Effects of Plant Hormones on Peroxidase Activity in Rice Leaf and Incidence of Rice Blast

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処理のイネ葉のパーオキシダーゼ活性といもち病発病に及ぼす影響

Abstract

The relationship between plant hormones and shift of peroxidase activity in the rice blast lesion was investigated using a compatible race-cultivar combination. Peroxidase activities in the leaf tissues were increased by the application of auxins, ethylene or their precursors, especially at the inoculated site. When kinetin was subsequently applied, the increase in the peroxidase activity by IAA or ethylene was enhanced, on the other hand, the increase by IAA or ethylene was suppressed by the subsequent application of ABA. The degree of rice blast incidence was suppressed by the application of auxins, ethylene or their precursors. The suppression of blast incidence by IAA or ethylene was strongly counteracted by the application of ABA prior to inoculation. The metabolic inhibitors such as formycin A, cycloheximide and blasticidin S caused considerable inhibition of IAA- or ethylene-induced increase in peroxidase. The hypothetical role of plant hormones on induced disease resistance of rice plant was proposed.

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Introduction

Many reports have revealed the participation of plant hormones in successive biochemical reactions^{3,16,25,29}, including the events in rice blast disease^{8,22,30}.

In previous paper from this laboratory the relationship between exogenously applied plant hormones and the degree of blast incidence was described¹⁵. The degree of blast incidence was suppressed by the application of auxins, tryptophan or ethylene (Ethrel® was used as an ethylene releasing agent) and stimulated by the application of kinetin or abscisic acid (ABA)¹⁵. The suppressive effect of indoleacetic acid (IAA) or ethylene was counteracted by the subsequent application of kinetin or ABA¹⁵.

These results suggest that plant hormones are implicated in host-parasite relationship in rice blast disease alike the other plant diseases and that there might exist an unidentified correlations between plant hormones as for the induction of resistance to rice blast disease.

Further, many studies have been also done as for the interactions between plant hormones and enzymes related to resistance reaction, such as peroxidase, phenylalanine ammonia-lyase and polyphenoloxidase^{2,3,25,29}.

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This paper deals with the effect of plant hormones on the peroxidase activity choosing as a parameter for resistance reaction in rice leaves inoculated with rice blast fungus.

Materials and Methods

Application of plant hormones and their precursors to rice plant. Indoleacetic acid (IAA), α -naphthalene acetic acid (NAA), Ethrel® (an ethylene releasing agent containing 39 % of 2-chloroethylphosphonic acid, Nissan Chemical Co.), kinetin, abscisic acid (ABA) were used as plant hormones. Tryptophan and methionine were used as precursors of IAA and ethylene, respectively. Spraying application of the above chemicals to the rice plant was carried out according to the methods previously described except otherwise stated¹⁵⁾.

Plants, fungus and inoculation. The cultivation of rice plant and the inoculation of blast fungus conidia (*Pyricularia oryzae*) to rice plants by spraying were performed as described previously¹⁵⁾. The other inoculation to rice leaves was performed by press-injury method^{7,18,33)}. Fifth leaf blades were used for the inoculation. A clot of filter paper powder moistened with the conidial suspension was placed on the injured spots^{7,18)}.

Application of plant hormones and antibiotics to the pieces of leaf blade. The 5th leaf blades of 6-leaf stage of rice seedlings were cut into pieces of approximately 10 mm lengths. Twenty pieces of leaf blades were soaked in the solution of IAA (50 ppm) or Ethrel (100 ppm) with or without antibiotics in vacuum. Vacuum infiltration was carried out by restoring the pressure slowly to atmospheric level during 45 minutes period¹⁴⁾. Formycin A (50 ppm), cycloheximide (10 ppm) and blasticidin S (50 ppm) were used as inhibitors of nucleic acid and protein synthesis respectively. The pieces of leaf blades infiltrated were transferred on moistened filter paper in petri dishes, inoculated with conidia of rice blast fungus and the subsequent incubation was carried out at 25 C for 48 hr under the illumination with plant growth fluorescent lamp (20W) from 30 cm height.

