STUDIES ON STERILITY MOSAIC DISEASE OF PIGEON PEA

II. Carbohydrate Metabolism of Infected Plants

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VIRUS infection causes considerable alteration in the carbohydrate metabolism of infected plants. A reduction in the chlorophyll content has been observed to reflect on the synthesis of carbohydrates by diseased plants. The adverse effect of virus infection on the carbohydrates metabolism has been reported by Dunlap (1930), Bolas and Bewley (1930), Cordingley *et al.* (1930) and John (1963). No information is however available on the carbohydrate metabolism of pigeon pea (*Cajanus cajan*) plants affected by the pigeon pea sterility mosaic virus. As part of the detailed study of this important disease, the present investigations were taken up and the results are reported here.

MATERIALS AND METHODS

Total chlorophyll.—The chlorophyll contents of healthy and diseased leaves were estimated by comparing with an artificial standard solution (Guthrie, 1928). The chlorophylls were extracted from healthy and diseased samples according to the official methods of Association of Official Agricultural Chemists. The intensity of the colour was read in a Klett-Summerson photoelectric colorimeter. Carotene and xanthophyll were analysed according to the method of Snell and Snell (1937).

Estimation of carbohydrates.—The various sugar constituents of dried samples of leaves collected at 6 A.M., 10 A.M., 2 P.M., and 6 P.M. were estimated by the method of Van der Plank (1936).

Chromatographic detection of carbohydrate.—The soluble sugars were extracted from various tissues using hot alcohol (80 per cent.). Known quantities were spotted on Whatman No. 1 filter-paper which was irrigated with *n*-butanol-acetic acid-water (4:1:5) solvent system for 18 hours

Benzidine in acetic acid was used to develop colour at 90° C. The area of the spots was measured and the quantity was expressed in sq. cm.

RESULTS

The total chlorophyll contents of healthy and diseased leaves of pigeon pea are given in Table I. The healthy leaves contained from 1·18 mg. to 1·46 mg. of chlorophyll per g. of tissue. The chlorophyll content of severely infected leaves was from 0·48 mg. to 0·63 mg. of chlorophyll per g. of leaf, the average being 0·535 mg. per g. of leaf. This indicates that the diseased leaves had only 39·1 per cent. of chlorophyll present in the healthy leaves.

TABLE I

Chlorophyll, carotene and xanthophyll content of healthy and diseased leaves

Name of the pigment	Healthy	Diseased
Chlorophyll	1·346 mg./g. of leaf	0·535 mg./g. of leaf
Carotene	0·1858 mg./g. of leaf	0·1635 mg./g. of leaf
Xanthophyll	100 parts	81·9 parts

Carotene and xanthophyll.—The content of carotene and xanthophyll from the healthy and diseased leaves was colorimetrically estimated. Carotene contents were determined by reference to a standard graph. Only a comparative estimation was possible in the case of xanthophyll and not determination of absolute quantities.

The difference between the carotene contents of healthy and diseased leaves was not considerable but still the difference was consistent. The healthy leaves on an average had 0·1858 mg. of carotene/g. of leaf whereas the diseased leaves had only 0·1635 mg. of carotene. In all the sets of samples analysed, the diseased leaves consistently showed a reduction in their carotene contents.

Taking the xanthophyll contents of healthy leaves as 100 parts, the relative concentration of xanthophyll from diseased leaves was assessed. This pigment was also found to be less in the diseased leaves. In general, virus infection caused a considerable amount of reduction in the content of pigments. The greatest amount of reduction was noticed in the chlorophyll followed by xanthophyll and carotene.

Chlorophyllase activity.—The dried solids after the removal of chlorophyll were made use of in determining the chlorophyllase activity (Willstatter and Stoll, 1928). The residue (0·5 g.) remaining after the extraction of plastid pigments, was added to the flasks containing chlorophyll solution in acetone and incubated for 24 hours followed by filtration through Buchner funnel. The solids remaining on the funnel were washed with diethyl ether and the ether-acetone extracts were shaken vigorously in the separating funnel. The pigments were transferred to the ether layer. The altered chlorophyll was extracted with 0·02 N KOH containing a few ml. of methyl alcohol and the solution was made up to a known volume. The intensity of the colour of the solution was measured with a Klett-Summerson photoelectric colorimeter. The amount of chlorophyll destroyed was assessed colorimetrically. The enzymatic activity of healthy, mild, moderate and severe type of infected leaves were determined.

