Studies on the Control of Plant Diseases by Amino Acid Derivatives*

(1) Effect of N-lauroyl-L-valine on rice blast

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本間保男**・志田俊郎***・見里朝正**： アミノ酸関連物質による植物病害の防除

(1) イネいもち病に及ぼす N-lauroyl-L-valine の影響

Abstract

Rice blast disease preventive activities of seven amino acid derivatives were examined by using rice seedlings of the 4- or 5- leaf stage. Because N-lauroyl-L-valine (No. 5) showed the most constant activity among them, it was used as the test compound in further experiments, and the following experimental results were obtained.

Lauric acid, L-valine and their mixture showed lower activities than that of No. 5.

In comparison with activities of optical isomers of valine, no significant difference was found among them.

Among various salts and esters of N-lauroyl-L-valine tested, only sodium and potassium salts were as effective as the free compound, whereas calcium salt, and methyl and ethyl esters had low preventive activity.

In case of substituting other amino acids for valine residue in N-lauroyl-L-valine, L-proline, L-methionine, L-leucine and DL-alanine derivatives were as effective as No. 5, whereas in case of replacing lauric acid (C₁₂) with other fatty acids, the protective effects reduced as increasing or decreasing the number of carbon atoms of substituted fatty acids.

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Introduction

Recently environmental pollution due to the used of agricultural chemicals has become a social problem. We have been engaged in the development of agricultural chemicals which may not bring about the possibility of environmental pollution. One of the reasons why conventional agricultural chemicals have caused environmental pollution is that they are not decomposed rapidly by soil microorganisms and plants. Generally, except antibiotics, they have chemical structures which do not exist in the natural world.

We have studied the utilization of several amino acid derivatives as agricultural chemicals. The derivatives consist of amino acids and fatty acids, and some compounds are condensates of amino acids with fatty acid, aryl-substituted fatty acids and fatty alcohols. Their linkage between amino acid and fatty moieties is amide or ester bond. Amino acids are components of proteins, and fatty acids and phenacetic acid is also component of living cells. Furthermore, amidase is widely distributed in microorganism and plant cells. When the amide or ester bond between amino acid and

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fatty acid is cleft by microorganism and plant cells, it is expected that the fragments may be rapidly metabolized, and that there is no residue problem about these chemicals.

van Andel reviewed the control of plant diseases by amino acids¹¹⁾, and Ohata *et al.*⁹⁾ described the effects on rice blast fungus by application of 17 amino acids, and observed inhibitory effects of some amino acids on the spore germination or the hyphal growth in the infected host cells. Egawa¹⁾ examined in detail the utilization of D-amino acid by plant pathogenic fungi.

On the other hand, fungicidal effects of fatty acids are well-known as their surfactant activities. Ishiyama *et al.*⁴⁾ reported that rhodan derivatives of fatty alcohols had protective effects on rice blast.

In this paper we describe the inhibitory effect of amino acid derivatives on rice blast diseases.

Materials and methods

A susceptible variety of rice plant "Jukkoku" was used throughout this work. After the surface of seeds was sterilized with 1:1000 "Uspulun" for an hour, they were rinsed in running tap water for an additional one hour, then placed in thermostat (25°C), and kept in humid chamber (100%) for four days. Germinated seeds were sown on plastic pots (8"×6") which were heaped with up soil containing fertilizer mixed as usual. They were then placed in a water tank, and grown in it for about 2 weeks. The seedlings at the 4- or 5- leaf stage were used as the test plant.

A strain of *Pyricularia oryzae* Cavara, the rice blast fungus kept in our laboratory was cultured in sterilized chaff medium (chaff 3 g, sucrose 0.2 g, yeast extract 0.01 g, soluble starch 0.05 g). Spores were collected from the medium, then suspended in tap water. One or two drops of "Dain" (Takeda Seiyaku K.K.) were added to the suspension as a spreading agent. The spore suspension (10^7 /ml) thus prepared was used as an inoculum source.

Amino acid derivatives used in this examination were all crystalline forms. These were dissolved in 0.5 ml of 80% ethanol and adjusted to appropriate concentrations with tap water.

