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Effect of Cassava Mosaic Virus on the Nitrogen Metabolism of Cassava (*Manihot esculenta* crantz.)

By

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ABSTRACT

Studies on the pathophysiology of the CsMV–infected cassava leaves with particular reference to nitrogen metabolism was taken up. The results showed a reduction in the total nitrogen content in the mosaic infected leaves at all sampling periods when compared to healthy and diseased leaves. Maximum total nitrogen was noted in samples collected in the early morning than in the samples taken subsequently. These observed diurnal fluctuations on the synthesis of various nitrogen fractions and the pattern of various nitrogen fraction contents were found to be more or less same in both healthy and diseased leaves, but the disease exaggerated the conditions either by increasing or decreasing the content. The C:N ratio also varied according to the time of sampling and most of the times the ratio was less in diseased samples than the healthy ones. Diseased leaves contained higher quantity of bound amino acids and the maximum level reached 15 days after grafting. Asparagine was found to accumulate more in the diseased leaves when compared to the healthy leaves.

INTRODUCTION

The physiology of cassava mosaic infected-plants in relation to the mineral and carbohydrate metabolism was reported by Alagianagalingam and Ramakrishnan, 1969, 1970). It has been proved by a number of research workers that virus infection has a profound influence on the nitrogen metabolism of the host plant (Diener, 1960; Harpaz and Applebaum, 1961; Bozarth and Diener, 1963; Sehgal and Boone, 1964: and Welkie *et al.*, 1969). In cassava a very preliminary work of Beck and Chant (1958) on the physiology of CsMV-infected cassava indicated that total nitrogen in the diseased cassava was low when compared to healthy ones. Hence detailed experiments on the nitrogen metabolism of CsMV - infected cassava were carried out and the results are presented in this paper.

MATERIALS AND METHODS

Estimation of various nitrogen fractions viz. total nitrogen, protein nitrogen, non-protein nitrogen, nitrate nitrogen and ammoniacal nitrogen were made in samples collected at 6 a.m. 10 a.m. 2 p.m. and 6 p.m. from 3-, 6-, and 9-month old crop. Cassava (variety T. 108) plants affected under field

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conditions were used for the experiment and the leaves of same age and position were selected for analytical purposes. Total nitrogen was estimated by the method of Jackson (1962), nitrate nitrogen by the method of Humphries (1956), protein, non-protein nitrogen and ammoniacal nitrogen by the method of Pregl (1945).

Bound amino acids were estimated in samples of 5, 10, 15, 20 and 25 days after grafting. One month old settlings were taken up for grafting purposes. The leaf samples were acid hydrolysed with 6N HCl for 20 hours by refluxing. The HCl present in the hydrolysate was evaporated on a water bath by frequent addition of distilled water. The residue containing amino acids was dissolved in 80 per cent ethanol. The amino acid estimation was carried out by following the methods of Block *et al.* (1955).

RESULTS AND DISCUSSION

i) Total Nitrogen :

In general, diseased leaves recorded lower values of total nitrogen at all sampling time and stages of the crop. Maximum and minimum quantity of total nitrogen was recorded in 6 a.m. and 6 p.m. samples of both healthy and diseased leaves respectively (Table 1).

i) Protein nitrogen :

Generally healthy samples recorded significantly higher protein nitrogen than the diseased leaves. The age of the crop had little effect over the protein nitrogen content of the crop. In both healthy and diseased plants peak values of protein nitrogen were recorded at 6 a.m. except in case of diseased samples of 9-month old crop. The protein nitrogen in 3-month old crop was higher always except in 6 a.m. samples, while in the other two stages the content was lower when compared to healthy samples (Table 1).

iii) Non-protein nitrogen :

In both healthy and diseased plants the non-protein nitrogen was influenced very much by the age of the crop and time of sampling. The minimum content of non-protein nitrogen was recorded in 3-month old crop which rose to a maximum quantity in 6-month old crop and then declined little in 9-month old crop. In the healthy leaves significantly higher values of non-protein nitrogen were noted in 6 a.m. samples of all ages and tended to reduce with advancement of the sampling time, while in diseased samples except in 10 a.m. samples of 9-month old crop, the content was found to be lower than in the healthy leaves (Table 1).

iv) Ammoniacal nitrogen :

Ammoniacal nitrogen significantly increased with the advancement of age. Maximum quantity of ammoniacal nitrogen was registered in 6 a.m. samples of 6. and 9-month old crop which generally decreased to a maximum level at 6 p.m. (Table 1). Generally, the diseased leaves always had lower ammoniacal nitrogen when compared to healthy samples.