TABLE II

Chlorophyllase activity of healthy and diseased leaves

No.	Healthy	Mild	Moderate	Severe
1	100	143·7	130·1	123·5
2	100	156·8	143·9	139·5

As a result of virus infection a sudden increase in chlorophyllase activity in leaves showing mild symptoms was noticed. The activity of the enzyme was maximum at this stage. Then the activity decreased with increase in severity of infection. But the activity was always higher in the diseased leaves than in the healthy leaves. It seems that virus infection induces an acceleration of chlorophyllase activity resulting in destruction of chlorophyll, but as the disease progresses the activity slows down presumably because of reduction in the substrate available.

Carbohydrate constituents.—The carbohydartes analysed were invert sugar, reducing sugar, sucrose, non-fermentable substances and starch. The results are presented in Table III. It may be observed that the total carbohydrate content of the diseased leaf was less than that of the healthy leaf.

It was however observed that the sucrose content of infected leaves collected at 6 A.M. was slightly higher than that of the comparable healthy sample.

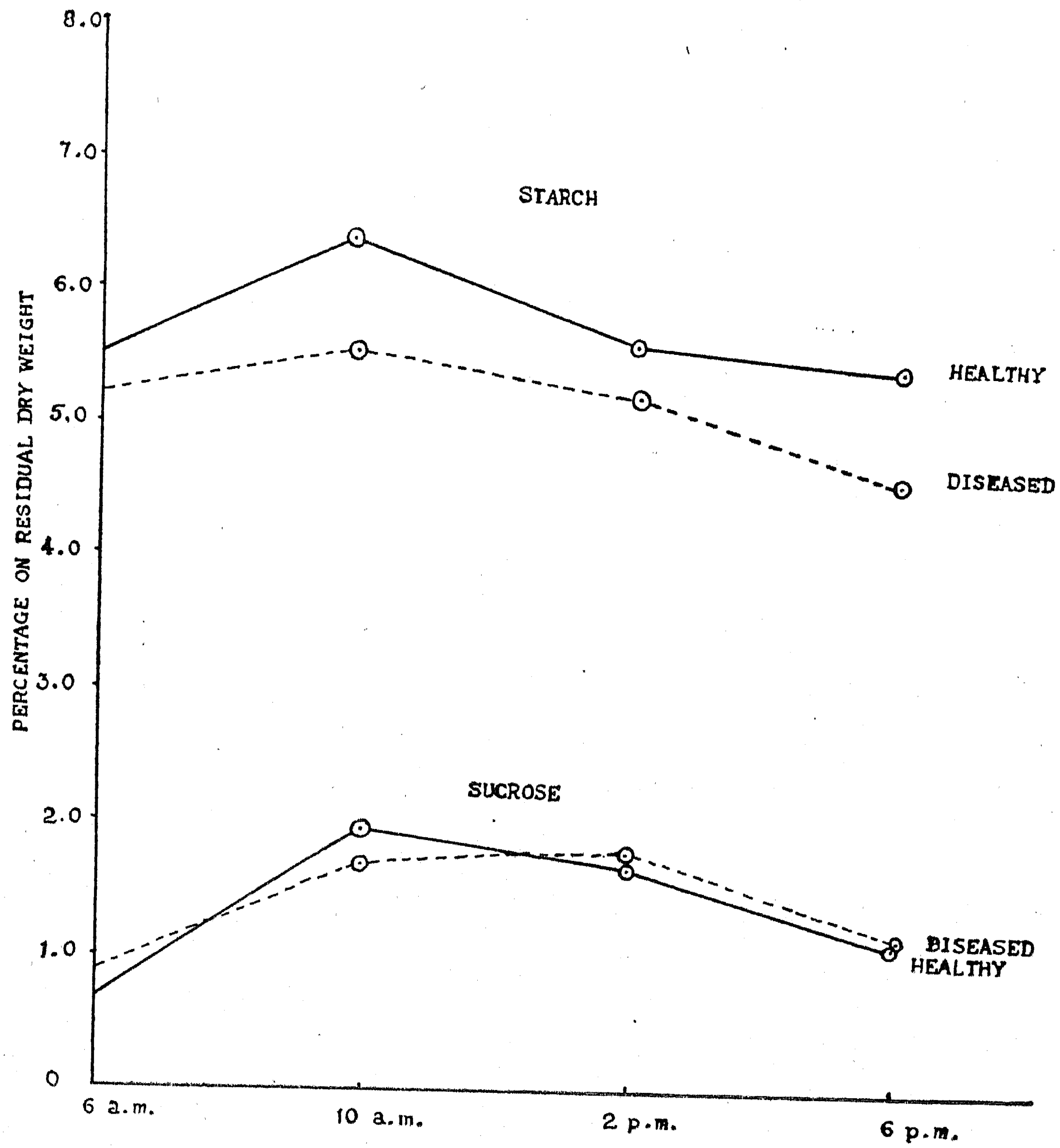

FIG. 1. Sucrose and starch contents of healthy and diseased leaves at 6 a.m., 10 a.m., 2 p.m. and 6 p.m.

