria. Further, decreases in area-ratio of cristae and in matrix density were not conspicuous even 12 hr after toxin treatment. These

Table 4. Results of ultrastructural observations and measurements made in chloroplasts of rice leaves treated with partially purified toxin(s) or water

Rice cultivar	Treatment	Treatment time (hr)	Number of chloroplasts examined	No. of osmiophilic globules/chloroplast (mean±SE)	Starch grains	
					Area-ratio of starch grains to chloroplasts (%) (mean±SE)	Number/chloroplast (mean±SE)
Asahi	Water	1	36	9.39±5.81	1.66±1.61	2.50±2.52
		6	36	8.58±4.96	1.84±1.86	1.56±1.56
		12	39	9.79±7.61	1.60±2.16	1.72±1.96
	Toxin	1	37	8.14±5.74	1.43±1.80	2.08±1.69
		6	34	9.68±7.11	2.03±2.66	1.15±1.31
		12	36	6.44±4.69	1.37±1.54	1.31±1.37
Himenomochi	Water	1	37	5.92±4.85	2.09±2.51	2.03±2.05
		6	32	6.91±4.91	0.24±0.49	0.38±0.61
		12	24	5.83±4.47	5.62±5.44 ^{a)}	2.50±2.21
	Toxin	1	30	9.03±5.01	3.84±3.40	2.67±1.97
		6	45	4.96±4.06	0.08±0.28	0.18±0.65
		12	30	6.73±3.92	10.38±6.56 ^{a)}	3.33±2.26

a) Indicated differences of each treatment were significant at $p < 0.01$ according to t -test.

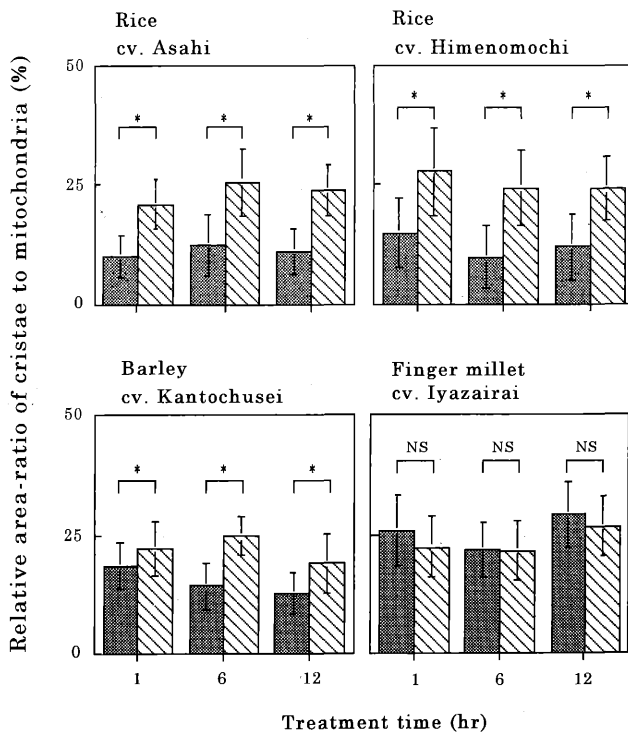


Fig. 1. Comparative area-ratio of cristae to mitochondria in rice (cvs. Asahi and Himenomochi), barley and finger millet leaves treated with the toxin(s) (1 mg/ml) (■) or water (□). The bars are standard errors, and asterisks indicate significant differences at $p < 0.01$ according to t -test.

slight changes of leaf mitochondria were also observed 12 hr after inoculation with *M. grisea* in rice leaf epidermal cells (data not shown). On the other hand, necrosis was not induced by *M. grisea* toxin(s) on rice (cvs. Asahi and Himenomochi) and barley (cv. Kantochusei) leaves. These results support the previous study⁵⁾ that the

Table 5. Comparative number of Golgi vesicles in cytoplasm within 0.5 μ m of the plasmalemma of rice leaves treated with partially purified toxin(s) or water

Rice cultivar	Treatment time (hr)	No. of Golgi vesicles in cytoplasm within 0.5 μ m of plasmalemma	
		Toxin	Water
Asahi	1	5.23	3.98
	6	4.59	4.31
	12	5.28	4.35
Himenomochi	1	3.85	3.10
	6	3.25	3.53
	12	4.71	4.77

pathologically important susceptibility-inducing activity of the toxin(s) is exerted non-phytotoxically.

Host-specific toxins (HSTs) from *Helminthosporium maydis* race T, *A. alternata* rough lemon pathotype and *A. alternata* tobacco pathotype are also known to attack mitochondria of host plants, as demonstrated by morphological analysis^{1,8,9)}. These HSTs caused mitochondria swelling, reduction of cristae and a decrease in electron density of the matrix. Further, the degree of mitochondrial damage by the HSTs increased with the time after treatment. Ultrastructural changes in rice mitochondria induced by the *M. grisea* toxin(s) were similar to those induced by *A. alternata* HSTs. However, the degree of mitochondrial damage by *M. grisea* toxin(s) did not increase with the time after treatment. Physiological and biochemical studies using isolated mitochondria are necessary to elucidate further the mode of actions of *M. grisea* toxin(s) and to compare *M. grisea* toxin(s) with HSTs.

