

イネいもち病罹病葉における脂質過酸化とスーパーオキシドディスムターゼ,カタラーゼ,ペルオキシダーゼ活性の経時的変化

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Time-Course Alteration of Lipid Peroxidation and the Activities of Superoxide Dismutase, Catalase and Peroxidase in Blast-Infected Rice Leaves*

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松山宣明**: イネいもち病罹病葉における脂質過酸化とスーパーオキシド
ディスムターゼ, カタラーゼ, ペルオキシダーゼ活性の経時的変化*

Key Words: rice blast, lipid peroxidation, superoxide dismutase, catalase, peroxidase,
active oxygen.

The toxicity of active oxygens such as $O_2^{\cdot-}$, $OH^{\cdot}$, 1O_2 , H_2O_2 and their participation in defence mechanism to parasites have been interested in animal diseases¹⁾. While, one of the active oxygen, superoxide radical $O_2^{\cdot-}$ which commonly generates from various biochemical reactions²⁾ has been much stressed, because of its special reference with the lipid peroxidation which may result in the deterioration of the membrane of organelles and cytoplasm^{3,5)} and may secondarily generate free radicals and harmful products to host and parasite cells¹⁻⁷⁾. In plant, close correlation between lipid peroxidation and decrease in chlorophyll content and/or increase in permeability of cells during senescence has been documented⁴⁾. Kato and Misawa⁵⁾ emphasized the close relation of the free radical formation at lipid peroxidation with the hypersensitive death of CMV-infected cowpea cells which resulted in a local lesion formation. Recently, a possible participation of the free radicals generated at lipid peroxidation in defence mechanism to rice blast disease was postulated⁶⁾. To certify this possibility, the degree of lipid peroxidation in blast-infected rice leaves was examined for the compatible and incompatible interactions. The activity of some enzymes which scavenge the toxicity of active oxygen during infection was also examined.

Rice cultivar Aichiasahi and two isolates of *Pyricularia oryzae* Cav., Ken 53-33 (race 137, compatible) and Ina 72 (race 031, incompatible) were used. Rice plants at 7th leaf stage were routinely spray-inoculated and top leaves were harvested at 0,

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1, 3, 5 and 7th day after inoculation. Determination of lipid peroxidation was conducted by the measurement of malondialdehyde, a major product of lipid peroxidation⁴⁾. Leaf sample (0.25g) was homogenized in 5 ml of 0.1 % trichloroacetic acid (TCA) with 1g of quartz sand in a mortar and centrifuged at $10,000 \times g$ for 10 min. To 1 ml of the supernatant 4 ml of 20 % TCA containing 0.5 % thiobarbituric acid (TBA) were added. The mixture was heated in boiling water for 30 min and then quickly cooled. After recentrifugation, the absorbance of the supernatant at 532 nm was read and the value for the non-specific absorption at 600 nm was subtracted⁴⁾.

Leaf tissue (0.5 g) was homogenized in 5 ml of 0.05 M phosphate buffer (pH 7.0) with 50 mg of polyvinylpyrrolidone and 1g of quartz sand in a mortar. The homogenate was centrifuged at $10,000 \times g$ for 10 min and the supernatant was used as an enzyme extract. Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Beauchamp and Fridovich⁷⁾. Fifty μ l of extract were added to 5 ml of the mixture (pH 7.8) containing 13 mM methionine, 0.1 mM EDTA, 0.75 mM NBT, 0.02 mM riboflavin and 0.02 mM NaCN. NaCN was added as the inhibitor of peroxidase and this substance did not affect the SOD activity at this concentration⁷⁾. The reaction mixture was kept at 20 C for 15 and 30 min under 15 W fluorescent lamp. The absorbance of reaction mixture was measured at 560 nm. The mixture which does not contain the enzyme and non-irradiated mixture were used as controls⁴⁾. Catalase was assayed by measuring the initial rate of disappearance of hydrogen peroxide⁸⁾. Ten μ l of extract were added to 5 ml of 0.03 % hydrogen peroxide in 0.05 M phosphate buffer (pH 7.0) and the optical density was measured at every 10 sec for 2 min at 240 nm. Peroxidase activity was assayed as previously reported elsewhere⁹⁾. Chlorophyll was extracted with 85 % acetone adding Na_2CO_3 , an inhibitor of chlorophyllase, and the content was measured at 663 nm.