Though the diseased leaves had higher sucrose content at 6 A.M. the healthy leaves synthesized sucrose at a more rapid rate showing a difference of 14·6 per cent. at 10 A.M. over that of diseased leaves. The peak accumulation of sucrose was reached much earlier in the healthy leaves than the infected leaves. The sucrose formed between 6 and 10 A.M. in the

TABLE III

The carbohydrate contents of healthy and diseased leaves expressed as percentages on residual dry weight basis

Time of sampling	Invert sugar		Reducing sugar		Non-fermentable substances		Sucrose		Starch		Total carbohydrates	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
6 A.M.	1·170	1·574	0·4827	0·5656	Trace	0·5725	0·587	0·908	5·49	5·22	7·73	7·79
10 A.M.	3·267	3·228	1·2530	1·4710	0·3734	0·6169	2·014	1·757	6·42	5·61	12·58	11·45
2 P.M.	2·476	2·859	0·6861	0·8644	0·1697	0·2723	1·790	1·995	5·71	5·54	10·49	10·98
6 P.M.	1·855	1·814	0·5231	0·6434	Trace	Trace	1·332	1·371	5·60	4·76	9·31	8·59

healthy leaves was 1·4 per cent. while the infected leaves produced only 0·8 per cent. sucrose. This reduction in the ability to synthesize appears to be indicative of the derangement of the photosynthetic ability of infected plants.

There were indications to show that the translocation of photosynthates from the leaf to the other parts of the plant is affected by virus infection. It was observed that in healthy plants 37·7 per cent. of the total carbohydrate formed was translocated while only 31·9 per cent. of the total carbohydrates was transported to other plant parts in diseased pigeon pea. If the individual sugars are examined, similar derangement in the translocation can be seen in the case of sucrose and starch. In the healthy plants 70·8 per cent. of sucrose formed was observed to be translocated whereas only 59·4 per cent. of sucrose was transported from the leaves in diseased plants. In the case of starch, the percentages of starch translocated in healthy and diseased plants were 14·4 and 6·9 respectively.

The formation and utilisation of starch in the healthy and diseased plants followed almost a similar trend as in the case of sucrose. The only difference noticed was that the healthy leaves showed a higher concentration of starch than the diseased leaves at 6 A.M. The trends of formation of invert sugars are similar in the healthy and diseased leaves with variations in the quantities. A remarkably high percentage of non-fermentable reducing substances was observed in the infected leaves, at 6 A.M., 10 A.M. and 2 P.M. while they were not detectable at 6 P.M. in both healthy and diseased leaves.

The total reducing sugars were found to be more in quantity in the diseased leaves. But a large proportion of the reducing substances were found to be non-fermentable substances. The translocation of reducing sugars was observed to be faster in the diseased leaves than in the healthy leaves. An increase of 17·5 per cent. in the translocation of reducing sugars was noticed in diseased plants (Table III). It is possible that the nature of the sugar translocated is different in the diseased plants.

The pattern of distribution of carbohydrates in leaves, petioles, stems and buds of healthy and diseased plants was traced by chromatography (Table IV).

TABLE IV

Carbohydrates of leaf, petiole, stem and bud of healthy and diseased plants

No.	Plant tissue analysed	Area of spot in sq. cm.		
		Sucrose	Glucose	Fructose
I.	Leaf:			
	(*a*) Healthy	4·27	5·06	1·68
	(*b*) Diseased	3·45	4·48	0·95
	Percentage of increase or decrease in diseased ..	−19·2	−11·4	−43·4
II.	Petiole:			
	(*a*) Healthy	2·43	3·16	..
	(*b*) Diseased	2·46	3·11	..
	Percentage of increase or decrease in diseased ..	+ 1·2	−1·5	..
III.	Stem:			
	(*a*) Healthy	2·44	2·04	..
	(*b*) Diseased	1·97	2·79	..
	Percentage of increase or decrease in diseased ..	−19·3	+36·7	..
IV.	Bud:			
	(*a*) Healthy	1·60	1·34	..
	(*b*) Diseased	1·34	1·48	..
	Percentage of increase or decrease in diseased ..	−16·25	+10·4	..

The quantities are expressed as sq. cm. of the areas of the spots occupied by each sugar in the chromatogram. Three kinds of sugars, namely sucrose, glucose and fructose, were detectable in the various parts analysed. Fructose was absent in all parts except the leaves in healthy and diseased plants. All the three sugars were found to be in lower concentration in the diseased leaves. This reduction in the carbohydrate contents has already been revealed by the chemical estimation of carbohydrates. The conclusion that the photosynthesis of infected plants is affected by virus infection resulting in the reduction of carbohydrate content is further supported by the results of this estimation by chromatography.