Literature cited

1. Aldrich, H.C., Gracen, V.E., York, D., Earle, E.D. and Yoder, O.C. (1977). Ultrastructural effects of *Helminthosporium maydis* race T toxin on mitochondria of corn roots and protoplasts. *Tissue Cell* 9: 167-177.
2. Arase, S., Kinoshita, S., Kano, M., Nozu, M., Tanaka, E. and Nishimura, S. (1990). Studies on host-selective infection mechanism of *Pyricularia oryzae* Cavara (2). Production of susceptibility-inducing factor(s) from germinating spores and their phytotoxicity. *Ann. Phytopathol. Soc. Jpn.* 56: 322-330.
3. Fujita, K., Arase, S., Hiratsuka, H., Honda, Y. and Nozu, M. (1994). The role of toxin(s) produced by germinating spores of *Pyricularia oryzae* in pathogenesis. *J. Phytopathol.* 142: 245-252.
4. Fujita, K., Arase, S., Honda, Y. and Nozu, M. (1995). Susceptibility-inducing factor(s) in ethyl acetate extract from germination fluids of *Pyricularia oryzae* as determinants of basic compatibility in rice blast. *Ann. Phytopathol. Soc. Jpn.* 61: 185-188.
5. Fujita, K., Arase, S., Honda, Y. and Nozu, M. (1995). Studies on host-selective infection mechanism of *Magnaporthe grisea* (Hebert) Barr (9). Possibility of phytotoxicity-non-dependent induction of susceptibility by susceptibility-inducing factor(s). *Ann. Phytopathol. Soc. Jpn.* 61: 194-196.
6. Heath, M.C. (1981). A generalized concept of host-parasite specificity. *Phytopathology* 71: 1121-1123.
7. Johal, O.S., Gray, J., Gruis, D. and Briggs, S.P. (1995). Convergent insights into mechanisms determining disease and resistance response in plant-fungal interactions. *Can. J. Bot.* 73 (suppl. 1): S468-S474.
8. Kodama, M., Park, P., Tsujimoto, T., Otani, H., Kohmoto, K. and Nishimura, S. (1990). Ultrastructural studies of an action site of host-selective AT-toxin produced by *Alternaria alternata* tobacco pathotype in tobacco leaf cells. *Ann. Phytopathol. Soc. Jpn.* 56: 637-644.
9. Kohmoto, K., Kondoh, Y., Kohguchi, T., Otani, H., Nishimura, S. and Scheffer, R.P. (1984). Ultrastructural changes in host leaf cells caused by host-selective toxin of *Alternaria alternata* from rough lemon. *Can. J. Bot.* 62: 2485-2492.
10. Park, P., Ohno, T., Nishimura, S., Kohmoto, K. and Otani, H. (1988). Golgi vesicles fused with invaginated plasma membranes in host cells treated with a host-specific toxin from the Japanese pear pathotype of *Alternaria alternata*. *Ann. Phytopathol. Soc. Jpn.* 54: 493-502.
11. Penelope, H. (1981). Ultrastructural effects. *In* Toxins in Plant Disease (Durbin, R.D. ed.), Academic Press, New York, pp. 449-475.
12. Uehara, T., Arase, S., Honda, Y., Park, P. and Nozu, M. (1997). Primary effects of *Magnaporthe grisea* toxin(s) on mitochondria of rice leaf cells. *Ann. Phytopathol. Soc. Jpn.* 63: 29-35.
13. Yaegashi, H. (1981). Studies on the perfect stage of *Pyricularia* species. *Bull. Tohoku Natl. Agric. Exp. Stn.* 63: 49-125.
14. Yamada, M., Kiyosawa, S., Yamaguchi, T., Hirano, T., Kobayashi, T., Kushibuchi, K. and Watanabe, S. (1976). Proposal of new method for differentiating races of *Pyricularia oryzae* Cavara in Japan. *Ann. Phytopathol. Soc. Jpn.* 42: 216-219.

和 文 摘 要

植原珠樹・荒瀬 栄・本田雄一・朴 杓允・野津幹雄：イネいもち病菌 (*Magnaporthe grisea*) 毒素による宿主および非宿主植物葉細胞のミトコンドリアの微細構造変化

イネいもち病菌が孢子発芽時に生成する基本的親和性成立に関与する毒素で、本菌の宿主植物であるイネおよびオオムギ葉と非宿主植物であるシコクビエ葉を1, 6または12時間処理し、電顕を用いてその作用を観察し画像解析により検討した。毒素による初期変性は処理1時間後から認められ、ミトコンドリアの基質は消失し、クリステ含有率は低下した。これらの毒素作用はミトコンドリアに特異的であり、他の細胞内器官には変化は認められなかった。この現象はオオムギおよびイネの表皮、葉肉および維管束細胞において認められ、さらにイネ葉では毒素生成菌株に感受性であるイネ品種と抵抗性であるイネ品種の双方のミトコンドリアに観察された。一方、非宿主植物のシコクビエではいずれの処理時間においても毒素による微細構造変化は認められなかった。毒素によるミトコンドリアの変性は、イネいもち病菌の宿主植物に種レベルでのみ認められ、毒素の感染誘導活性とよく一致した。これらの結果は、毒素による宿主細胞のミトコンドリア変性はイネいもち病菌が宿主植物との間に基本的親和性を確立するために必要不可欠な現象であることを示唆している。

