

ポプラ葉さび病感受性に対する栄養条件の影響に関する研究(III)

アミノ酸との関係について

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論 文

Studies on the Susceptibility to Poplar Leaf Rust Influenced
by Different Nutrient Conditions (III).

Changes in Free Amino Acid Contents

Kazuo SUZUKI* and the late Osamu CHIBA**

ポプラ葉さび病感受性に対する栄養条件の影響に関する研究 (III)

アミノ酸との関係について

鈴木和夫*・故千葉 修**

要 旨: ポプラ葉さび病菌 (*Melampsora larici-populina* KLEB.) に対する感受性と宿主の遊離アミノ酸含有量との関係を、宿主の養分欠乏条件下において調べ、次のような結果を得た。

ポプラの葉に含まれる遊離アミノ酸の全含有量は、N及びP欠乏処理区において著しく減少した。また、各々のアミノ酸の絶対量はばらつきを示すが、全アミノ酸含有量に対する割合(%)にはほとんど変動がなく、すべての処理個体においてアラニン、セリン、グルタミン酸、スレオニン、アスパラギン酸が主要な割合を占めた。

感染によって全アミノ酸含有量は、高い罹病率の値を示す対照区及びK欠乏処理区において著しく減少するが、罹病率の低いN及びP欠乏処理区においては、ほとんど変化を示さなかった。

一方、感染に伴う各々のアミノ酸の構成割合(%)は、N欠乏処理区を除き、各々の養分欠乏条件下で特異的な変化を示した。

Summary: To determine the relation between the susceptibility to a poplar leaf rust, caused by *Melampsora larici-populina* KLEB., and the amino acid content of leaves, qualitative and quantitative changes of amino acid contents were investigated under the condition of nutrient deficiency.

Though total amounts of amino acids in leaves were remarkably reduced by nutrient deficiency, except for potassium deficiency, each component of amino acids in leaves was not reflected in its ratio to the total content. From the results, it is suggested that the amino acids composition is not responsible for the susceptibility except for the total amount of amino compounds.

The infection of leaves gave rise to a pronounced increase or decrease in some amino acids, viz. alanine, aspartic acid, glutamic acid and threonine. These were major components of the free amino acids not only in rusted leaves but also in uninfected ones. A remarkable decrease in alanine and an increase in aspartic acid are commonly observed in the rusted leaves, though the changes in other amino acids such as glutamic acid, valine and arginine varied with the treatments. From these results, it is suggested that the change in amino acid metabolism through the rust development is specific to each treatment under the condition of nutrient deficiency.

Introduction

In the previous paper¹⁾, it was reported that the sugar content of leaves of the host was affected by nutrition, and the relationship between the sugar content of leaves due to different nutrient conditions and the susceptibility to the leaf rust was discussed. In addition to sugars, contents of some amino acids

also have been found to have a close relationship to the susceptibility of host plants^{2,3)}. SAMBORSKI & SHAW⁴⁾ showed that treatment with maleic hydrazide broke the resistance of Khapli wheat to stem rust, and that the change of reaction type was associated with a marked increase in free amino acid contents. The fact that the nitrogen nutrition of the plant has an effect on its susceptibility

* 東京大学農学部 Fac. of Agr., Univ. of Tokyo, Tokyo

現勤務先: 農林省林業試験場九州支場 Present address: Kyushu Branch, Gov. For. Exp. Sta., Kumamoto

** 農林省林業試験場 Gov. For. Exp. Sta., Tokyo

indicates that nitrogen metabolism plays an important role in the disease development.

Correlation between the amino acid content and the susceptibility of the host plants is often considered to support GARBER's nutrition-inhibition hypothesis⁵⁾ that a correlation between a high level of an amino acid and susceptible reaction, or between a low level and resistant reaction would be due to the stimulatory effect or indispensability of the amino compounds for fungus development. This consideration has given rise to many investigations of the qualitative and quantitative changes in the composition of the free amino acids in plant tissue during infection.

As to a poplar leaf rust, one of the authors observed considerable differences in amino acid contents among several poplar clones and correlations between their contents and the susceptibility to the leaf rust, using paper chromatography⁶⁾.

The present investigation was undertaken to determine if qualitative and quantitative changes of amino acids content of leaves would be attributable to the changes of susceptibility of host plants due to unbalanced nutrition, and to observe the changes in free amino acid contents of leaves of healthy and diseased plants under different nutrient conditions.