Extraction and assay of enzyme. Forty-eight hr after the inoculation with conidia of blast fungus, the inoculated leaf blades or the inoculated pieces of leaf blades were collected for extraction of peroxidase. When the leaf blades were inoculated after press-injury method, the tissues surrounding the inoculated site and those exterior to the surrounding zone were collected separately⁷⁾. The collected 32 leaves, 20 pieces or 64 leaf tissues were homogenized at 0-5 C using a Polytron (Kinematica, Switzerland) with 40 times volume (w/v) of 0.1M acetate buffer (pH 5.0). The homogenates were centrifuged at $13,000 \times g$ for 10 min. The supernatants were used as the enzyme source. Peroxidase activity was assayed by the modified guaiacol method⁷⁾ at least 5 times for each sample.

Results

Effect of plant hormones on the increase in peroxidase activity and the incidence of blast

Each 100 ppm aqueous solution of IAA, NAA, tryptophan, methionine or Ethrel was

Fig. 1. Peroxidase activity in the leaf blades treated with auxins, ethylene (Ethrel) or their precursors and the incidence of rice blast on them. The degree of blast incidence was indicated as disease index calculated from the number of progressive type lesion (control=1.0). The means and standard errors of peroxidase activity were indicated ($n=5$, $\alpha=0.05$). IAA: indoleacetic acid, NAA: α -naphthalene acetic acid, Trp: tryptophan, Met: methionine, Cont.: water.

sprayed on the leaf blades of rice seedling at 4 hr before inoculation. The peroxidase activities in the leaf blades at 48 hr after inoculation and the degree of blast incidence (indicated as disease index, calculated from the number of progressive type lesion, control=1.0) at 10 days after inoculation are shown in Fig. 1.

The peroxidase activities in the leaf blades were increased by treatment with the plant hormones or their precursors. They were more increased by inoculation with conidia of rice blast fungus than the treatment alone. The incidences of progressive type lesion were suppressed by treatment with hormones and their precursors, as well as previously given¹⁵⁾. The lesions of blast on treated leaves were mostly stagnation type, about 75-80% of lesions on the treated leaves were brown spots, on the contrary 79.2% of lesions on the control leaves were progressive type in this experiment. It was roughly observed that negative correlation existed between the peroxidase activities and the degrees of blast incidences.

Interaction between hormones

IAA or Ethrel (each 100 ppm) was sprayed at 24 hr and kinetin or ABA (each 20 ppm) was sprayed at 4 hr before inoculation on rice seedlings to determine the effect of each hormone upon rice blast disease. IAA or Ethrel was used with or without kinetin or ABA to determine the interaction between those plant hormones. The inoculation was carried out by press-injury method on 5th leaf blades. Peroxidase activity 48 hr after inoculation and degrees of blast incidences 15 days after inoculation in leaf tissues treated with plant hormones are shown in Table 1.

Peroxidase activities in the leaf tissues were increased by the application of IAA, Ethrel, kinetin or by the inoculation of rice blast fungus, and were more increased by the combination of hormone application and the inoculation. A higher enzymic activity

Table 1. Peroxidase activities in the leaf tissues treated with the plant hormones

Treatment	Collected part ^{a)}	IAA ^{b)} 100 ppm			Ethrel ^{b)} 100 ppm			Control (water ^{b)})		
		Non-inoculated	Inoculated		Non-inoculated	Inoculated		Non-inoculated	Inoculated	
		Peroxidase	Peroxidase	Blast	Peroxidase	Peroxidase	Blast	Peroxidase	Peroxidase	Blast
Kinetin ^{c)} 20 ppm	A	0.221 ^{d)} (± 0.009) ^{e)}	0.348 (± 0.008)	+ ^{f)}	0.246 (± 0.009)	0.445 (± 0.008)	-~+	0.222 (± 0.005)	0.258 (± 0.010)	++~+++
	B	0.141 (± 0.004)	0.230 (± 0.004)		0.179 (± 0.006)	0.270 (± 0.008)		0.150 (± 0.009)	0.177 (± 0.005)	
	C	0.103 (± 0.004)			0.113 (± 0.005)			0.100 (± 0.004)		
ABA ^{c)} 20 ppm	A	0.180 (± 0.007)	0.245 (± 0.007)	+++	0.192 (± 0.004)	0.254 (± 0.006)	++~+++	0.171 (± 0.010)	0.178 (± 0.008)	+++
	B	0.121 (± 0.006)	0.172 (± 0.010)		0.133 (± 0.007)	0.159 (± 0.010)		0.112 (± 0.005)	0.095 (± 0.005)	
	C	0.072 (± 0.007)			0.076 (± 0.008)			0.063 (± 0.004)		
Control (Water ^{c)})	A	0.229 (± 0.006)	0.291 (± 0.005)	+~++	0.275 (± 0.008)	0.356 (± 0.007)	-~+	0.149 (± 0.008)	0.200 (± 0.005)	+++
	B	0.145 (± 0.010)	0.169 (± 0.005)		0.169 (± 0.006)	0.213 (± 0.010)		0.077 (± 0.004)	0.132 (± 0.003)	
	C	0.079 (± 0.006)			0.101 (± 0.005)			0.061 (± 0.006)		

a) A: Press-injured center and its surrounding zone.