The resistant symptom (brown spots) appeared at 2nd-3rd day after inoculation, while, susceptible symptom (whitish-gray spots) appeared at 3rd-4th day and enlarged with time. Appearance of symptoms reflected in the chlorophyll content (Table 1). The decrease in the content appeared first in incompatible interaction. It has been reported that the decrease in the content could be, at least in part, due to the

Table 1. Time-course alteration of chlorophyll content after inoculation

Compatibility	Days after inoculation				
	0	1	3	5	7
Check	1.041	0.968	1.255	1.219	1.129
Incompatible	—	0.820	1.074	1.031	0.894
Compatible	—	0.906	1.163	0.988	0.888

Chlorophyll content was shown by an optical density at 663 nm. Resistant symptom appeared at 2nd-3rd day, while, susceptible symptom appeared at 3rd-4th day after inoculation. Cultivar: Aichiasahi. Inocula: Ken 53-33 (compatible) and Ina 72 (incompatible).

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destruction of chlorophyll¹⁰⁾ and/or the deterioration of chloroplast membrane⁴⁾ by the lipid peroxidation. Therefore, the rapid peroxidation of lipid in incompatible case was anticipated. As shown in Table 2, the lipid peroxidation was observed at 24 hrs after inoculation to be equal in both compatible and incompatible interactions. After 3rd day the peroxidation eminently increased in compatible interaction. SOD and catalase which scavenge $O_2\cdot$ and H_2O_2 generated at lipid peroxidation were activated in inoculated leaves (Tables 3 and 4) and the activities were slightly high in compatible interaction at the latter stage of infection. The higher activities of SOD and catalase may bring the better circumstance for the parasite¹¹⁾. However, it is obscure whether or not such difference of activities is meaningful in the compatibility between the host and parasite. The alteration of peroxidase activity was distinctly different between compatible and incompatible interactions (Table 5). The rapid increase in the activity was observed in incompatible interaction and this result agreed with previous report⁹⁾. Since peroxidase generates free radicals^{12~14)}, the possibility of direct participation of peroxidase in incompatibility may exist. However, the contradictory results have been also documented^{9, 15~18)}. More studies will be needed for this subject.

Thus, the occurrence of the lipid peroxidation in infected leaves and the existence of SOD in rice leaves were firstly documented in this experiment. However, the degree of the lipid peroxidation was, unexpectedly, high in compatible interaction.

Table 2. The alteration of lipid peroxidation after inoculation

Compatibility	Days after inoculation				
	0	1	3	5	7
Check	0.138	0.168	0.168	0.166	0.140
Incompatible	—	0.214	0.224	0.170	0.134
Compatible	—	0.218	0.268	0.246	0.316

Number shows optical density at 532nm. The larger number means the higher content of malondialdehyde (a product of lipid peroxidation).

Table 3. Time-course of superoxide dismutase (SOD) activity after inoculation

Compatibility	Days after inoculation				
	0	1	3	5	7
Check	24	25	41	41	43
Incompatible	—	33	41	37	51
Compatible	—	33	39	49	54

The activity of SOD was shown as an inhibition percent of photochemical reduction of nitro blue tetrazolium. The larger number means the higher activity.

Table 4. Time-course of catalase activity after inoculation

Compatibility	Days after inoculation				
	0	1	3	5	7
Check	90	87	85	75	62
Incompatible	—	87	99	79	60
Compatible	—	90	91	89	53

Catalase activity was shown as an index. The decrease of optical density at 240 nm in 2 min was measured, multiplied by 10^3 and used as an index. The larger number means the higher activity.

Table 5. Time-course of peroxidase activity after inoculation

Compatibility	Days after inoculation				
	0	1	3	5	7
Check	1.35	1.46	1.62	2.06	1.64
Incompatible	—	2.02	2.72	2.75	2.82
Compatible	—	1.91	2.70	2.92	2.89

Peroxidase activity was shown by an optical density at 430 nm after the reaction with o-phenylenediamine and hydrogen peroxide at pH 4.7⁹⁾. The larger number means the higher activity.

Further, the activities of the scavengers, SOD and catalase, in the early stages of infection were not distinctly different in compatible and incompatible interactions. Therefore, accumulation of evidences to clarify whether or not the lipid peroxidation and active oxygens may participate, directly or indirectly, in the defence mechanism to rice blast disease is necessary.

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