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The Effect of Infection with Papaya Leaf Reduction Virus on the Total Nitrogen and Carbohydrate Content of Papaya Leaves

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Introduction

Virus infection generally results in the drastic bio-chemical and physiological changes in the host plants. These changes might be used in setting up the characteristic of the disease as a supplement to the symptomatology. Plant virus diseases have been divided into two types, i. e. the mosaic type and yellow type of plant virus diseases (DUNLAP 1930). The mosaic type of plant virus diseases are characterised by increased and decreased total nitrogen and carbohydrate contents respectively, whereas, decreased total nitrogen and increased carbohydrate contents are the characteristics of yellow type of plant virus diseases (DUNLAP 1930). Changes in the nitrogen (DUNLAP 1930; HILLS & MCKINNEY 1942; COMMONER, SCHIEBER & DIETZ 1953; ORLOB & ARNY 1961; FARAKAS & SZIERMAI 1969; JENSEN 1969) and carbohydrate (CAMPBELL 1925; DUNLAP 1930; MALHOTRA 1931; WATSON & WATSON 1951; ORLOB & ARNY 1961; JOHN 1963; NARAYANASWAMY & RAMKRISHNAN 1965; REDDY 1966 and KHATRI & CHENULU 1969) contents have been reported for several different plant hosts infected with different viruses. But for the study of ORLOB & ARNY 1961 with barley yellow dwarf virus and KHATRI & CHENULU 1969 with cowpea mosaic virus, little work has been done on the effect of systemic virus infection on the total nitrogen content and carbohydrate content of plants. It is, therefore, worthful to investigate changes in total nitrogen and carbohydrate contents in the course of disease development due to papaya leaf reduction virus infection.

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Material and methods

Papaya leaf reduction virus (PLRV) was first described by SINGH 1969. PLRV was used as the parasite and young healthy seedlings of *Carica papaya* L. cv. 'Washington' as the host. Plants were grown in pots containing soil-composed mixture. Inoculations were made when the seedlings were 15-days old. The virus inoculum was prepared by macerating leaf tissue in a mortar adding 0.05 M phosphate buffer (pH7.0) at the rate of one ml. for every gm. of tissue. Healthy vigorously growing 15 days old plants were inoculated by usual sap inoculation method using carborundum as an abrasive. Every care was taken to inoculate the plants as uniformily as possible. Control plants were treated similarly but with the buffer solution only.

Estimation: Composite samples were collected on the 5th, 10th, 15th, 20th, 25th and 30th day after inoculation and dried to constant weight in

Table 1

Total nitrogen content (mg/g dry wet.) in leaves of healthy and PLRV-infected plants of *C. papaya*, cv. 'Washington' at various intervals after inoculation

Plant	Days after inoculation					
	5	10	15	20	25	30
Healthy	51.17	52.20	51.78	51.76	51.89	51.97
Inoculated	52.21	57.23	68.11	68.00	69.54	76.01

an oven at 65° C. The total nitrogen was determined from the powdered dried material by the conventional micro-Kjeldahl method (A.O.A.C. 1960). The various carbohydrate constituents were extracted from the powdered dried material by boiling on a water-bath for half an hour in different grades of alcohol starting from 90 percent followed by 70 percent, 50 percent, 40 percent, 30 percent and finally in water. The supernatant from all the extracts was combined and filtered through Whatman No. 42. Residue was dried at 65° C and preserved for starch estimation. The combined extract was clarified according to the A.O.A.C. 1960 method. Reducing sugars were estimated by the SOMOGYI 1952 method. Total sugars were estimated on the acid hydrolysed extract by the above method. Non-reducing sugar content was obtained by substracting the value of reducing sugar from total acid hydrolysable sugars and multipied by 0.93 to correct for water taken up during hydrolysis. Starch content was estimated on the basic of acid hydrolysed extract of the residue obtained by the above method and multiplying the value by 0.9. Paper chromatography was employed for separation and identification of sugars, by the technique

described by BLOCK, DURRUM & ZWEIG 1955. One dimensional chromatography on Whatman No. 1 paper was improved with butanol, glacial acetic acid 10 ml, trichloroacetic acid 4 gm, water 10 ml and alcohol 80 ml) sprayed with Benzidine-acetic acid reagent (Benzidine 500 mg, acetic acid 10 ml., trichloroacetic acid 4 gm., water 10 ml. and alcohol 80 ml.) and dried in a oven at 80° C for two minutes. The sugars were identified with respect to their Rf values as compared to those of known sugars.

Determination of virus concentration: The first visible symptom of PLRV infection, i. e. vein-clearing of the young leaves followed by the development of translucent areas adjoining the veins, appear 5 to 7 days after inoculation. The dilution end point of the virus lies between 1 : 100 and 1 : 1,000 (SINGH 1969). As PLRV infects papaya plants only and no

Table 2

Reducing sugar, non-reducing sugar and starch contents (mg/g dry wet.) in leaves of healthy and PLRV-infected plants of *C. papaya*, cv. 'Washington' at various intervals after inoculation

Days after inoculation	Reducing sugar		Non-reducing sugar		starch	
	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
5	37.54	34.21	20.17	26.71	41.65	39.98
10	36.83	32.11	28.76	20.13	39.48	27.67
15	36.14	32.08	27.15	19.54	40.17	30.71
20	34.86	29.97	25.68	18.07	39.87	20.78
25	33.67	29.03	22.19	16.73	40.11	20.53
30	33.35	25.57	19.87	15.74	41.72	19.36

local lesions are formed (SINGH 1969), virus concentration was determined by the usual dilution end point method. Part of leaves collected for the estimation was used to determine the virus concentration on 5th, 10th, 15th, 20th, 25th and 30th day after inoculation. Healthy *C. papaya* seedlings again of cv. 'Washington', were used as the test plants and were observed for 20 days after inoculation. Results obtained are presented in Table 3.

