

<Original Article>

A COMPARATIVE STUDY OF FREE AMINO ACIDS IN HEALTHY
AND VIRUS DISEASED CHINESE DATE TREE.

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洪淳佑·河永七: Virus에 感染된 데후나무의 罹病葉과 健全葉에 있어서의 游離 amino酸의 定性的 比較.

ABSTRACT

Hong, Soon-Woo and Yung-Chil, Hah (Dept. of Botany, Seoul National University, Seoul, Korea.) A comparative investigation of free amino acids in healthy and virus diseased Chinese date tree. Kor. Jour. Bot. 4(1)9~12 1961: A comparative investigation of free amino acids content in healthy check and virus diseased leaves of Chinese date tree, *Zizyphus jujuba Mill var. inermis Rhed.*, was carried out by authors throughout the growing season of 1959 and 1960 from June to October.

The methods of qualitative analysis of free amino acids applied in this experiment is followed by Moore and Stein.^{1,2} Free amino acids determined in this experiment are shown in Fig. 1 and Table 1. As the figure and the table are shown, three more amino acids such as glutamine, asparagine and histidine are detected in the diseased material. The additional amino acids which are known as diamines in diseased leaves are conspicuous. It is presumed that the diamine might be increased by the self-reproduction of the virus in cooperation with certain enzymes which are carrying out the protein metabolism in the host protoplast in contrast with the healthy checks which is carrying out normal protein metabolism.^{3,4,5} From the histological point of view, the facts of phloem degeneration or necrosis in diseased leaves, it seems to interrupt to move free amino acids from roots to leaves and it possibly takes place an excessive production of NH_3 which is diaminated by the metabolism of nitrogen compounds in such conditioned leaves.^{6,8} Therefore, it is also presumed that additional diamino acids are accumulated in diseased leaves.

There are no change of amino acids in both materials of this plant throughout the growing season qualitatively, and this result agrees with the paper of Knight.⁹

INTRODUCTION

Early papers, Bawden and Pirie,^{3,4} and Grien,⁵ have reported that viruses are produced in the protoplast of host plants with the cooperation of certain enzymes and further-more the process of the enzymatic inducement are also interacted with protein components of host plants.^{10~11}

So it is naturally presumed that plant virus which is physicochemically composed of ribonucleic acid and virus protein has the function to produce different compositions of proteins in host protoplast primarily to originate from the virus infection. These, however, are not presented in normal plants.^{18~22}

Thus, these fields of studies have been studied by many biochemists, more comprehensively and the biochemical results are also being applied to the diagnosis of virus diseases.

Authors have, therefore, attempted to study some amino acid analysis in healthy and diseased leaves of these economic plants to look for biochemical specifics of the virus disease. These studies are followed by the author's previous works^{6,7} on the characteristics of external morphological symptoms and the internal anatomical characteristics of the virus disease which has spread throughout most parts of South Korea since 1958.

MATERIAL AND METHOD

The materials used in this experiment, the healthy and virus infected leaves of Chinese date tree which was planted in Seoul, Korea, have been collected every month during the growing periods of the plants from 1959 to 1960. The collected materials are washed with distilled water and dried in a dry oven at 80°C.

The method of qualitative analysis of amino acids in these two kinds of leaves applied to this experiments was followed by Moore and Stein.^{1,2}

And the materials which are extracted in 80% ethanol for 24 hrs, are centrifugated at 2,500 rpm., and the supernatant of the solutions are collected. Adding a few drops of 10% trichloroacetic acid into these solutions at 80°C, the soluble proteins are precipitated. The solutions are washed 3 times with ether to defat from them and filtered. And then these are concentrated on a water-bath at $80 \pm 3^\circ\text{C}$ until it became like paste. After these, redistilled water is added up to 2 ml of total volume of the sample.

Spotting papers are developed in a jar ($20 \pm 3^\circ\text{C}$) with to dimensions; a solvent for one dimension is water saturated phenol and the other n-buntanol 4, acetic acid 1 and water 5. After drying the developed papers, all amino acids spots which are reacted by spraying 0.25% acetone solution of ninhydrine and 0.2% acidic acetone solution of isatine on the papers. These are identified by comparing Rf values and different color reactions with those of standard amino acids at the same time as samples do. The individual colors which are identified are shown in Table 1.

RESULT AND DISCUSSION

As the free amino acids determined in this experiment are shown in Fig. 1 and Table 1, those are identified 13 sorts of free amino acids and two pigments in healthy check, and three more amino acids which are diamino acids such as glutamine, asparagine and histidine in the diseased material. The additional amino acids which are known as diamines in diseased leaves are conspicuous. It is presumed that the diamine might be increased by the self-reproduction of the virus in cooperation with certain enzymes which are carrying out the protein metabolism in the host protoplast in contrast with that the healthy checks are carrying out normal protein metabolism.^{3,4,10,22}

From the histological point of view, the facts of phloem degeneration or necrosis in diseased leaves, it seems to interrupt to move free amino acids from roots to leaves and it possibly takes place an excessive production of NH_3 which is diaminated by the metabolism of nitrogen compounds in such conditioned leaves.^{6,7,8} Therefore, it is also presumed that additional diamino acids are accumulated in diseased leaves.