B: Exterior zone to the surrounding zone.

C: Healthy leaf (noninjured-noninoculated).

b) Sprayed at 24 hr before inoculation.

c) Sprayed at 4 hr before inoculation.

d) The mean of peroxidase activity ($\Delta OD_{420}/\text{min}$).e) Standard error of peroxidase activity ($n=5$, $\alpha=0.05$).

f) The degree of blast incidence. Blast incidences were ranked into 5 degrees based on the lesion sizes; Control (water-treated, inoculated: +++), moderate (+~+++), no lesion (-).

was observed at the injury-inoculated center and its surrounding zone than at the exterior zone. From these observations, in the case of spraying inoculation (Fig. 1), the augmented activity in the leaves treated with IAA or Ethrel might be due to the locally increased activity at the invaded sites.

On the other hand, the activities in the leaf tissues treated with ABA were observed to be lower than those treated with water.

When IAA or Ethrel was sprayed and kinetin was subsequently sprayed at 24 hr and 4 hr before inoculation respectively, the activity was more increased than that induced by IAA, Ethrel or kinetin alone, especially at the inoculated center and its surrounding zone. When ABA was subsequently applied, the increase in the activity by the treatment of IAA or Ethrel was suppressed.

The degree of blast incidence was suppressed by the treatment of IAA or Ethrel and was not by ABA alike the previous observation¹⁵⁾. The blast suppression by IAA and Ethrel was effectively counteracted by treatment of ABA prior to inoculation. On the contrary, the suppression was scarcely counteracted by kinetin. The similar correlations between plant hormones (and its precursors) and the peroxidase activity or the blast incidence were observed in repeated experiments, although the level of peroxidase activity was shifted due to difference of culture lot of the plant used in each experiment.

Fig. 2. Peroxidase activity in the leaf pieces infiltrated with IAA or Ethrel in the presence of antibiotics.

The means and standard errors were indicated ($n=5$, $\alpha=0.05$). Cont.: water, CHI: cycloheximide, BcS: blasticidin S, FMA: formycin A.

Fig. 3. Time course of the increase of peroxidase activity in the leaf pieces infiltrated with Ethrel.

1: Ethrel, 2: Control (water), 3: Control (detached intact leaf blades which were not cut into pieces, being only naturally senescent).

Effect of antibiotics on peroxidase activity enhanced by IAA or Ethrel

Peroxidase activities in the leaf pieces which were infiltrated with IAA, Ethrel or water in the presence of formycin A, cycloheximide or blasticidin S are shown in Fig. 2. In this system, peroxidase activity was increased by the treatment of IAA or Ethrel and also by the inoculation with conidia of rice blast fungus as in the case of seedling test (Fig. 1 and Table 1): When formycin A, cycloheximide or blasticidin S was applied to the leaf pieces, the increase in peroxidase activity in the leaf pieces by the treatment of IAA or Ethrel was strongly inhibited.

Fig. 3 shows the time course of the increase in peroxidase activity in the leaf pieces which were infiltrated with Ethrel or water. From 4 hr after infiltration, the peroxidase activity in the

leaf pieces treated with Ethrel was increased. Consequently, at 48 hr after-infiltration, the peroxidase activity in the leaf pieces treated with Ethrel was highly increased up to 1.44 times or 2.26 times than those treated with water or than those of detached intact leaf blades which were not cut into pieces, and being only naturally senescent on the

moistened filter paper under the illumination.

These results might suggest that the induction of peroxidase in the leaf blade treated with IAA or Ethrel depended on *de novo* synthesis of enzyme-protein involving the RNA system¹²), and that the synthesis of protein began in the leaf blades within at least 4 hr after IAA or Ethrel treatment.

