

**THE EFFECTS OF PAPAYA RINGSPOT VIRUS
INFECTION ON THE NITROGEN METABOLISM OF
Carica papaya L.: PART I. PROTEIN AND
NON-PROTEIN NITROGEN CONTENTS IN LEAVES.**

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Abstract

The effects of papaya ringspot virus (PRSV) infection, on protein and non-protein nitrogen contents in the leaves of the host, *Carica papaya* L. were examined. The protein and non-protein nitrogen contents were affected by the virus, but the total nitrogen content remained significantly unchanged when compared to those of healthy leaves. The changes in the levels of non-protein and protein were first detected in the mechanically inoculated leaves in three hours of inoculation. About one and a half times increase in non-protein and a corresponding decrease in protein nitrogen contents were detected in the inoculated leaves after three hours of inoculation. After 12 weeks of inoculation, a three fold increase in non-protein and a corresponding decrease in protein nitrogen contents were detected in the systemically infected leaves, when compared to those of healthy leaves. Similar effects of PRSV infection were observed in naturally infected field plants.

Key words: PRSV, *Carica papaya* L, protein nitrogen, non-protein nitrogen, mechanically inoculated, systemically infected

1. Introduction

Papaya (*Carica papaya* L.) is widely grown in tropical and sub tropical countries for its delicious and nutritious fruit. A number of viruses have been reported to cause diseases in papaya (Cook, 1972), out of which the PRSV has been identified as the most disastrous one that limits papaya production (Lindner *et al.*, 1945; Conover, 1946; Capoor and Verma, 1948;

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Jensen, 1949; Herold and Weibel, 1962; Singh, 1969; Story and Halliwell, 1969; Zetler, 1970; Wang *et al.*, 1978; and Lana, 1980). The occurrence of PRSV in papaya, in Sri Lanka has been reported by Senevirathna *et al.*, (1984) and Salim and Wijendra, (1995). Although, the symptomatology, etiology and the biochemical properties of the virus have been thoroughly studied, only a few information is available on the metabolic changes brought about by the virus in its host. In this study we have examined the effect of PRSV infection on the protein and non-protein nitrogen contents of the leaves of *C. papaya*, inoculated mechanically and under naturally infected field conditions.

2. Materials and methods

Source of virus isolate:

Isolate designated as PRSVs was obtained from the infected leaves of a matured, fruit bearing papaya plant, growing in the premises of the University of Sri Jayewardenepura. This isolate was maintained on *Carica papaya* L. by mechanically inoculating to young papaya seedlings (variety not known).

Mechanical inoculation:

Infected leaf tissues of papaya with clear "virus" symptoms were ground in 0.05M cold potassium phosphate buffer (pH 7.5) at the rate of 1:3 w/v and extracts were rubbed on test plants (healthy papaya seedlings kept in dark for 24h) dusted with 100 mesh carborundum. Inoculated plants were maintained in insect-protected cages, kept in a green house at $30 \pm 2^\circ\text{C}$. Papaya seedlings with five to six leaf stage (three weeks after transplanting) were selected for inoculation and leaves designated as 1, 2, 3 and 4 were inoculated as indicated in Fig.1. The leaves of healthy controls were also dusted with carborundum and rubbed only with the inoculation buffer.

Sampling for analysis:

The experiment had two treatments: (1) leaves inoculated with PRSVs. (2) treated only with inoculation buffer ("healthy") as described above.

Leaf samples for analysis were collected at time intervals: 1 $\frac{1}{2}$ hours, 3 hours, 24 hours, 2 weeks, 4 weeks and 12 weeks of inoculation. After 1 $\frac{1}{2}$ hrs, 3 hrs and 24 hrs of inoculation only the inoculated leaves (1, 2, 3, 4,) were taken for analysis, as it was confirmed by ELISA (enzyme linked immunosorbent assay) test that the upper leaves, (Fig. 1) were free from virus. Samples collected after 2, 4 and 12 weeks of inoculation, contained the upper leaves (I, II, III, IV) with or without prominent symptoms.

At each time interval of sampling, similar leaves from different sets of five plants were collected (five replicates for each time interval) and the leaves of an individual plant were pooled and 2.5g of the pooled sample was used for analysis.