Having quantitatively compared the amino acids of healthy check with those of diseased to calculate the densities and the sizes of colored spots on developed papers, it can be said that there are volumetrically a little more free amino acids in healthy check than diseased one except threonine. However, it could not be known why more free amino acids and less threonine are in healthy leaves.

And though we have studied a change of amino acids in both materials of this plant throughout the growing season qualitatively, there was no change at all in both of materials. This result is agreed with the papers of Knight.⁹

摘 要

Virus에 感染된 대추나무의罹病葉과健全葉內에 含有되어 있는 free amino acids를 Paper Chromatography로 檢出하여 본 結果 罹病葉에는健全葉보다 더 많은 種類의 amino acids를 含有하고 있음을 알았다. 그 種類數로는健全葉에서 13種을 檢出 할 수 있는데 罹病葉에서는 3種의 diamine系 즉, glutamine, asparagine 및 histidine이 더 檢出되어健全葉보다 3種이 더 많은 16種의 free amino acids를 檢出 할 수 있는 것이 特異한 現象이다.

이는 virus가 宿主 原形質內에 侵入함으로써 宿主의 蛋白質 및 酵素系를 攪亂하여 元來의 蛋白質 및 free amino acids와는 定性的으로 다른 것을 만들어 낼 수 있다고 推定 할 수 있다.

또한 組織學的으로 볼 때는 罹病葉 篩管部의 一部가 閉塞 또는 破壞되는 경우가 觀察되고 있는데 이 事實로 미루어 봐서 物質의 移動에 影響을 받은 일에 있어서는 含窒素物質의 代謝로 생길 수 있다고 생각되는 過剩 NH_3 가 作用함으로써 diamine系의 amino acids가 罹病葉에 더 많이 만들어져 蓄積되어 있지 않은가 생각된다.

그리고 全 生長期間을 통해서 月別 變化도 아울러 調査해 보았으나 年中 變化는 두 材料 共히 無하였다. 이는 Knight(1947)의 Tobacco mosaic virus 罹病葉의 實驗에서 얻은 것과 一致하였음을 알았다.

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Fig. 1. Paper chromatogram of free amino acids in virus diseased leaves of Chinese date tree.

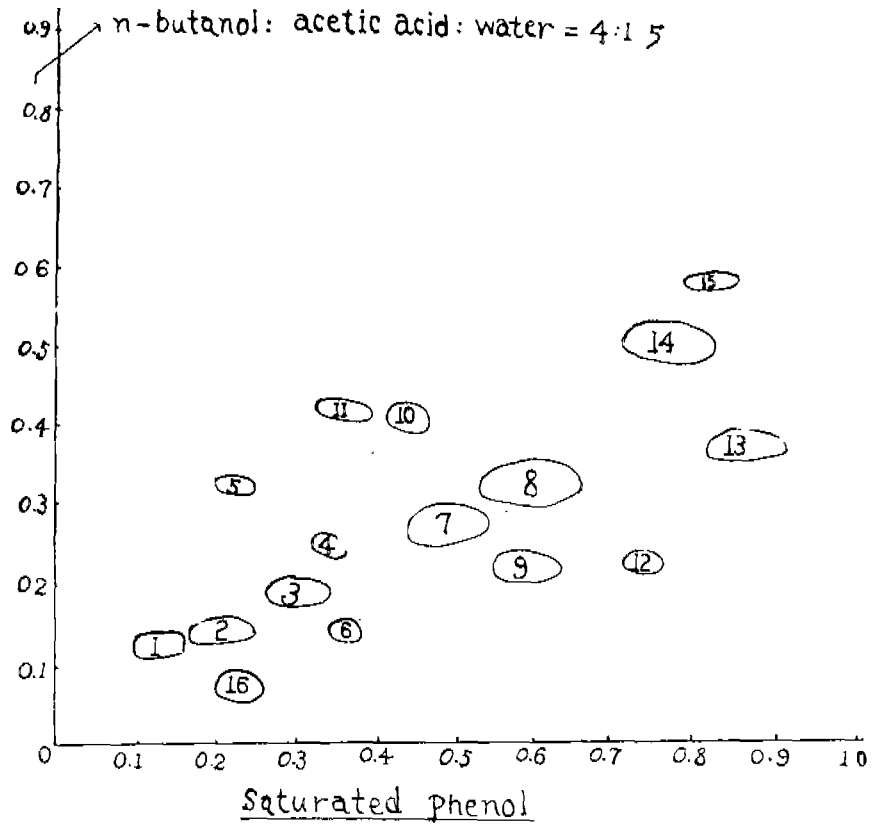


Table 1. Paper chromatogram of free amino acids in healthy checks and diseased leaves of Chinese date tree.

Free amino acids	Healthy check	Diseased check	Color
1. L-aspartic acid	++	+	blue purple
2. L-glutamic acid	++	+	purple
3. DL-serine	+++	+++	purple
4. glycine	+	+	red purple
5. Unknown	+	+	purple
6. L-asparagine		+	brown
7. DL-threonine	+++	++++	red purple
8. alanine	+++	+++	purple
9. glutamine		++	purple
10. pigment (yellow green)	++	++	
11. pigment (yellow green)	++	++	
12. histidine		+	grey purple
13. L-proline	++++	+++	yellow
14. valine	++++	++++	purple
15. leucine	+	+	purple
16. L-cystine	++	++	red purple