Explanation of plates**Plate I**

1. Normal mitochondria in vascular bundle cells of Asahi leaves treated with water for 12 hr. Mitochondrial matrix existed. Scale bar=0.5 μ m.
2. Modified mitochondria (arrowheads) in a vascular bundle cell of Asahi leaves treated with the toxin(s) for 12 hr. A decrease in number of cristae and in electron density of matrix were observed. Scale bar=0.5 μ m.
3. Normal mitochondria (arrowheads) in a vascular bundle cell of Himenomochi leaves treated with water for 6 hr. Mitochondrial matrix existed. Scale bar=0.5 μ m.
4. Modified mitochondria (arrowheads) in vascular bundle cells of Himenomochi leaves treated with the toxin(s) for 1 hr. Modified mitochondria were observed. Scale bar=0.5 μ m.

Plate II

1. Normal mitochondria in vascular bundle cells of barley leaves treated with water for 6 hr. Scale bar=0.5 μ m.
2. Modified mitochondria (arrowheads) in vascular bundle cells of barley leaves treated with the toxin(s) for 6 hr. A decrease in number of cristae and in electron density of matrix were observed. Scale bar=0.5 μ m.
3. Normal mitochondria (arrowheads) in vascular bundle cells of finger millet leaves treated with water for 12 hr. Scale bar=0.5 μ m.
4. Normal mitochondria (arrowheads) in vascular bundle cells of finger millet leaves treated with the toxin(s) for 12 hr. Mitochondrial matrix existed. Scale bar=0.5 μ m.

Abbreviations: M, mitochondrion; N, nucleus; Pd, plasmodesmata.

Plate I

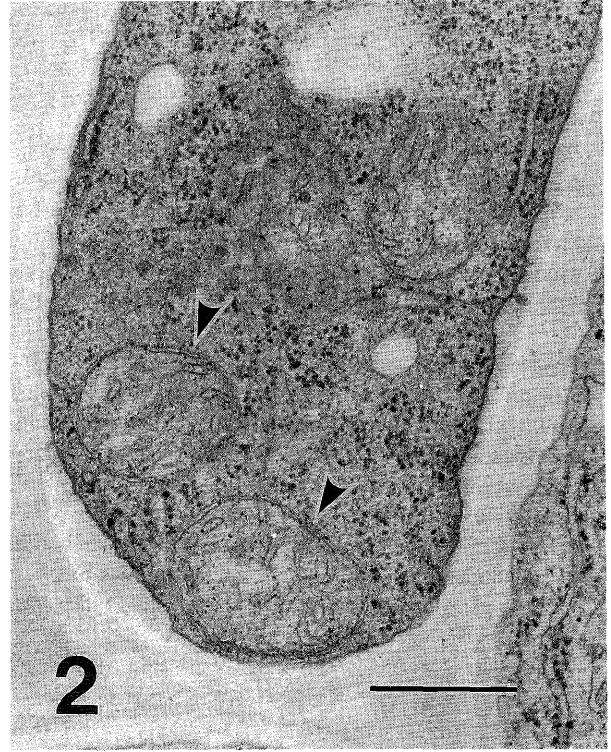
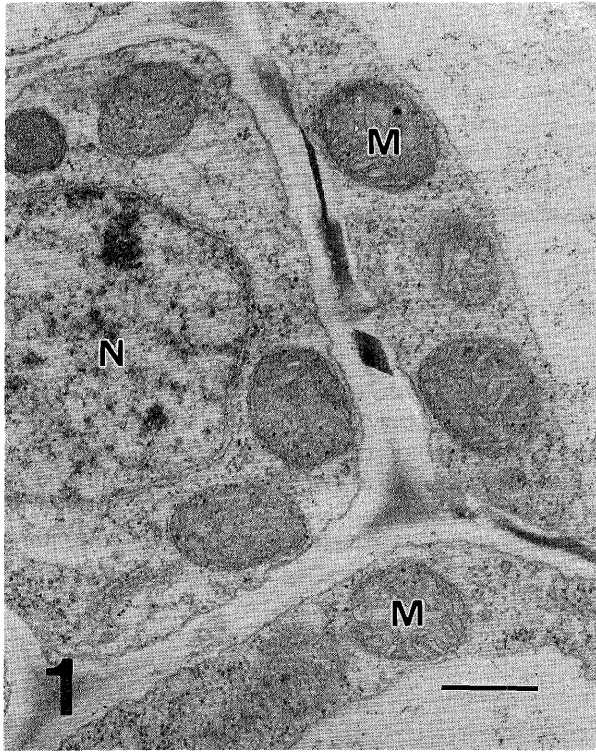


Plate II