Results

From the data presented in Table 1 it is evident that PLRV infection greatly increased total nitrogen content in papaya leaves. This increase in total nitrogen is higher after the 10th day of inoculation. It was also observed that concentration of the virus in the leaves of inoculated plants increased upto the 15th day, then after it remained stationary upto the 30th day after inoculation (Table 3).

Data on reducing sugar content are presented in Table 2. The reducing sugar content decreased continuously with the time in both healthy and PLRV inoculated plants. However, leaves of inoculated plants had lesser amount of reducing sugar at all stages following inoculation than the corresponding leaves of uninoculated healthy plants. This decrease in reducing sugar content is not much significant. Infection also caused decrease in non-reducing sugar at all intervals (Table 2). It is also evident from the results in Table 2 that PLRV infection caused decrease in starch content at all intervals after inoculation. However, percentage of reduction in starch content was more pronounced in the later stage of plant growth, i. e. after 15 days of inoculation (Table 2).

Nature of sugars: Four kinds of sugars namely, glucose, fructose, sucrose and galactose were detected. There was no qualitative difference

Table 3

Number of plants infected out of ten inoculated with the sap from leaves of *C. papaya*, cv. ‚Washington' plants infected for different periods

Dilution	Inoculum taken from leaves of plants infected for different periods in days					
	5	10	15	20	25	30
Undiluted	1	6	10	10	10	10
1 : 10	0	6	10	10	10	10
1 : 100	0	2	8	8	8	8
1 : 1,000	0	0	0	0	0	0

in the type of sugars present in the leaves of healthy and infected plants at any stage of disease development.

Discussion

The present study reveals that PLRV infection increased total nitrogen content in papaya leaves (Table 1). Increased total nitrogen content have also been reported in other host-virus combinations (Dunlap 1930; Commoner & al. 1953; John 1963; Jensen 1969). It was also observed that virus concentration attained the maximum value on the 15th day after which it remained stationary (Table 3). The first visible symptom, i. e. vein clearing appeared on the 5th day of inoculation. PLRV-infected papaya plants are very much stunted, bear thin thread like leaves and bleed very less latex on injury (Singh 1969). Now it is obvious that increase in total nitrogen content in PLRV- infected papaya plants is more pronounced in the later stage of disease when the virus has attained the maximum concentration (Table 1 and Table 3). As PLRV concentration remained stationary after 15th day of inoculation (Table 3), it is concluded that after 15th day more virus protein than virus nucleic acid is formed.

Mosaic type of virus diseases reduce carbohydrate contents in infected plants (DUNLAP 1930). Reduced carbohydrate contents have been reported in different host-virus combinations by different workers (TRUE & HAWKINS 1928; BOLAS & BEWELY 1930; DUNLAP 1930; CORDINGLY & al. 1934; JOHN 1963; NARAYANASWAMY & RAMAKRISHNAN 1965; GORDON 1966; KHATRI & CHENULU 1969). PLRV infection also decreased the carbohydrate contents in the leaves of *C. papaya* cv. 'Washington' plants. The decrease in reducing and non-reducing sugars and starch contents in infected plants could occur either due to decreased rate of synthesis of these compounds or that break down is inhanced due to increased respiration or due to both (NARAYANSWAMY & RAMKRISHNAN 1964). Increased respiratory rates in the leaves of PLRV-infected papaya plants have been observed (SINGH 1971). RISCHKOV 1943 further suggested that accumulation of virus is accompanied by an expenditure of carbohydrates. Thus according to criterion used by DUNLAP 1930 the present disease should be kept in the group of mosaic type of plant virus diseases.

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Summary

Changes in the total nitrogen and carbohydrate contents were studied in PLRV-infected leaves of *C. papaya*, cv. 'Washington' plants at various intervals after inoculation. The total nitrogen content increased at all stages of disease development, however, this increase was more pronounced at the later stage of infection. The reducing and non-reducing sugars and starch contents were reduced as a result of PLRV infection at all stages of disease development and their was no qualitative difference in the types of sugars present in the leaves of healthy and infected plants at any stage of disease development.

Zusammenfassung

Blätter von *Carica papaya* L. cv. 'Washington', mit PLRV (= Papaya leaf reduction virus) infiziert, ändern den gesamten Stickstoff- und Kohlehydratinhalt zu verschiedenen Zeiten nach der Impfung. Der gesamte Stickstoffgehalt erhöht sich in allen Abschnitten der Krankheitsentwicklung, am deutlichsten im letzten. Der Inhalt an reduzierendem und nicht reduzierendem Zucker sowie an Stärke sinkt als Ergebnis der PLRV-Infektion in allen Krankheitsabschnitten. Es ergab sich kein qualitativer Unterschied in den Zuckertypen zwischen gesunden und infizierten Pflanzen irgendeines Krankheitsstadiums.

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