Studies on the Physiology of Chilli (*Capsicum annuum* L.) Plants affected by Potato Virus Y. III. Effect on Nitrogen Metabolism

by

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In the earlier papers (Jeyarajan and Ramakrishnan, 1968, 1971) the effects of infection by potato virus Y on some aspects of the host physiology were presented. The results of further work on N metabolism of chilli (*Capsicum annuum* L.) plants affected by PVY are presented in this paper.

Materials and Methods: The free amino acids were extracted from fresh plant materials and separated by descending two dimensional partition paper chromatography (Block *et al.*, 1955) and quantitatively estimated spectrophotometrically (Selman *et al.*, 1961). The content of total N was estimated by the Kjeldahl's method. Protein N was determined after preliminary extraction of the dried and powdered leaf material in a Soxhlet's apparatus with 75% ethanol. Non-protein N was calculated by difference. Amide N was estimated by the method described by Shewan (1938) and nitrate reductase activity by the method of Eckerson (1931).

Results: 1. *Various fraction of N:* The contents of total N, nitrate N, ammoniacal N, amide N, protein N and non-protein N are presented in Table 1.

TABLE 1. *Forms of nitrogen and activity of nitrate reductase in healthy and PVY infected chilli leaves*

Constituents *	Healthy	Infected	Infected (%) Healthy	C.D. (P=0.05)
Total nitrogen	2.76	3.48	126.0	0.34
Nitrate nitrogen	0.40	0.18	45.0	0.10
Ammoniacal nitrogen	Trace	Trace	—	N.S.
Protein nitrogen	1.62	2.36	145.6	0.38
Non-protein nitrogen	1.14	1.11	97.3	N.S.
Amide nitrogen	0.82	1.28	156.0	0.10
Nitrate reductase activity	1.11	1.32	119.5	0.08

* Nitrate reductase activity expressed as mg of nitrate produced by the enzyme per 100 g of tissue fluid. Nitrogen fractions expressed as per cent of dry matter. N.S. – Not significant.

There was no difference in ammoniacal N and non-protein N contents of healthy and infected leaves. The infected leaves contained 26% more total N

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than healthy leaves. Protein N and amide N increased by 46 and 56% respectively in infected leaves over the healthy ones. But there was 55% reduction in nitrate N content in infected leaves.

2. *Activity of nitrate reductase:* Since nitrate reductase is the first enzyme to act on nitrate during its assimilation in higher plants the activity of this enzyme was estimated in healthy and diseased plants. The results are expressed as mg of nitrate produced by the enzyme from 100 g of tissue fluid (Table 1). It was found that infected leaves showed 19.5% more activity of this enzyme than healthy leaves.

3. *Free amino acids:* Having found a marked increase in the protein content of infected leaves the free amino acids were estimated in healthy and infected plants. The results are presented in Table 2. In general, the leaf, petiole, stem and flower buds from infected plants contained more of free amino acids than the corresponding parts of healthy plants.

TABLE 2. *Free amino acid content of healthy and PVY infected chilli plants (µg per g of fresh material)*

Amino acid or amide	Leaf		Petiole		Stem		Flower bud	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Cystine	—	73.8	—	—	—	—	24.3	33.5
Asparagine	33.8	69.7	17.6	39.8	18.6	20.9	Trace	Trace
Aspartic acid	64.7	107.0	42.9	40.7	62.1	48.7	—	39.3
Glycine	227.6	337.2	—	—	—	—	82.9	70.8
Glutamic acid	Trace	34.45	—	27.9	—	16.6	Trace	47.4
Arginine	—	—	—	—	—	—	29.0	31.0
Tyrosine	—	—	—	33.3	—	19.8	—	37.5
Tryptophan	84.9	82.6	25.7	30.8	—	29.7	—	—
Methionine	56.3	55.1	37.8	—	—	—	—	—
Proline	558.3	312.1	18.2	—	17.9	27.9	47.7	40.9
Hydroxy proline	247.0	447.7	—	—	—	—	—	—
Alanine	65.2	85.4	20.2	—	21.1	—	—	—
Leucine	76.9	110.3	50.7	66.3	55.6	67.6	—	—
Valine	—	64.6	—	—	—	—	—	22.6
Serine	—	—	—	—	—	—	42.8	50.1
Glutamine	53.4	89.4	25.5	44.3	25.7	31.0	Trace	Trace
Total	1468.1	1869.4	238.6	283.1	201.0	262.2	226.7	373.1
Infected/Healthy	127.3%		118.6%		130.4%		164.5%	

4. *Total amino acids:* The total amino acid content was estimated chromatographically after hydrolysing the peptides with 6 N hydrochloric acid for 20 hours. The results are presented in Table 3. The contents of glycine, alanine, leucine, aspartic acid, asparagine, glutamic acid, glutamine, cystine; arginine, tyrosine and hydroxy proline were found to be greater in infected

leaves than in healthy ones. Leucine, isoleucine, glutamic acid, glutamine and tyrosine were found in greater concentrations in infected petiole than in healthy ones. In infected stem the content of glutamic acid, tyrosine, tryptophan and proline increased while that of arginine decreased. In flower buds isoleucine, asparagine and arginine contents decreased and aspartic acid, glutamic acid and tyrosine content increased due to infection.