The rice plant seedlings grown on pots as described were placed on a turntable. At a distance of about two meters from the table, the chemical preparations were sprayed by a spray gun. After two days or two hours, the spore inocula were applied to the seedlings with spray gun, inoculated plants were maintained for three days under moist conditions. The number of lesions of the most diseased leaf of a stalk were counted on the fourth day after inoculation, and the average number of lesions per leaf was calculated for twenty stalks. The preventive value was calculated according to the following equation.

$$\text{preventive value (\%)} = \frac{\text{number of lesions per untreated leaf} - \text{number of lesions per treated leaf}}{\text{number of lesions per untreated leaf}} \times 100$$

Results

1. Comparison of preventive values of various amino acid derivatives As shown in Table 1, N-lauroyl-glycine, N-lauroyl-L-valine, dodecyl-DL-alaninate·HCl, dodecyl-DL-valinate·HCl, N-phenacetyl-glycine, N- α -naphthylacetyl-L-valine and N- α -naphthylacetyl-L-leucine had protective activity against rice blast disease among about 150 amino acid derivatives tested. The chemical structures of seven active amino acid derivatives are shown in Table 2. N-lauroyl-L-valine was used in further experiments, because this compounds had the most constant activity.

2. Protective effect of components of N-lauroyl-L-valine Further investigation on the inhibitory activity of components N-lauroyl-L-valine was carried out. As shown in Table 3, L-valine, lauric acid and their mixture were scarcely effective.

From this experiment, it is deduced that the amide linkage connecting the fatty acid with the

Table 1. Effective amino acid derivatives on rice blast

Amino acid derivatives	Concentration (ppm)	Preventive value (%)		Phyto- toxicity
		Two days ^{a)}	Two hours ^{a)}	
N-Lauroyl-glycine	500	22	41	—
	1000	55	51	—
	2000	66	61	—
N-Lauroyl-L-valine	500	65	75	—
	1000	70	88	—
	2000	84	97	—
Dodecyl-DL-alaninate · HCl	500	69	72	—
	1000	70	75	—
	2000	80	86	—
Dodecyl-DL-valinate · HCl	500	44	75	—
	1000	76	72	—
	2000	80	83	—
N-Phenacetyl-glycine	500	23	44	—
	1000	45	57	—
	2000	69	66	—
N- α -Naphthylacetyl-L-valine	500	46	55	—
	1000	50	67	—
	2000	61	78	—
N- α -Naphthylacetyl-L-leucine	500	50	50	—
	1000	57	68	—
	2000	75	81	—
None	0	0	0	—

a) Time till inoculation after amino acid derivatives treatment.

amino acid is necessary for protection of rice from blast disease.

3. The effect of isomers, salts, and derivatives of N-lauroyl-L-valine on rice blast disease The protective effect of isomers of N-lauroyl-valine was examined on rice blast disease (Table 4). When the inoculation was made two days after fungicide application, the preventive value of D-isomer was slightly higher than that of the compounds containing L-, and DL-isomers. There was no remarkable difference between L- and DL-isomers. In case of inoculation two hours after fungicide application, the preventive values of L- and D-isomers were slightly higher than that of DL-isomer.

Various salts of N-lauroyl-L-valine were also tested. When two days interval was interposed between fungicide application and inoculation, sodium salt was a little more protective than the others. Sodium and potassium salts were remarkably protective in comparison with calcium salt if inoculated on the same day.

Both methyl and ethyl esters of N-lauroyl-L-valine were a little effective on rice blast disease.