Discussion

The lines of experimental evidence described herein indicated that auxins, ethylene and their precursors augmented the resistance of rice plant against rice blast fungus. Those also indicated that the induced resistance was closely associated with an increase in the peroxidase activity in the treated rice leaves. It might be able to consider that the increase of peroxidase activity mainly takes place at invaded sites (Table 1), as a result of *de novo* synthesis of enzyme-protein (Fig. 2).

Auxins were well known to stimulate ethylene production in plants^{1,12,21,26,34,35}). Further, it was observed that auxin and ethylene markedly stimulated the induction of some enzymes such as peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase and cinnamic acid 4-hydroxylase^{3,9,17,19,25,29,31,32}).

From above lines of evidence, it was quite possible that the shift in augmentation of peroxidase with auxin was at least in a consequence of the stimulation of ethylene biosynthesis following auxin treatment^{12,35}).

A hypothetical scheme for induced resistance involving plant hormone in rice plant is proposed as follows^{23,24}); the inducers within plant pathogenic microorganism^{5,6})—receptor of the inducers on protoplasmic membrane of rice plant cell²⁰)—IAA operating system—ethylene operating system—activation of enzymes related to resistance reaction—formation of the chemical barrier such as phytoalexin, antimicrobial substance and/or the physical barrier such as lignin accumulation—expression of disease resistance. It might be plausible that there are at least two regulatory systems in the above pathway of successive resistance reactions. One is the negative feedback system in which IAA is degraded by increased peroxidase¹¹). The other is the amplifying system in which ethylene biosynthesis is accelerated by the increased peroxidase^{12,27}) and the ethylene formed by itself, alike autocatalytic response^{12,26}).

It was observed in the present study that kinetin enhanced the IAA- or Ethrel-induced increase in peroxidase within the rice tissues. This observation might be related to the synergistic effect of kinetin on IAA-induced ethylene production^{4,10}). In this study, kinetin did not show any tendency to stimulate the occurrence of rice blast, despite stimulative effect was observed in previous experiment¹⁵). The unidentical effect of kinetin may be caused by difference of inoculation procedures in the two experiments, that is, by the spraying¹⁵) and the press-injury method.

It was also observed that ABA counteracted the augmentation of peroxidase activity by IAA or ethylene and also the induced resistance against rice blast by IAA or ethylene, besides ABA inhibited the growth of rice plant. Since ABA was known to depress RNA synthesis in plant¹³) and the counteraction of ABA on the blast-suppressive effect of IAA and ethylene was not observed when ABA was treated at 24 hr after inoculation¹⁵), ABA might act in the beginning of the induction of resistance by IAA, ethylene or blast fungus.

The shift of plant hormones including IAA, ethylene and ABA in rice leaf tissues should be quantitatively surveyed when the leaf tissue is inoculated with blast fungus conidia or is treated with the inducers in blast fungus^{5,6)}.

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和 文 摘 要

植物ホルモン類処理のイネ葉のパーオキシダーゼ活性と
いもち病発病に及ぼす影響

岩田道顕・関沢泰治・岩松寿子・鈴木幸雄・渡辺哲郎

イネを植物ホルモン類で処理した場合のイネ葉組織中におけるパーオキシダーゼ活性の変動と、いもち病発病との関係について調べた。オーキシシン類、エチレンあるいはそれらの前駆物質で処理すると、パーオキシダーゼ活性が増加した。その増加は、接種部位で著しかった。IAA あるいはエチレン処理後に、カイネチンを処理すると、それぞれ単独よりもパーオキシダーゼ活性が増加することが認められた。一方、IAA あるいはエチレン処理後に、アブジン酸で処理すると、IAA あるいはエチレンによるパーオキシダーゼ誘導作用は抑制された。いもち病の発病は、オーキシシン類、エチレンあるいはこれらの前駆物質によって抑制された。IAA あるいはエチレン処理後に、アブジン酸で処理すると、IAA あるいはエチレンによる発病抑制作用は発現しなかった。IAA あるいはエチレンによるパーオキシダーゼ誘導作用は、フォルマイシンA、シクロヘキシイミドおよびプラストサイジンSによって阻害された。したがってこの誘導作用は、酵素蛋白質の *de novo* 合成を伴う反応であると考えられる。イネいもち病における誘導抵抗性に、植物ホルモン類が重要な役割を果たしているものと思われる。