TABLE 1. Different forms of nitrogen in leaves of healthy and mosaic infected cassava plants (Nitrogen as % on dry wt. basis)

Kinds of nitrogen	Age of Crop in months	6 a.m. sample		+ Per cent increase − or decrease over healthy	10 a.m. sample		+ Per cent increase − or decrease over healthy
		Healthy	Diseased		Healthy	Diseased	
Total	3	4.605	3.595	−21.94	3.581	3.442	−3.89
nitrogen	6	5.093	4.839	−2.11	4.644	4.362	−6.08
	9	5.242	4.793	−8.57	4.718	3.520	−25.40
Protein	3	4.215	3.333	−20.93	3.093	3 139	1.48
nitrogen	6	4.157	4.600	10.65	3.925	3.675	−6.37
	9	4.168	4.045	−3.65	3.980	2.577	−35.36
Non-prntein	3	0.210	0.136	−35.24	0.240	0.156	−35.00
nitrogen	6	0.419	0.366	−12.65	0.394	0.375	−4.83
	9	0.359	0.269	−25.07	0.254	0.629	147.63
Ammoniacal	3	0.180	0.120	−33.34	0.241	0.150	−37.76
nitrogen	6	0.494	0.360	−27.13	0.422	0.367	−13.04
	9	0.685	0.474	−30.81	0.479	0 314	−34.45
Nitrate	3	0.150	0.123	−18.00	0.699	0.150	−78.55
nitrogen	6	0.600	0.250	−58.34	0.440	0.251	−42.96
	9	0.829	0.749	−9.66	0.699	0.674	−3.58

v) Nitrate nitrogen

Maximum quantity of nitrate nitrogen was observed in 9-month old crop and minimum in 3-month old crop. Samples collected in the forenoon had higher nitrate nitrogen than in both healthy and diseased leaves were

Table 1 (Continued)

Kinds of nitrogen	Age of crop in months	2 p.m. sample		+ Per cent increase or decrease — over healthy	6 p.m. sample		Per cent increase + or decrease — over healthy
		Healthy	Diseased		Healthy	Diseased	
Total	3	3.498	3.530	0.91	3.516	3.445	—2.02
nitrogen	6	4.688	4.605	—1.78	4.477	4.357	- 2.69
	9	4.194	4.093	—2.41	4.494	3.145	—30.02
Protein	3	3.180	3.245	2.04	2.726	3.120	14.45
nitrogen	6	4.075	4.036	—0.96	3.906	3.919	0.33
	9	3 471	4.390	26.47	4.001	2.622	—34.47
Non protein	3	0.195	0.150	—23.08	0.183	0.125	—31.70
nitrogen	6	0.289	0.283	- 2.08	0.255	0.209	—18.04
	9	0 239	0.224	—6.28	0.209	0.254	21.53
Ammonical	3	0.120	0.135	12.50	0.195	0.150	—23.08
nitrogen	6	0 364	0.263	—27.75	0.362	0.232	- 35.91
	9	0.574	0.479	—16.56	0.299	0.239	—30.07
Nitrate	3	0.650	0.573	—11.85	0.449	0.150	—66.60
nitrogen	6	0.159	0.160	0 62	0.159	0.157	—1.26
	9	0.674	0.150	—77.75	0.673	0.648	- 3.72

recorded in 6 a.m. samples of 6-and 9-month old crop (Table 1). Irrespective of the time of sampling, the diseased leaves recorded lower nitrate nitrogen when compared to healthy leaves.

Carbohydrate: Nitrogen ratio (C:N ratio):

The C:N ratio was found to be low in diseased samples at 6 a.m. at all stages of the crop when compared to

TABLE 2. Carbohydrate/Nitrogen ratio in healthy and diseased cassava leaves of 3-, 6-, and 9- month old plants.

Time of sampling	Age of the crop in months	Total Carbohydrates (% on dry wt. basis)		Total nitrogen (% on dry wt basis)		Carbohydrate/ Nitrogen ratio		Per cent increase or decrease over healthy
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	
6 A.M.	3	10.338	5.867	4.605	3.595	2.25	1.64	−27.12
	6	14.741	11.452	5.093	4.839	3.01	2.37	−21.27
	9	13.238	8.138	5.242	4.793	2.51	1.70	−32.26
10 A.M.	3	18.103	14.841	3.581	3.442	5.10	4.31	−16.50
	6	9.544	9.185	4.644	4.362	2.05	2.12	3.41
	9	16.995	13.910	4.718	3.520	3.59	3.94	9.74
2 P.M.	3	15.923	18.906	3.495	3.530	4.56	5.36	17.51
	6	7.970	8.805	4.688	4.605	1.63	1.91	17.17
	9	13.845	11.578	4.194	4.093	3.30	2.95	−10.31
6 P.M.	3	14.880	16.009	3.516	3.445	4.26	4.64	−8.92
	6	11.144	9.927	4.477	4.357	2.54	2.27	−10.63
	9	13.142	8.026	4.494	3.145	2.84	2.50	−11.98

the corresponding healthy leaves. But on the other hand the ratio was found to be higher especially in 3-and 6-month old crop between 10 a.m. and 2 p.m. (Table 2).