4. The effect of various N-lauroyl-amino acids on rice blast disease The effects of N-lauroyl derivatives of various acids at concentrations of 2000 ppm on rice blast were investigated (Table 5). When the inoculation was made two days after fungicide application, N-lauroyl-L-leucine, L-proline, L-methionine, L-valinate, DL-alanine, L-isoleucine, and D-phenylalanine showed considerable protective activity. In case of the inoculation two hours after fungicide application, sodium N-lauroyl-L-valinate,

Table 2. Effective amino acid derivatives on rice blast

N-Lauroyl-glycine	$\text{CH}_3-(\text{CH}_2)_{10}-\text{CONH}-\text{CH}_2-\text{COOH}$
N-Lauroyl-L-valine	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH}-\underset{\text{NH}-\text{CO}-(\text{CH}_2)_{10}-\text{CH}_3}{\text{CH}}-\text{COOH}$
Dodecyl-DL-alaninate · HCl	$\begin{array}{c} \text{CH}_3-\text{CH}-\text{COOC}_{12}\text{H}_{25} \\ \\ \text{NH}_2 \cdot \text{HCl} \end{array}$
Dodecyl-DL-valinate · HCl	$\begin{array}{c} (\text{CH}_3)_2-\text{CH}-\text{CH}-\text{COOC}_{12}\text{H}_{25} \\ \\ \text{NH}_2 \cdot \text{HCl} \end{array}$
N-Phenacetyl-glycine	
N-α-Naphthylacetyl-L-valine	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH}-\underset{\text{NH}-\text{CO}-\text{CH}_2-\text{Naphthalene}}{\text{CH}}-\text{COOH}$
N-α-Naphthylacetyl-L-leucine	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH}-\text{CH}_2-\underset{\text{NH}-\text{CO}-\text{CH}_2-\text{Naphthalene}}{\text{CH}}-\text{COOH}$

Table 3. Effects of L-valine, lauric acid and their mixture as the components of N-lauroyl-L-valine on rice blast

Components of N-Lauroyl-L-valine	Concentration (ppm)	Preventive value (%)		Phyto- toxicity
		Two days ^{a)}	Two hours ^{a)}	
Lauric acid	2000	7	28	—
L-Valine	2000	27	21	—
Lauric acid + L-Valine	2000	0	32	—
N-Lauroyl-L-valine	2000	84	90	—
Blasticidin S	10	75	96	—
None	0	0	0	—

^{a)} Time till inoculation after treatment.

Table 4. Effects of isomers, derivatives, and salts of N-lauroyl-L-valine on rice blast

Chemical	Concentration (ppm)	Preventive value (%)		Phyto- toxicity
		Two days ^{a)}	Two hours ^{a)}	
N-Lauroyl-L-valine	2000	75	98	—
N-Lauroyl-DL-valine	2000	76	88	—
N-Lauroyl-D-valine	2000	82	95	—
Sodium N-Lauroyl-L-valinate	2000	81	98	—
Potassium N-Lauroyl-L-valinate	2000	71	94	—
Calcium N-Lauroyl-L-valinate	2000	71	58	—
Methyl N-Lauroyl-L-valinate	2000	29	56	—
Ethyl N-Lauroyl-L-valinate	2000	20	27	—
Blasticidin S	10	78	90	—
None	0	0	0	—

^{a)} Time till inoculation after treatment.

Table 5. Effects of N-acylated amino acid residue in N-lauroyl-L-valine on rice blast

N-Acylated amino acid residue	Concentration (ppm)	Preventive value (%)		Phyto- toxicity
		Two days ^{a)}	Two hours ^{a)}	
N-Lauroyl-glycine	2000	70	59	—
N-Lauroyl-L-leucine	2000	91	88	—
N-Lauroyl-L-isoleucine	2000	68	73	—
N-Lauroyl-L-phenylalanine	2000	67	79	—
N-Lauroyl-L-lysine	2000	0	30	—
N-Lauroyl-L-glutamic acid	2000	48	73	—
N-Lauroyl-L-threonine	2000	12	79	—
N-Lauroyl-L-aspartic acid	2000	38	67	—
N-Lauroyl-L-alanine	2000	50	92	—
N-Lauroyl-L-proline	2000	87	98	—
N-Lauroyl-L-methionine	2000	77	96	—
N-Lauroyl-L-serine	2000	13	46	—
N-Lauroyl-DL-tryptophane	2000	56	88	—
N-Lauroyl-DL-alanine	2000	70	89	—
N ₂ -Lauroyl-L-arginine	2000	62	38	—
N ₂ -Lauroyl-L-histidine	2000	46	27	—
Sodium N-Lauroyl-L-valinate	2000	75	100	—
Blasticidin S	10	69	100	—
None	0	0	0	—

^{a)} Time till inoculation after treatment.