Leaf samples, naturally infected with PRSV in the field were taken from 1-2 $\frac{1}{2}$ years old papaya plants, growing in different climatic zones of Sri Lanka: wet zone (Colombo, isolates designated as PRSV1, PRSV2, PRSV3, PRSV4 and PRSV5), intermediate zone (Kurunegala, PRSV-k) and dry zone (Dambulla, PRSV-d). These virus isolates have caused mosaic symptoms and blisters in leaves, and the reduction of leaf lamina to various degrees in their respective mother plants. As controls, leaf samples were collected from healthy plants apparently of similar age. All leaf samples taken for analysis were tested for the presence of PRSV by an indirect procedure of ELISA test (Lommel *et al.*, 1982)

Determination of Nitrogen Content:

Fractionation of nitrogen was carried out as described by Petereburski (1968). For the determination of protein and non-protein nitrogen, proteins in the samples were deposited by treating them with 10% trichloro acetic acid (TCA) and were centrifuged at 3000 rpm. The nitrogen content of both supernatant and the pellet was determined by the micro-kjeldahl procedure.

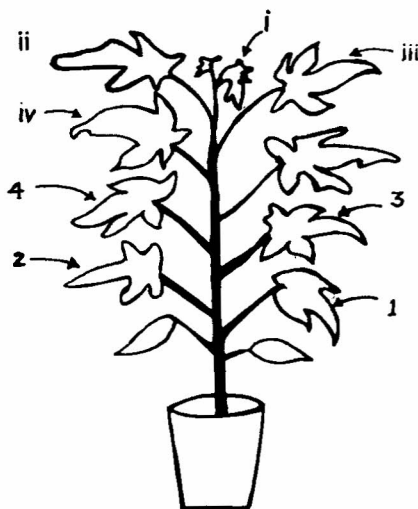


Figure 1. Inoculated and uninoculated leaves of papaya for sampling. Leaf positions: 1, 2, 3 and 4 were inoculated when the seedlings were of 5-6 leaf stage. The leaves designated as: i (non-expanded, young leaf next to the apex), ii, iii, and iv showed symptoms after 5 weeks of inoculation.

3. Results

The results of protein and non-protein nitrogen contents of the healthy and PRSVs infected papaya leaves are presented in Table 1 and Figure 2. According to these results, a relatively higher percentage of non-protein nitrogen (0.75%) was observed in the inoculated leaves with PRSVs, after 3 hours of inoculation when compared to that of healthy leaves (0.57%). This was accompanied by a reduction in the percentage of protein nitrogen from 0.70% in the control to 0.46% in the inoculated leaves, while the total nitrogen content remained relatively unchanged.

Similar tendency was observed throughout the testing period, and the difference in the protein and non-protein contents in the controls and diseased was increased gradually with the time of inoculation. After 12 weeks of inoculation, a three fold increase in non-protein and a corresponding decrease in protein nitrogen content were observed in the infected leaves (Table 1 and Figure 2).

As indicated in Table 2, similar results were obtained for samples collected from naturally infected field plants.

Table 1. The contents of protein, non-protein and total nitrogen (%) in *Carica papaya* leaves as affected by the PRSVs infection.

Time after inoculation	Nitrogen, %		
	Non-protein	Protein	Total
3 hours			
PRSVs	0.75	0.46	1.21
Healthy	0.57	0.70	1.27
24 hours			
PRSVs	0.78	0.31	1.09
Healthy	0.56	0.72	1.28
2 Weeks			
PRSVs	1.05	0.83	1.88
Healthy	0.82	1.84	2.66
4 Weeks			
PRSVs	2.28	1.76	4.04
Healthy	1.04	3.14	4.18
12 Weeks			
PRSVs	2.93	1.10	4.03
Healthy	0.93	3.32	4.25