Bound Amino Acids :

The results of the bound amino acids obtained from graft-inoculated and the healthy counter part are presented in the Table 3. The results indicated that the diseased plants contained higher quantities of amino acids ght from 5th day after grafting. Both in healthy and diseased plants a peak was noted on the 15th day, however, in diseased plants the magnitude of difference was greater. The diseased

TABLE 3. Bound amino acid contents of graft - inoculated and healthy cassava (Expressed in mg/g of fresh tissue)

Aminoacids	Days after grafting									
	5		10		15		20		25	
	H	D	H	D	H	D	H	D	H	D
1*	110	120	...	520	180	270	...	290	120	440
2	10	30	...	20	10	36	10	20	14	120
3	...	...	...	...	...	40	...	...	...	600
4	...	...	...	...	...	38	...	100	...	110
5	...	1850	950	440	1960	1670	380	1670	380	750
6	...	950	...	1040	...	1920	...	...	...	...
7	...	1220	160	...	330	...	...	1640	...	520
8	50	...	...	90	70	50	96	...	40	74
9	400	...	350	1180	...	1140	560	...	350	1560
10	352	252	285	240	500	700	310	72	386	300
11	1974	...	1000	1000	360	530	830	540	100	880
2	12	980	170	200	140	110	250	220	190	100
3	42	1400	10	470	...	280	170	430	700	570
4	...	...	...	...	...	1400	...	1740	...	...
15	2950	6802	2925	5235	3550	8184	2606	6722	2280	6024

* Aminoacids : 1. Glycine 2. Alanine 3. Isoleucine 4. Leucine 5. Valine 6. Serine 7. Aspartic acid 8. Glutamic acid 9 Asparagine 10. Cystine 11. Methionine 12. Tyrosine 13. Tryptophan 14. Proline 15. Total

leaves contained qualitatively more number of amino acids. The healthy leaves had the following eight amino acids: glycine, alanine, valine, aspartic acid, glutamic acid, cystine, methionine and tyrosine, while in diseased leaves

in addition to these, five more amino acids viz. isoleucine, leucine, serine, aspargine, tryptophan, and proline were present. Amino acids like valine serine, asparagine and proline were found to be in remarakably higher quantities in diseased leaves when compared to other amino acids besides glycine, cystine and methionine.

Cassava mosaic-infected cassava leaves contained lower total nitrogen at all three stages of the crop and at all the four sampling times. This is in close agreement with the earlier findings of Beck and Chant (1958) in CsMV-infected cassava; Rhiakhovsky and Fedulaev (1941) in cereals affected by wheat mosaic and Wann and Blood (1933) in curly top virus infected tomato. The higher quantities of protein nitrogen in younger crop and on the other hand, lower quantities of non-protein nitrogen, ammoniacal nitrogen and nitrate nitrogen are of usual occurrence in virus infected plants. Commoner and Nehari (1953) observed that during the synthesis of TMV, non-protein nitrogen was withdrawn from the host's pool and this may explain the significant reduction in non-protein nitrogen in CsMV infected cassava leaves also. Commoner and Dietz (1952) proposed a hypothesis that the deficiency of ammonia indicated that the synthesis of bulk of TMV protein must occur *de novo* from ammonia and nitrogen free carbon sources rather than by condensation of intact amino acid and amide residue. In the present study the lower ammoniacal nitrogen in CsMV-infected cassava might be due to the utilization of ammoniacal nitrogen for virus synthesis.

The C:N ratio was found to be low in 6 a.m samples collected at all stages of the crop. But the ratio tended to increase when the day advanced and especially with 3-month and 6-month old crop. The observation of Beck and Chant (1958) on the C:N ratio in cassava collected at a particular sampling time was higher and they explained it due to rapid break down of organic nitrogen compounds. The present study indicated the variation in C:N ratio at different times might be due to the diurnal fluctuation in the carbohydrate and nitrogen metabolism.

In the present study the amino acid content was found to be more in the diseased leaves when compared to healthy leaves. This is in close agreement with the findings of Thankappan and Chako (1970) in CsMV-infected cassava. Maximum quantity of amino acids were found both in healthy and diseased leaves on 15th day after grafting. The content of glycine, asparagine, serine, cystine, valine, methionine, tryptophan and proline was found to be more in the diseased leaves. There are reports to show that one or more amino acids were

found in increased quantity in virus-infected plants. Roberts and Ramasarma (1952) observed the accumulation of serine, threonine and proline in plants infected by turnip yellow mosaic. Similarly an increased concentration of glutamic acid, glutamine, serine, asparagine, amino butyric acid and proline were found in PVY-and PVX-infected tobacco (Bozarth and Diener, 1963). These reports corroborate the present findings. It is interesting to note that asparagine was found to be more in CsMV-infected cassava in 10th, 15th and 25th day after grafting. It is well known from literature that asparagine and glutamine participate in the biosynthesis of purine, pyrimidine and nucleotides which are essentially required for virus synthesis (Webster, 1959). Asparagine accumulation has been correlated with the development of disease in case of PSMv-infected pigeon pea (Narayanasamy, 1963) and multiplier disease affected strawberry (Sehgal and Boone, 1964). Hence the significance of the presence of higher asparagine content in the mosaic-infected cassava is well understood from the observations of the earlier workers.

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