N-lauroyl-L-proline, and L-methionine had preventive values near 100 per cent, and N-lauroyl- DL-alanine, DL-tryptophane, L-leucine, L-phenylalanine, L-threonine, and L-isoleucine had protective effects above 70 per cent. The results suggest that these compounds may be used practically as potential agricultural chemicals.

5. The effects of various N-acyl-L-valine complexes on rice blast disease Experimental results mentioned above showed that there were remarkable differences in protective effect among complexes of lauric acid and various amino acids. As a subsequent experiment, the effects of complexes of L-valine and various fatty acids were investigated.

As shown in Table 6 and Fig. 1, when the inoculation was made two days after fungicide appli-

Table 6. Effects of N-acyl radical in N-lauroyl-L-valine on rice blast

N-Acyl radical	Concentration (ppm)	Preventive value (%)		Phyto- toxicity
		Two days ^{a)}	Two hours ^{a)}	
N-Caprinoyl-L-valine	2000	40	27	—
N-Myristoyl-D-valine	2000	31	19	—
N-Undecelenoyl-L-valine	2000	17	69	—
N-Stearoyl-L-valine	2000	5	0	—
N-Palmitoyl-L-valine	2000	0	56	—
N-Propionoyl-L-valine	2000	29	41	—
N-Behenoyl-L-valine	2000	11	0	—
N-Octanoyl-L-valine	2000	23	70	—
N-Oleoyl-L-valine	2000	39	0	—
N-Lauroyl-L-valine	2000	74	91	—
Blasticidin S	10	65	87	—
None	0	0	0	—

a) Time till inoculation after treatment.

Fig. 1 Relationship between number of carbon atoms in N-acyl radical of N-lauroyl-L-valine and inhibitory effect on rice blast

cation, lauroyl derivatives was the most active among ten fatty acid derivatives tested. Furthermore octanoyl and oleoyl derivatives had considerable inhibitory effect on rice blast.

In case of the inoculation two hours after fungicide application, lauroyl derivative was the most effective, and octanoyl and undecelenoyl derivatives had also some protective effect. These results show that N-lauroyl-L-valine was always most active.

Discussion

Many researchers have investigated the effects of several amino acids on the plant diseases¹¹⁾. van Andel classified the mode of action of amino acids into two types. One hypothesis suggests that amino acid has a direct effect on the fungus, and metabolism to involve amino acid in the host is proposed as another possibility¹¹⁾. Though many papers supported his hypothesis, they described only discovery of phenomena or its experimental evidence in laboratory.

It is considered that agricultural chemicals which can be decomposed by microorganisms do not cause environmental pollution. Our attention was given to various components of living organisms such as amino acids, fatty acids, sugars and nucleic acids, and we examined the possibility of their utilization as pollution-free fungicides. Their effects on control of plant diseases were repeatedly tested not only *in vitro* but also *in vivo*, and we found that some amino acids derivatives had inhibitory effect on rice blast disease. One of the most active amino acid derivatives, N-lauroyl-L-valine, was selected as the test compound, and not only its effect in the field, but also the mechanism of protection and decomposition by microorganisms¹⁰⁾ were investigated.

N-lauroyl-L-valine, a complex of lauric acid and L-valine combined by amide linkage, exists scarcely in the natural world. In order to clarify the activity of N-lauroyl-L-valine, therefore, the activities of lauric acid and L-valine were compared with N-lauroyl-L-valine, and a little activity was found in two compounds.

Ohata *et al.*⁹⁾ studied effects of 17 amino acids on both the growth of germ tube and of hyphae of rice blast fungus within invaded tissues, and pointed out that several amino acids had some inhibitory effect against the pathogen. But in their experiment L-valine did not show any activity against at the concentration of 1800 ppm supporting our results *in vivo*. Both free lauric acid and L-valine were not active to rice blast disease, but the amide derived from the two compounds had remarkable inhibitory effect. In addition to that, the amide linkage would be easily broken by *Pseudomonas aeruginosa* (AJ 2116)¹⁰⁾ and therefore, it would not remain as original compound under natural environment. From the point of view described above, we are able to consider it as one of the new types of agricultural chemicals.

