

STUDIES ON THE STERILITY MOSAIC DISEASE OF PIGEON PEA

III. Nitrogen Metabolism of Infected Plants

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ABSTRACT

A decrease in the chloroplastic protein and a slight increase in the cytoplasmic protein were seen in the diseased leaves. There was no appreciable quantitative difference in the amino-acid contents of proteins of healthy and diseased plants. The total nitrogen content of diseased leaves showed a progressive increase over healthy during the day starting from the morning till evening. The free amino-acids of diseased leaves showed variations both in quality and quantity. Alanine, asparagine, arginine and aspartic acid were found to be in higher concentrations in diseased leaves, the increase being proportional to the severity of disease symptoms. The presence of two unidentified amino-acids was detected only in diseased leaves.

The amino-acids, alanine, asparagine, aspartic acid and arginine which were in very high concentrations in diseased leaves at 6 a.m. were observed to be either completely absent or present only in very small amounts at 6 p.m. A decrease in the C/N ratio resulted due to virus infection. This reduction could be attributed to the reduction in the carbohydrate content and increase in nitrogen content of diseased leaves.

INTRODUCTION

CHANGES in carbohydrate metabolism of pigeon pea plants infected with sterility mosaic disease were discussed in an earlier communication (Narayanaswamy and Ramakrishnan, 1965). The nitrogen metabolism of pigeon pea plants suffering from the same disease is discussed in this paper.

MATERIALS AND METHODS

Nitrogen.—The total nitrogen content was estimated by Kjeldahl's method and is expressed as percentages on dry weight.

Leaf proteins.—The protein fractions of healthy and diseased leaves were separated by the method of Yemm and Folkes (1953). The amino-acid constituents of the chloroplastic and cytoplasmic proteins thus separated were determined by chromatography. The amino-acids were eluted with a mixture of alcohol and buffer and readings were taken in a Beckman DU Spectrophotometer at the specified wavelengths of light as recommended by Selman *et al.* (1961).

Free and bound amino-acids.—The free amino-acids were extracted by grinding the samples (1 g.) with 80 per cent alcohol in a mortar and centrifuging at 3,000 rpm for 10 minutes. Three volumes of chloroform were added to the supernatant solution and the top layer containing the amino-acids was separated. This solution was evaporated to dryness *in vacuo* and the contents were dissolved in 1 ml. of 80 per cent alcohol. The contents of these solutions were analysed chromatographically.

The total amino-acids were estimated by hydrolysing the samples (1 g.) with 6 N HCl for 20 hours. The acid was evaporated on a water-bath and then the solution was dried *in vacuo*. The amino-acids were dissolved in 1 ml. of 80 per cent alcohol and separated by two-dimensional chromatography using *n*-butanol-acetic acid-water and phenol saturated with buffer solvent systems.

RESULTS

Leaf proteins.—The chloroplastic and cytoplasmic proteins were quantitatively assessed. It may be observed that the protein content of the leaf was modified due to virus infection. The chloroplastic protein content of diseased leaves was about 64 per cent. of the healthy leaves while the cytoplasmic protein content was higher by 16.6 per cent.

The total nitrogen content of healthy and virus infected leaves of pigeon pea was estimated at 4-hour intervals during the day from 6 a.m. to 6 p.m. and the results are presented in Table I. It may be seen that the diseased leaves contained higher percentage of nitrogen at all periods of sampling than the comparable healthy leaves.

The nitrogen content steadily increased from 6 a.m. to 6 p.m. in both healthy and diseased leaves. The increase appeared to be slightly faster in diseased than in healthy leaves.

Amino-acid contents of chloroplastic and cytoplasmic proteins.—In the chloroplastic protein, the contents of amino-acids did not show any quali-

TABLE I

Nitrogen contents of healthy and diseased plants during day

Time of sampling	Percentage of total nitrogen		Percentage of increase in diseased
	Healthy	Diseased	
6 a.m.	3.332	3.948	18.4
10 a.m.	3.584	4.116	14.8
2 p.m.	3.640	4.228	16.1
6 p.m.	3.724	4.536	21.8

tative differences between healthy and diseased plants. However, the chloroplasts in diseased leaf contained considerably lesser quantities of valine and serine (Table II). In the cytoplasmic proteins of healthy and diseased leaves,

