Rishitin及びその代謝産物rishitin-M-1及びM-2の抗菌性及びジャガイモ組織に対する毒性

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Toxicity of Rishitin, Rishitin-M-1 and Rishitin-M-2 to *Phytophthora infestans* and Potato Tissue.

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石栗幸男* · 冨山宏平* · 村井章夫** · 勝井信勝** · 正宗　直**：Rishitin 及びその代謝産物 rishitin-M-1 及び M-2 の抗菌性及びジャガイモ組織に対する毒性

Abstract

Rishitin and rishitin metabolites were tested for their antifungal activities to spore germination and mycelial growth of *Phytophthora infestans*, and for toxicity to potato tuber tissues. The antifungal activities of rishitin-M-1 and rishitin-M-2 to races 0 and 1 of *P. infestans* were about one tenth that of rishitin. Treatment of tuber tissue C. V. s Rishiri, Irish cobbler and Pentland ace with rishitin caused injury and darkening of the treated tissue at about 5×10^{-3} M, but treatments with rishitin-M-1 and rishitin-M-2 had little effect on the tuber tissue even at 10^{-2}M, the highest concentration tested. Little difference in the toxicities to the fungi and the tuber tissue was observed between rishitin-M-2 and its epimer: (11S)- rishitin-M-2. These results suggest that metabolism of rishitin to rishitin-M-1 and rishitin-M-2 may protect the healthy potato tissue from toxic effect of accumulated rishitin. These phenomena suggest that a mechanism exist in healthy plant tissue for the detoxification of the phytoalexin.
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Introduction

The sesquiterpene phytoalexins of potato plants, rishitin, lubimin etc., play an important role in disease resistance of potato[1,6,10,11,12]. However, it is still not clear how the induction of their synthesis, accumulation and disappearance occurs. In previous papers[2,3,9], it was reported that aged slices of potato tuber could metabolize rishitin. We also reported on the chemical identification of the ether-soluble metabolites of rishitin, and named them rishitin-M-1 and rishitin-M-2 (Fig. 1). It was

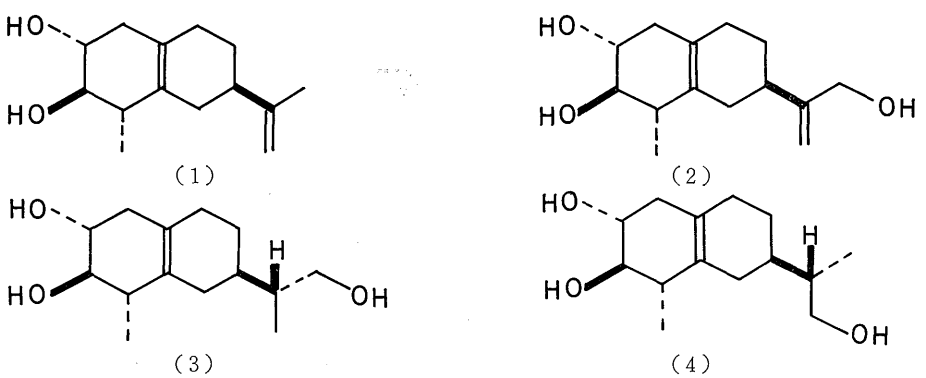

Fig. 1. Chemical structures of (1) rishitin, (2) rishitin-M-1, (3) rishitin-M-2 and (4) (11S)-rishitin-M-2.

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presumed that the balance between production and metabolism of rishitin might determine the rate of rishitin accumulation. It has been known that rishitin is not only toxic to *P. infestans,* but also at high concentrations to potato tissue[4]. It is important to know whether the rishitin metabolites are toxic to *P. infestans,* and potato tissue or not.

In this paper, we report the toxicities of rishitin, rishitin-M-1, rishitin-M-2 and an epimer of the latter: (11S)-rishitin-M-2 to *P. infestans,* and potato tuber tissue.

Materials and methods

Preparations of compounds tested. Rishitin isolated from tuber of potato cultivars "Rishiri" infected by race 0 of *Phytophthora infestans* according to the method previously reported[7,11], and final purification was effected by formation of crystallin bis [3,5-dinitrobenzoate], followed by hydrolysis of the benzoate and distillation in vacuo. Rishitin-M-1[3,9], rishitin-M-2[3,9] and (11S)-rishitin-M-2 were prepared by the method of Murai et al.

Spore germination test. The compounds were dissolved in sterile distilled water containing 20 % acetone. Race 0 and race 1 of *P. infestans* were grown on the cut surfaces of potato tubers C. V. Irish cobbler. Conidia obtained from the mycelial mats were suspended in water containing 2×10^{-4}M $CaCl_2$ and allowed to germinate at 4 C. Then, the zoospore suspension was shaken (110 strokes per min) for 50 min at 24 C, resulting in encystment of zoospores. A drop of encysted zoospore suspension (20μl of 20×10^6 spores/ml) was added to 20μl of the compounds solution placed on a glass slide. Germination percentages of spores were estimated under a microscope after incubation under moist conditions in the dark for 2 h at 18 C.

Mycelial growth test. Droplets containing 20 μl of encysted spore suspension of *P. infestans* were germinated on glass slides for 1 h at 18 C. To them was added 20μl of the test compounds. The mycelial growth was determined after incubation in a dark at 18 C for 2 h.

Potato-tuber slice test. Tissue cylinders (16 mm in diameter, 0.5 mm in thickness) were cut out with a cork borer from the central parenchyma of potato tubers, and then cut into discs. The discs were washed with running water for 5 min and kept on a moist filter paper in a plastic box at 18 C for 10 h and then used. Twenty μl of the each test compound was placed on the tuber disc. Effects of the compounds on the discs were observed 24 h after incubation in a dark at 18 C.