Materials and Methods

Tests were carried out using a susceptible clone, *P. deltoides* × *P. nigra caudina*, in June 1969. As in the previous paper⁷⁾, plants of every clone were cultivated separately in the complete nutrient solution, or three kinds of nutrient solutions, each lacking one of the essential elements, in a green house at 20~25°C. After about two months, leaves of one or two plants in every treatment were collected for analysis at noon, and then the leaves of other plants were inoculated with uredospores of *Melampsora larici-populina*. The 5th and 6th leaves from the top were used for analysis and inoculation. Methods of inoculation and rating of disease severity were the same as those in the previous experiment⁷⁾. These infection coefficients were 7.8 for the complete nutrition, 2.3 for the nitrogen deficiency, 6.1 for the potassium deficiency and 3.6 for the phosphorous deficiency, respectively.

Freshly harvested plant tissues were weighed, and immediately dried at 100°C for 5 minutes to inactivate enzymes, and then they were homogenized with 80% ethanol. Each macerate was filtered and the residue was washed with 80% ethanol. The proteins were precipitated by addition of an equal volume of 1% picric acid and then removed by centrifugation. The supernatant was passed

through a column of Dowex 1 × 8 Cl⁻ (200-400 mesh) to remove picric acid. Aliquot of effluent was placed on each of the 15-cm and 70-cm ion exchange resin columns and then analyzed for basic, neutral, and acidic amino acids. 30-50°C system for basic and 40-50°C system for neutral and acidic amino acids were used⁸⁾. Amino acids were determined by the JLC-3BC Liquid Chromatograph, Japan Electron Optics Laboratory.

Results and Discussion

Among 22 amino compounds in healthy and/or diseased poplar leaves, 16 were identified as known amino acids.

Total contents of amino acids in the poplar leaves before inoculation showed the largest amount in the complete nutrient leaves and the smallest in the nitrogen deficient leaves, as seen in Table 1. In spite of the great difference in the absolute amounts, the percentage composition of each amino acid to the total contents of amino compounds was nearly constant in every treatment (Table 2). For example, alanine was by far the most abundant amino acid in healthy plants. And even though the absolute amount ranged from 14.0 to 44.8 × 10⁻⁸ mole/g fresh weight, its relative amount was 37~52% of the total amino acids for every case of nutrient condition.

This fact suggests that the amino acid composition in poplar leaves does not show any appreciable change after the culture under the nutrient deficient conditions for about two months. In this respect, DURZAN & STEWARD⁹⁾ reported that nutrient deficiency affected both the total amount and relative composition of amino acids in leaves of coniferous plants after the culture for one year and a half.

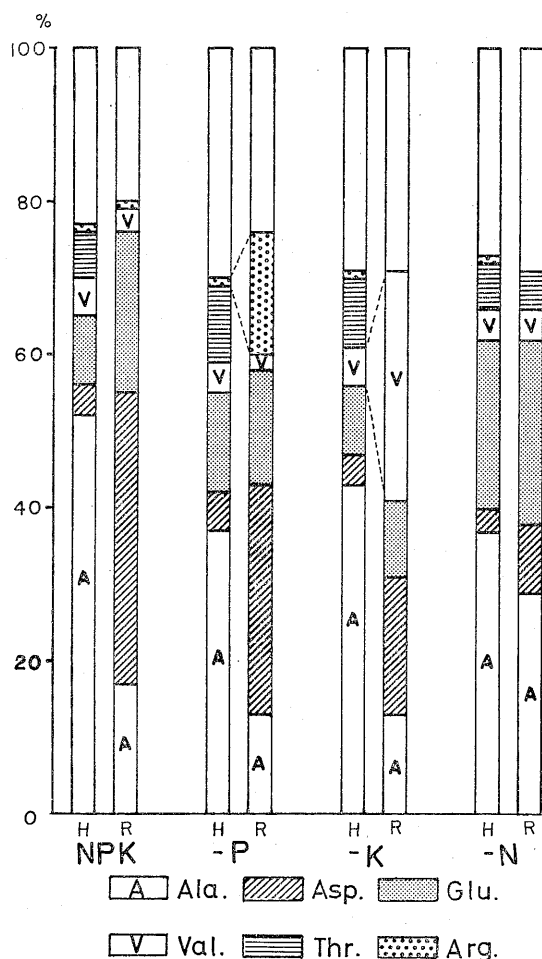
Three weeks after inoculation, the rusted leaves of the complete nutrient and potassium deficient plants showed remarkable decreases in total content of amino acids, while those in the other treatments, such as phosphorus and nitrogen deficiency, did not show any appreciable changes (Table 1). Such differences may be partly attributable to disease severity as described in the previous paper⁷⁾. In contrast, SHOW & COLOTELO¹⁰⁾ found