TABLE II

Amino-acids of chloroplastic and cytoplasmic proteins of healthy and diseased leaves

Name of amino-acid	Chloroplastic Protein		Percentage of decrease in diseased	Cytoplasmic protein		Percentage of decrease in diseased
	Healthy $\mu\text{g./100 mg.}$	Diseased $\mu\text{g./100 mg.}$		Healthy $\mu\text{g./100 mg.}$	Diseased $\mu\text{g./100 mg.}$	
Cystine ..	71.6	74.3	..	43.0	44.0	..
Arginine ..	3.3	3.3	..	2.0	2.0	..
Asparagine ..	41.6	40.0	..	2.0	2.0	..
Serine ..	6.6	3.3	-50.0	4.0	3.0	..
Glycine ..	63.3	63.3	..	37.0	38.0	..
Aspartic acid ..	61.6	60.0	..	36.0	36.0	..
Glutamic acid ..	51.6	51.6	..	30.0	32.0	..
Proline ..	76.6	75.0
Alanine ..	31.6	30.0	..	17.0	18.0	..
Valine ..	56.6	40.0	-29.3	22.0	18.0	-18.1
Phenylalanine ..	65.0	60.0	-7.7

proline and phenyl alanine were absent. Except for valine which was less by 18·1 per cent in diseased leaves, the rest of the amino-acids were found in more or less similar concentrations in healthy and diseased leaves.

Free amino-acids of diseased leaves.—The free amino-acid content of healthy, mild, moderate and severe type of diseased leaves was studied. The presence of the following amino-acids was detected: cystine, arginine, glycine, aspartic acid, glutamic acid, alanine, histidine, asparagine, phenylalanine, methionine in healthy leaves and two unidentified amino-acids in diseased leaves.

TABLE III
Free amino acids of healthy and diseased leaves

Name of amino-acids	$\mu\text{g./100 mg. of leaf}$				Percentage of increase in severely diseased leaves
	Healthy	Mild	Moderate	Severe	
1. Cystine	44·0	45·0	45·0	46·0	...
2. Arginine	4·0	6·0	12·0	15·0	+300·0
3. Glycine	35·0	36·0	36·0	36·0	...
4. Aspartic acid	28·0	38·0	38·0	39·0	+ 39·3
5. Alanine	18·0	21·0	28·0	30·0	+ 66·6
6. Histidine	41·0	40·0	40·0	40·0	...
7. Asparagine	21·0	42·0	41·0	45·0	+114·3
8. Phenylalanine	37·0	37·0	36·0	36·0	...
9. Methionine	32·0	32·0	33·0	33·0	..
10. Glutamic acid	30·0	30·0	32·0	33·0	..
11. Unidentified	9·84	11·69	..
12. Unidentified	4·01	5·32	...

Virus infection induced considerable changes in quantities of some of the free amino-acids. There was no change in the concentration of cystine, glycine, histidine, phenylalanine, methionine and glutamic acid due to infec

tion. Appreciable increases in the concentration of arginine, aspartic acid, alanine and asparagine were observed in the diseased leaves. Arginine content in the diseased leaves increased proportionally to the increase in the severity of disease. The increases in arginine, aspartic acid, alanine and asparagine content in severely diseased leaves were 300, 39.3, 66.6 and 114.3 per cent respectively. The quantities of two ninhydrin positive substances detected in diseased leaves increased with increase in severity of infection.