In all the experiments described above, 20% acetone was used as a control for the toxicity tests.

Results and discussion

The minimum concentration which completely prevented germination (M. C. P. G.) and medium effective doses (ED_{50}) of rishitin and its metabolites for inhibition of the spore germination are shown in Table 1. The ED_{50} of rishitin was 3.8×10^{-4} M for both race 0 and race 1 of *P. infestans,* a value slightly higher than the value 2.2×10^{-4}M reported previously[4]. This might be due to difference in the experimental condition. The ED_{50}s of rishitin-M-1 and -M-2 were about one tenth that of rishitin. The inhibitory effects of rishitin-M-1 and rishitin-M-2 on hyphal growth of race 0 and race 1 were also about one tenth that of rishitin (Table 2).

Table 1. Inhibition of encysted zoospore germination of *Phytophthora infestans* by rishitin, rishitin-M-1, rishitin-M-2 and (11S)-rishitin-M-2

Compounds	*P. infestans*			
	race 0		race 1	
	M. C. P. G[a]	ED_{50}[b]	M. C. P. G	ED_{50}
Rishitin	7.5×10^{-4}	3.8×10^{-4}	7.5×10^{-4}	3.8×10^{-4}
Rishitin-M-1	1.0×10^{-2}	5.1×10^{-3}	1.0×10^{-2}	5.0×10^{-3}
Rishitin-M-2	7.5×10^{-3}	4.2×10^{-3}	7.5×10^{-3}	4.1×10^{-3}
(11S)-Rishitin-M-2	7.5×10^{-3}	4.6×10^{-3}	7.5×10^{-3}	4.1×10^{-3}

a : minimum concentration (Mol) which completely prevented germination.
b : Concentration (Mol) which reduced germination by 50%

Table 2. Inhibition of mycelial growth of *Phytophthora infestans* by rishitin, rishitin -M-1, rishitin-M-2 and (11S)-rishitin-M-2

Compounds	Races of *P. infestans*	Concentration of cpds ($\times10^{-4}$M)					
		0	5	10	25	50	100
Rishitin	race 0	11.8[a]	2.7	0	0	0	0
	race 1	9.4	3.1	0	0	0	0
Rishitin-M-1	race 0	11.6	—	—	12.4	5.5	0
	race 1	11.6	—	—	12.5	8.7	0
Rishitin-M-2	race 0	12.3	—	—	12.8	5.8	0
	race 1	10.3	—	—	10.4	3.6	0
(11S)-rishitin-M-2	race 0	10.6	—	—	9.8	5.0	0.3
	race 1	9.6	—	—	6.3	3.3	0

a : Mycelial growth (μm/hr)

Table 3. Effect of rishitin, rishitin-M-1, rishitin-M-2 and the (11S)-rishitin-M-2 on the potato tuber slices

Compounds	Potato	Concentration of cpds ($\times10^{-3}$M)			
		1	2.5	5	10
Rishitin	Rishiri	—	—	+[a]	++[a]
	Irish cobbler	—	±[a]	++[a]	++[a]
	Pentland-ace	—	—	+[b]	++[b]
Rishitin-M-1	Rishiri	—	—	—	—
	Irish cobbler	—	—	—	—
	Pentland-ace	—	—	—	—
Rishitin-M-2	Rishiri	—	—	—	—
	Irish cobbler	—	—	—	—
	Pentland-ace	—	—	—	—
(11S)-rishitin-M-2	Rishiri	—	—	—	—
	Irish cobbler	—	—	—	—
	Pentland-ace	—	—	—	—

a : Potato tuber tissue was dead and darkened.
b : Potato tuber tissue was dead but not darkened.
—: Non-injured, +: injured slightly, ++: injured greatly.

Treatment with rishitin at a concentration of 5×10^{-3}M caused injury and darkening of the tuber tissue of C. V. s Rishiri, Irish cobbler and Pentland-ace. Irish cobbler was most sensitive to rishitin as signs of injury appeared at 2.5×10^{-3}M. The cells of Pentland ace were killed at concentration of more than 5×10^{-3}M, but darkening of the injured tissue did not occur.

Rishitin-M-1 and rishitin-M-2 were not toxic to potato tuber slices of all cultivars even at 10^{-2}M, the highest concentration tested. In all the experiments described above, little difference was observed between the toxicities of rishitin-M-2 and its epimer: (11S)-rishitin-M-2.

It has been known that some of the phytoalexins could be detoxicated by hydroxylation[12]. The present experiments showed that hydroxylation of rishitin at isopropenyl group resulted in reduction of toxicity to *P. infestans* and potato tissue.

These results suggest that the metabolism of rishitin to rishitin-M-1 and rishitin-M-2 may protect the healthy tuber tissue from the toxic effect of accumulated rishitin.

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和 文 摘 要

Rishitin 及びその代謝産物 rishitin-M-1 及び M-2 の抗菌性及びジャガイモ組織に対する毒性

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ジャガイモのファイトアレキシン rishitin はジャガイモ塊茎組織によって rishitin-M-1 及び M-2 に転換

される。ジャガイモ疫病菌 *Phytophthora infestans* の race 0, race 1 の胞子発芽及び発芽管長伸長に対する rishitin-M-1 及び M-2 の抗菌性は rishitin の約 1/10 であった。またジャガイモ塊茎組織に対して rishitin では 5×10^{-3}M で毒性があったが，rishitin-M-1, M-2 は 10^{-2}M で毒性がなかった。以上から健全組織における rishitin の両物質への転換は生理学的には除毒的効果をもつと云ってよい。