It was shown by Kuč *et al.*⁵⁾ that there were different effects of various amino acids and their isomers on plant diseases, such as, apple scab. They proved that apple leaves treated with D- and DL-phenylalanine could have a greater resistance in resistant varieties Russian and Geneva to all three races, but those treated with L-phenylalanine did not become more resistant^{(2),(3),(5),(6),(7),(8),(9)}. On the other hand, susceptible varieties Jonared, McIntosh and so on, did not induce resistance after infusion with D- or DL-phenylalanine, but the reduction of degree of susceptibility was found. Slight differences of protective effect on rice variety "Jukkoku" were found after treatment with N-lauroyl-D-valine, N-lauroyl-DL-valine and N-lauroyl-L-valine, and the order of protective effects was N-lauroyl-D-valine, N-lauroyl-L-valine and N-lauroyl-DL-valine (Table 4). Because the remarkable difference of preventive values reported by Kuč *et al.* is not observed in our experiment, it may be necessary to examine further whether the results *in vivo* agree with those results *in vitro* or not.

Sodium, potassium and calcium salts of N-lauroyl-L-valine had different preventive values as

shown in Table 4. Especially, calcium salt showed lower protective effect than others. One of the reasons for the low activity may be attributed to the water insolubility of this compound. On the other hand, N-lauroyl-L-valine has some chelating action, so it seems that it may catch calcium ion in epidermis of the rice plant. The formation of calcium salt may reduce the protective effect of the free compound. It is necessary to study this phenomenon further.

The effects of amino acid residues in various N-lauroyl-amino acid complexes were examined. Among them, L-leucine, L-proline, L-methionine and DL-alanine complexes had high protective effect (Table 5). Some amino acid residues which showed high protective activity in our experiments also inhibited the growth of invaded hyphae into the epidermal tissues of leaf sheath⁹⁾. These results suggest that some kind of amino acids play an important role in inhibitory effect on the rice blast fungus. Relationship between the number of carbon atom of amide radicals in fatty acid-L-valine complexes and their inhibitory activities was shown in Fig. 1. The activity was the highest in lauric acid (C₁₂), but as the number of carbon atoms in fatty acid molecules increases or decreases, the activity reduces markedly. This result suggests that the chain length of fatty acids is important to the activity of these derivatives. It is not clear whether the phenomena were due to the physical property of fatty acids or not. But it was demonstrated that in case of direct action of fatty acids on rice blast fungus, preventive activity varied according to the numbers of carbon atoms.

The control of plant diseases by such naturally occurring materials would be very important since a pretty number of synthetic agricultural chemicals have stable structures and properties under the natural circumstances.

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和文摘要

アミノ酸関連物質による植物病害の防除

(1) イネいもち病に及ぼす N-lauroyl-L-valine の影響

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約150種のアミノ酸誘導体について、4—5葉期のイネ苗を供し、そのいもち病防除効果を検討した結果、7種類の有効物質を得た。その中から N-lauroyl-L-valine を選び、その化学構造と抗いもち病活性について検討した結果を得た。

N-lauroyl-L-valine の構成成分である lauric acid, L-valine の単独または混合では、いもち病防除効果はきわめて低いことが判明した。

アミノ酸の異性体の中で活性を比較すると $D \geq L > DL$ の順であったが、それらの間には顕著な差は認められなかった。

N-lauroyl-L-valine の Na, K, Ca 塩およびメチル、エチル各エステルの間では、Na, K 塩だけが遊離酸とあまり差がなかったが、その他のものはきわめて防除効果が低かった。

valine を他のアミノ酸で置き換えた場合には、L-proline, L-methionine, L-leucine, DL-alanine などに L-valine と同程度の効果が認められた。いっぽう lauric acid (C_{12}) を他の脂肪酸で置き換えた場合には、脂肪酸部分の炭素数が C_{12} より増加あるいは減少するにつれて防除効果が低下した。