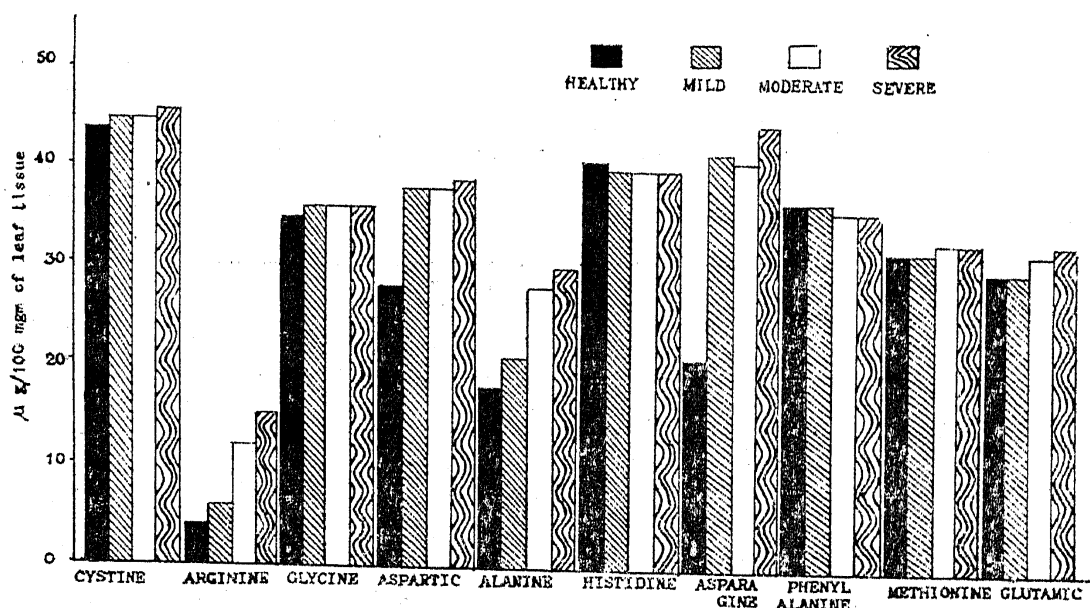


FIG. 1. Free amino-acid contents of healthy, mild, moderate and severe types of diseased leaves.

Total amino-acids.—The following amino-acids were detected in the healthy and diseased leaves by two-dimensional chromatography: cystine, aspartic acid, serine, asparagine, glycine, tyrosine, tryptophane, glutamic acid, alanine, arginine, histidine, methionine, proline, phenylalanine, leucine, isoleucine and one unidentified amino-acid. There was no qualitative difference in the amino-acids of healthy and diseased tissues.

The contents of cystine and histidine did not vary in the healthy and diseased samples. A reduction was noted only in the contents of serine, asparagine and methionine in the diseased sample over that of healthy. Aspartic acid, glycine, tryptophane, glutamic acid, alanine and proline were in slightly higher quantities in diseased leaves. Tyrosine, arginine and phenylalanine, however, were higher by 300, 205 and 21.5 per cent in diseased leaves.

Variation in the free amino-acid contents during the day.—It is known that light has a definite influence on the free amino-acid content of plants

and that protein synthesis is favoured by daylight while its breakdown occurs in the night. The changes in the amino-acid contents of the healthy, mild, moderate and severe type of infected leaves were studied at the beginning of the day (6 a.m.) and the evening (6 p.m.). The results are presented in Table V. It may be observed that virus infection had brought about considerable modifications in the amino-acid pattern.

TABLE IV
Total amino-acids of healthy and diseased pigeon pea

Name of the amino-acid	$\mu\text{g./100 mg. of leaf}$		Percentage of increase (+) or decrease (–) over healthy leaves
	Healthy	Diseased	
1. Cystine	225	225	—
2. Aspartic acid	200	210	+ 5.0
3. Serine	30	20	– 33.3
4. Asparagine	215	205	– 4.6
5. Glycine	190	195	+ 2.6
6. Tyrosine	35	145	+314.2
7. Tryptophane	205	210	+ 2.4
8. Glutamic acid	165	170	+ 3.03
9. Alanine	110	115	+ 4.5
10. Arginine	40	130	+225.0
11. Histidine	205	205	—
12. Unidentified	—
13. Methionine	170	165	– 2.9
14. Phenylalanine	190	250	+ 31.5
15. Proline	230	245	+ 6.5
16. Leucine and Isoleucine	125	280	+124.0

TABLE V

*Free amino-acid content of healthy and diseased leaves during day
in $\mu\text{g.}/100\text{ mg.}$ of tissue*