Table 1. Absolute amounts of free amino acids per gram fresh weight in poplar leaves of healthy (H) and rusted (R)

	(10 ⁻⁸ mole)	
	H	R
NPK	86.0	53.6
-P	69.7	68.3
-K	84.3	39.1
-N	38.3	38.7

Table 2. Absolute amounts of free amino acids per gram fresh weight (10^{-3} mole) and ratio of each amino acid to the total amount (%)

	NPK		-P		-K		-N	
	10^{-3} mole	%	10^{-3} mole	%	10^{-3} mole	%	10^{-3} mole	%
Aspartic acid	3.3	4	3.6	5	3.3	4	1.0	3
Threonine	5.1	6	7.3	10	8.0	9	2.2	6
Serine	8.4	10	11.1	16	12.9	15	5.4	14
Glutamic acid	7.4	9	8.8	13	7.3	9	8.3	22
Glycine	0.0	0	0.0	0	0.0	0	1.1	3
Alanine	44.8	52	25.6	37	36.4	43	14.0	37
Valine	4.2	5	2.9	4	3.8	5	1.6	4
Cystine	+	0	0.8	1	0.7	1	0.3	1
Methionine	2.0	2	0.8	1	1.4	2	+	0
Iso-Leucine	2.6	3	2.0	3	1.8	2	0.9	2
Leucine	4.4	5	2.6	4	4.0	5	1.4	4
Tyrosine & Phenylalanine	2.7	3	2.3	3	2.6	3	1.3	3
Histidine	0.3	0	0.7	1	0.6	1	0.4	1
Lysine	0.3	0	0.2	0	0.6	1	0.2	1
Arginine	0.5	1	1.0	1	0.8	1	0.2	1
Total	86.0	100	69.7	100	84.3	100	38.3	100



that in the case of the rust infection on Little Club wheat, free amino acids increased by approximately 4 times with the rust development within 9 days after inoculation.

The infection of leaves resulted in a pronounced increase or decrease in some amino acids, viz. alanine, aspartic acid, glutamic acid and threonine, which were major components of the free amino acids, not only in rusted leaves but in infected ones.

A remarkable decrease in alanine and threonine, and an increase in aspartic acid are common phenomena after infection except for the case of nitrogen deficiency (Fig. 1). The changes in the other amino acids are dependent on each treatment. In the diseased leaves with complete nutrition, the contents of glutamic acid increased twice from 9 to 21%. In the potassium deficient leaves, the content of valine increased strikingly by six times, from 5 to 30%. And in the phosphorus deficient leaves arginine increased from 1 to 16%.

It is worth while to note that the increase of valine or arginine was observed characteristically in the potassium or the phosphorus deficient leaves after infection. Such changes in amino acid level seem to reflect changes in the activity of the enzymes concerned with amino acid metabolism⁽¹⁾, though the significance of these phenomena is not yet known.

On the other hand, infection caused little change in amino acid content in the case of nitrogen de-

Fig. 1. Ratio of each amino acid (alanine, aspartic acid, glutamic acid, valine, threonine and arginine) to total amounts of amino compounds in poplar leaves of healthy (H) and rusted (R)

ficient leaves. This is supposed as a phenomenon resulting from the restriction of fungus development within host tissues under the condition of nitrogen deficiency.

From these results, it is suggested that the changes in amino acid metabolism through the rust development is specific to nutrient condition.

Generally, phenolic compounds and other aromatic metabolites are known to increase in concentration after infection^{12,13}. In this experiment the poplar clone used seems to contain a very small amount of synthetic substrates of phenolic compounds, such as tyrosine, tryptophane and phenylalanine. This consideration is also based on the fact that heavy chlorosis is produced on leaves by the infection without necrosis. Therefore, clones producing heavy necrosis after infection must be applied as test materials to the further study of this problem.

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訂正

本誌第56巻5号の論文「DARCY 則に基づく自由地下水についての一考察」中、以下の誤りがありましたので訂正します。

	誤	正
155 p (3) 式	$\epsilon = \frac{\partial y}{\partial t} + \frac{\partial q}{\partial x} = P$	$\epsilon \cdot \frac{\partial y}{\partial t} + \frac{\partial q}{\partial x} = P$
156 p (13) 式	$\frac{k_y}{\mu} \left(\frac{\partial p}{\alpha y} + \rho g \cos \alpha \right) + q_y = 0$	$\frac{k_y}{\mu} \left(\frac{\partial p}{\partial y} + \rho g \cos \alpha \right) + q_y = 0$
156 p (19) 式のうへ	$dh'/dx = \sin \alpha$	$dh'/dx = -\sin \alpha$