TABLE 3. *Total amino acid content of healthy and PVY infected chilli plants (μg per g of fresh material)*

Amino acid or amide	Leaf		Petiole		Stem		Flower bud	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Cystine	60.0	126.1	—	—	46.5	52.1	83.4	88.4
Aspartic acid	92.6	124.0	69.9	71.4	62.1	54.0	52.9	73.9
Glycine	233.1	356.9	2.7	3.7	Trace	Trace	86.8	80.7
Glutamic acid	48.1	93.5	24.6	63.8	14.0	39.2	42.9	84.2
Arginine	29.6	71.2	22.7	23.7	37.5	27.6	95.7	69.5
Tyrosine	27.3	42.5	18.8	55.7	29.7	48.7	49.0	62.0
Tryptophan	116.2	117.8	25.7	30.8	9.7	35.5	—	—
Methionine	114.5	121.0	37.8	3.9	28.9	30.0	17.8	15.2
Phenylalanine	—	—	18.5	11.6	—	—	49.3	43.2
Proline	583.1	330.0	18.2	—	17.9	27.9	63.6	51.0
Hydroxy proline	247.0	447.7	—	—	—	—	—	—
Alanine	65.2	85.4	20.2	—	21.1	—	—	—
Leucine	76.9	110.3	50.7	66.3	55.6	67.6	—	—
Isoleucine	34.9	33.9	12.6	19.3	17.2	20.9	35.1	19.2
Valine	—	64.6	—	—	—	—	—	22.6
Serine	—	—	—	—	—	—	42.8	50.1
Glutamine	53.4	89.4	25.5	44.8	25.7	31.0	Trace	Trace
Asparagine	90.6	138.6	47.7	59.3	63.9	65.0	32.8	21.9
Total	1872.5	2352.9	395.6	459.8	429.8	499.5	652.1	681.9
Infected/Healthy	125.6%		114.7%		115.2%		104.5%	

Discussion: The increase in the total N content noted in the present study corroborates the reports made on mosaic viruses. It is known that most of the N in leaf occurs in the form of protein. In the present study the increase in total N was found to be due to increase in protein N. As multiplication of the virus in plant tissues involves the synthesis of virus proteins, disturbances in the N metabolism of infected leaves can naturally be expected. The increased amounts of free amino acids, amides and total N in infected leaves seem to have led to an increased rate of protein synthesis through condensation of amino acids. Obviously the enhanced rate of respiration reported earlier (Jeyarajan and Ramakrishnan, 1968) provided the increased energy requirements for this higher rate of protein synthesis.

It is possible that the protein for the synthesis of virus particles is derived in two ways namely a diversion of the normal plant proteins themselves through the activity of enzymes and also by the increased activity of nitrate reductase which ultimately leads to the production of greater amounts of amides in infected than in healthy plants. That the nitrogen for TMV synthesis is actually derived from normal plant protein has been shown by Takahashi (1941).

Increases in the amide N content and activity of nitrate reductase (Table 1) were found in infected leaves. It has been established that nitrate is assimilated via ammonia which is directly incorporated into organic nitrogenous substances via glutamine in higher plants. The increased activity of nitrate reductase in diseased leaves indicates the formation of greater amounts of nitrite. Since nitrite is not tolerated beyond a certain limit by the plant it must be converted into ammonia and subsequently amides. The presence of an enzyme capable of reducing nitrite in plants has been demonstrated by Sanderson and Cocking (1964). Hence it can be safely concluded that by the increased activity of nitrate reductase, greater amounts of amides were ultimately synthesised in infected than in healthy plants as evidenced by the increase in the amide N content and the amides asparagine and glutamine.

The mechanism which brings about the increase in amino acids in virus-infected plants is not fully understood at present. Amino acids occupy an important position in plant metabolism both in the free form and as constituents of proteins. It was shown by Meneghini and Delwiche (1951) that virus is formed from one or more N compounds such as amino acids which undergo more rapid exchange of N than does the extractable protein of the cell. The amides asparagine and glutamine are implicated in the biosynthesis of purine, pyrimidine, nucleosides and nucleotides. Further asparagine is a more favourable material for protein synthesis than amino acids. Hence the increased content of these amides in infected plants play a vital role in nucleic acid metabolism and biosynthesis of virus itself. The increase in total amino acids (Table 3) may be due to the higher protein content of infected leaves or hydrolysis of virus protein itself.

By applying leucines to the root zone of healthy plants Steinberg (1952) simulated 'frenching' in tobacco and Woltz and Jackson (1960, 1961) produced yellow strap leaf of *Chrysanthemum morifolium* Ram. both of which are physiological disorders producing symptoms akin to those produced by the present virus on chilli. Woltz and Jackson (1961) found in chrysanthemum leaves naturally affected by yellow strap leaf, 100% greater free amino acid content than the normal leaves. When D- and L-leucine and DL- alloisoleucine were applied to the root zone of *Capsicum frutescens*, they found green netting of veins to be produced in leaves. Further work on these lines will throw more light on the relationship between amino acid changes and symptom expression in virus infections.

Summary: In chilli (*Capsicum annuum* L.) leaves infected by PVY, the content of total N, protein N and amide N increased and nitrate N decreased over that in healthy leaves whereas that of ammoniacal N and non-protein N was not affected. The activity of the enzyme nitrate reductase increased in diseased leaves. There were marked changes in the amino acid content of diseased leaves and the significance of these biochemical changes is discussed.

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