Name of the amino-acid	Healthy		Mild		Moderate		Severe	
	6 a.m.	6 p.m.	6 a.m.	6 p.m.	6 a.m.	6 p.m.	6 a.m.	6 p.m.
Cystine ..	45.0	—	45.0	—	45.0	—	46.0	—
Arginine ..	4.0	2.0	8.0	—	12.0	—	15.0	—
Asparagine ..	22.0	20.0	45.0	20.0	46.0	18.0	48.0	20.0
Glycine ...	36.0	36.0	38.0	—	38.0	—	38.0	—
Aspartic acid ...	28.0	28.0	40.0	—	40.0	—	41.0	—
Glutamic acid ..	30.0	30.0	31.0	31.0	32.0	31.0	35.0	31.0
Alanine ..	19.0	16.0	21.0	—	30.0	—	31.0	—
Histidine ..	41.0	40.0	41.0	40.0	41.0	40.0	42.0	40.0
Phenylalanine ..	38.0	38.0	38.0	37.0	38.0	37.0	39.0	37.0
Methionine..	32.0	33.0	33.0	31.0	33.0	31.0	33.0	31.0
Unidentified ..	—	—	—	—	+	—	+	—
Unidentified ..	—	—	—	—	+	—	+	—

In the healthy leaf the amino-acid content at 6 p.m. was lower than at 6 a.m. Infection had apparently no significant effect on the content of glutamic acid, phenylalanine, histidine and methionine. In the infected leaf the concentrations of arginine, asparagine, aspartic acid and alanine were high at 6 a.m. These amino-acids also appeared to be utilised more rapidly in the diseased leaf during the day so that all of them except asparagine could not be detected at 6 p.m. and the latter was also reduced considerably. Apparently no change in the behaviour of cystine was brought about by infection.

The two unidentified amino-acids were not present in the healthy and mild type of infected leaves both at 6 a.m. and 6 p.m. Moderate and severe types of infected leaves showed their presence only in the morning. The two unidentified amino-acids were absent at 6 p.m. in moderate and severe type of diseased leaves.

Carbohydrate/nitrogen ratio.—The variations in the carbohydrate/nitrogen ratio of healthy and diseased leaves were studied at 6 a.m., 10 a.m., 2 p.m. and 6 p.m.

TABLE VI

Percentages of total carbohydrates and total nitrogen and C/N ratio

Time of sampling	Total carbohydrate per cent		Total nitrogen per cent		C/N	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
6 a.m.	7.83	7.79	3.332	3.948	2.34	1.97
10 a.m.	12.58	11.45	3.584	4.116	3.51	2.78
2 p.m.	10.49	10.98	3.640	4.228	2.85	2.59
6 p.m.	9.31	8.59	3.724	4.536	2.50	1.87

The ratio was found to be decreased in the diseased plants at all times of sampling. The trend was similar in the healthy and diseased samples, though the nitrogen contents of the healthy and diseased leaves showed a steady increase from morning to evening.

DISCUSSION

There was a tendency for increase in the free amino-acid contents of infected pigeon pea leaves. The increase in the concentration of arginine, alanine, aspartic acid and asparagine was found to be proportional to the severity of the disease in pigeon pea leaves. John (1963) reported that the number and content of free amino-acids increased with increase in severity of mosaic symptoms. Pipecolic acid, proline and arginine have been observed to be present in higher concentration in Western \times diseased cherry leaves (Diener, 1960).

The reasons for the accumulation of amino-acids in the plants have been found to be many. Accelerated carbohydrate breakdown, increased proteolytic activity and low potassium content are some of the reasons. The accumulation of amino-acids in virus diseased plants was considered to be an indication of a check to the growth and normal protein synthesis (Selman *et al.*, 1961). Hayashi (1962) attributed it to the enhanced amino-acid

activating system of diseased plants. The low potassium content, higher proteolytic and diastatic activity may be responsible for the observed increase in the amino-acid contents of infected pigeon pea leaves (Narayanaswamy, 1963).

In the study of diurnal variations in the free amino-acid content of infected plants, it was observed that arginine, alanine, aspartic acid and asparagine were present in very high concentrations at 6 a.m. but they were either totally absent or present in very small concentrations at 6 p.m. John (1963) showed that the amino-acid content of plants exposed to light was reduced. Commoner and Nehari (1953) reported that a transitory deficiency of both amides and amino-acids occurred during the period of rapid virus synthesis. The diurnal variation observed in the free amino-acid content of infected pigeon pea leaves may indicate that protein synthesis is at a faster rate in infected plants resulting in conspicuous deficiency in the nitrogen pool, due to the removal of amino-acids and amides possibly for the synthesis of virus protein.

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