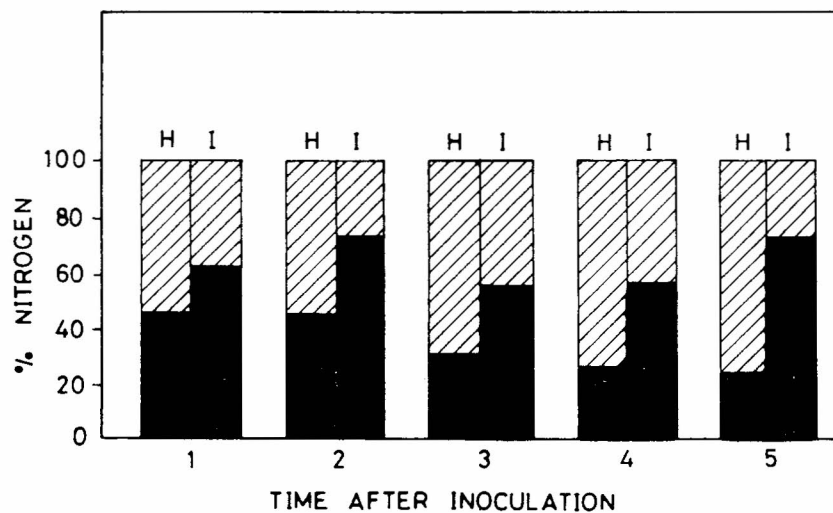


Figure 2. Nitrogen contents (total expressed as 100%) in the leaves of healthy and PRSVs infected papaya leaves.

1 - 3 hours ; 2 - 24 hours; 3 - 2 weeks;
 4 - 4 weeks ; 5 - 12 weeks
 H-healthy ; I - infected
 ▨ - protein ; ■ - non - protein

Table 2. The contents of protein, non-protein and total nitrogen (%) in the leaves of *Carica papaya*, naturally infected in the field with PRSV

Sample	Nitrogen, %		
	Non-protein	Protein	Total
PRSV3	2.10	2.24	4.34
*HEALTHY	1.65	3.14	4.79
PRSV5	3.02	0.98	4.00
*HEALTHY	1.01	3.23	4.24
PRSV-k	2.90	1.12	4.02
*HEALTHY	1.09	3.04	4.13
PRSV-d	1.68	0.93	2.61
*HEALTHY	0.62	1.92	2.54

*Healthy samples were collected from plants apparently of similar age as those of infected ones and tested by ELISA.

4. Discussion

Findings of the present investigation reveal that PRSV infection caused change in the contents of non-protein and protein nitrogen without affecting the total nitrogen in leaves of *C. papaya*. In mechanically inoculated leaves with PRSVs, these changes were first observed, after 3 hours but not after 1½ hours of inoculation which indicates that, the virus requires about three hours for the process of infection.

A slight decrease observed in the protein nitrogen content after 3 hours of inoculation, may be probably due to the breakdown of host proteins, induced by the virus. The increase in the non-protein nitrogen which accompanied the decrease of protein nitrogen content in leaves, may be probably due to the accumulation of free amino acids as a result of the hydrolysis of host proteins. As the total nitrogen content remained significantly unchanged it appears that the virus had not directly induced the synthesis of amino acids that were required to build its capsid protein, but has had obtained by the hydrolysis of the protein already present in the leaf tissue.

The increased breakdown of proteins (by three fold) at 12 weeks of inoculation, may perhaps be due to the active synthesis of the virus in the systemically infected leaves, which were collected for analysis at this stage. The virus concentration has been reported to be high in the systemically infected leaves than in inoculated leaves (Walkey, 1985). Therefore, the present results indicate that the PRSV reached a high titer in papaya within 4-12 weeks of inoculation in the systemically infected leaves. This is also reflected by the fact that the symptoms started appearing in the uninoculated upper leaves only after 4-5 weeks of inoculation. Gonsalves and Ishii (1980) also reported that PRSV reached a highest titer in the systemically infected leaves in 3-4 weeks of inoculation in *Cucurbita pepo* L.

However, it has been reported that the changes in total nitrogen, protein nitrogen and soluble nitrogen in leaves infected with virus were erratic and no general trend in changes of these have been observed (Saksena, 1983). Some investigators have reported higher levels of total nitrogen, protein nitrogen and alcohol soluble nitrogen in the virus infected plants whereas, others have reported low levels of protein nitrogen in the virus infected leaves as compared to healthy ones. The effect of virus on the nitrogen contents in leaves seemed to be a characteristic of a particular host-virus combination (Saksena, 1983).

In the present study for the combination, PRSV-papaya, the effect of infection on the levels of non-protein and protein nitrogen were consistent throughout the testing period and a similar tendency was observed in the samples collected from the field despite the age of the host and localities where the hosts were growing. In view of this, it is important of further studying the effects of PRSV on the fractional composition of non-proteinous nitrogen in papaya.

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