The petioles of the healthy and diseased plants had sucrose and glucose. There was no appreciable difference in the contents of the sugars present in the healthy and virus diseased petiole samples. The presence of sucrose and glucose was detectable in the healthy and diseased stems. There was a decrease in the sucrose content of the diseased stem while an increase in the quantity of glucose was noticeable. This increase in the glucose content of the diseased stem becomes important while considering the nature of the sugar that is translocated. The detection of high glucose content in the diseased stem substantiates the observation already made that the nature of the sugar translocated may be altered due to virus infection. It was observed that there was no considerable difference in the carbohydrate contents of healthy and diseased buds.

DISCUSSION

A consideration of the effect of virus infection on the chlorophyll is important in the discussion of carbohydrate metabolism of virus infected plant. A reduction up to 60 per cent. in the chlorophyll content of infected pigeon pea leaves was noticed. Decrease of chlorophyll contents of tobacco infected by TMV (Peterson, 1931) *Abutilon* infected by chlorosis virus (Euler *et al.*, 1930) and *Dolichos lab lab* infected by *Dolichos* enation mosaic virus (John, 1963) has been reported.

The lower chlorophyll contents of the diseased plants may be due either to a stimulation of normal cell enzymes that affect chlorophyll or utilization of plastid protein for the synthesis of virus protein (Bawden, 1956). The reduction in chlorophyll content may be due to enhanced activity of chlorophyllase as observed in pigeon pea leaves affected by virus. Peterson and Mckinney (1938) demonstrated that enhanced activity of chlorophyllase in tobacco was induced by yellow mosaic, mild dark green mosaic and mild mosaic viruses but not by common TMV. The low chlorophyll content of virus-infected pigeon pea leaves may also indicate the diversion of chlorophyll protein as suggested by Woods and Dubuy (1941) and Bald (1942).

The low cholorophyll content of infected pigeon pea leaves evidently leads to poor photosynthesis and subsequently to lower carbohydrate synthesis. Mosaic type of virus diseases have been reported to cause a reduction in the carbohydrate contents (Dunlap, 1930). Such a reduction in the carbohydrate content has been observed in spinach blight disease (True and Hawkings, 1928) in aucuba mosaic infected tomato (Bolas and Bewley, 1930) in TMV infected tobacco (Cordingley *et al.*, 1934) and Dolichos enation mosaic infected *Dolichos lab lab* (John, 1963).

The initial higher sucrose content in the morning in diseased tissues indicates a possible block in the translocation of the products of photosynthesis. An inhibition in translocation of sugars has been reported by Holmes (1931) in TMV infected tobacco tissues and Barton-Wright and McBain (1932) in leaf roll infected potatoes, Barton-Wright and McBain (1933) in potato affected by paracrinkle virus and Sreenivasaya and Sastri (1928) in sandal spike disease. Viruses causing mosaic diseases affect the spread of hydrolysis of starch and its translocation (Bawden, 1956). Sreenivasaya and Sastri (1928) considered that the impaired translocation of carbohydrates was due to calcium deficiency. Wynd (1943) suggested that the retarded translocation of carbohydrates might either be due to changes in the permeability of cells or the influence of virus infection on carbohydrates.

Sucrose was found to be the translocatory sugar in healthy plants while the sugar of translocation in diseased plants appeared to be glucose. The chromatographic detection of high amount of glucose in diseased stem and high quantity of sucrose in healthy stem indicates that the nature of sugar translocated in the infected plant is probably changed by virus infection. This kind of alteration in the nature of sugar translocated has been recorded by Barton-Wright and McBain (1932) in the case of leaf roll virus infected potatoes.

The decrease in the total carbohydrate contents in diseased plants may be due to retardation in the synthesis of carbohydrates, faster breakdown of carbohydrates to serve as substrates for the increased respiration and conversion of carbohydrates into amino acids which are ultimately used for protein formation.

SUMMARY

The reduction in the contents of chlorophyll in virus diseased pigeon pea leaves was observed to be as high as 60·9 per cent. Carotene and xanthophyll contents of diseased leaves also showed a decrease. The activity of chlorophyllase was increased due to virus infection. The total carbohydrate contents of diseased leaves were reduced. The synthesis of sucrose in diseased leaves was at a lower rate than in the healthy. This reduction in the ability to synthesize sucrose indicated a derangement of photosynthetic activity in diseased plants. Evidences were obtained to show that the translocation of sugars was reduced and that the nature of sugars translocated was altered in the diseased plants.

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