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THE EFFECT OF PAPAYA RINGSPOT VIRUS INFECTION ON THE NITROGEN METABOLISM OF *Carica papaya* L. : PART II. COMPOSITION OF FREE AMINO ACIDS IN THE LEAVES

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Abstract

Leaves of mechanically inoculated *Carica papaya* L. plants with papaya ringspot virus (PRSV) were examined for free amino acids by using paper, thin layer and high pressure liquid chromatography (HPLC) techniques. Seven amino acids: aspartic acid, glutamic acid, serine, arginine, threonine, alanine and glycine were detected by paper and thin layer chromatography and another eight amino acids: histidine, methionine, isoleucine, leucine, lysine, tyrosine, valine and penylalanine were detected by HPLC. A two fold increase in the concentration of serine, alanine, arginine, threonine and aspartic acid was observed in the leaves infected with PRSV. Methionine, valine histidine and tyrosine contents in the infected leaves were decreased but no significant differences were found in the contents of glutamic acid, isoleucine and leucine, These changes were detected in the inoculated leaves as early as three hours of inoculation and same tendency was observed at the later stages of disease development.

Similar changes in free amino acids were observed in the field plants naturally infected with PRSV, despite their age and different climatic conditions of growth.

Key words: *Carica papaya* L., PRSV, Mechanically inoculated, Free amino acids, Paper chromatography, Thin layer chromatography, HPLC

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1. Introduction

Papaya (*Carica papaya* L.), a fruit crop grown in the tropics have been reported to be infected by a number of viruses (Cook, 1972). A mosaic disease caused by PRSV is one of the most destructive diseases of papaya in many countries including Sri Lanka (Lindner *et al.,* 1945; Conover, 1946; Capoor and Verma, 1948' Jensen, 1949 a; Herold and Weibel, 1962; Singh, 1969' Story and Halliwell, 1969; Cook and Zetler, 1970; Wang *et al.,* 1978; Lana, 1980; Seneviratne *et al.,* 1984; and Salim and Wijendra 1995). The symptomatology, spread of the disease and the biochemical properties of PRSV have been extensively described, however, only a few reports are available on the metabolic changes brought about by the virus in the host. Previously we reported the effect of PRSV infection on the non-protein and protein content in papaya leaves (Wijendra *et al.,* 1995). In this study, we have examine the free amino acid composition in the leaves of 'healthy' and naturally infected papaya plants with PRSV. Further, leaves of mechanically inoculated papaya seedlings also were examined for the composition of free amino acids at different stages of disease development.

2. Materials and methods

Source of virus isolate and mechanical inoculation:

Isolate designated as PRSVs was collected and inoculated mechanically to the first four leaves of young papaya seedlings (variety not known) as previously described by us (Wijendra *et al.,* 1995).

Sampling procedure:

In mechanically inoculated plants, leaf samples for analysis were collected $1^1/_2$ hours, 3 hours, 24 hours, 2 weeks, 4 weeks and 8 weeks of inoculation. As confirmed by the ELISA (enzyme linked immunosorbent assay) test that the upper leaves were free of virus, only the inoculated leaves were taken for analysis after $1^1/_2$ hrs, 3 hrs and 24 hrs of inoculation. Samples collected after 2,4 and 8 weeks of inoculation contained uninoculated, upper leaves as described in Wijendra *et al.,* (1995), with or without prominent symptoms.

At each interval of sampling, similar leaves were collected from a set of five different plants. The leaves of an individual plant were pooled and 5g of the pooled sample was used for analysis.

Leaf samples, in the field were taken form $1\text{-}2^1/_2$ years old papaya plants naturally infected with PRSV, which were growing in home gardens of different climatic zones of Sri Lanka. The samples infected with wet zone

isolates: PRSV1, PRSV2, PRSV3, PRSV4 and PRSV5, the dry zone isolates: PRSV-d and the isolate of intermediate zone : PRSV-k described by Wijendra *et al.* (1995) were used for the analysis of free amino acids.

All leaf samples taken for analysis were tested by an indirect ELISA procedure (Lommel *et al.,* 1982) for the presence of PRSV, using an antiserum produced against a Hawaiian isolate of PRSV (kindly supplied by Dr. D Gonsalves, Cornell University, USA).

Determination of Free Amino Acids:

Five grams of fresh leaves were extracted by Draper (1976) procedure. The amino acids in extracts were analysed by ascending paper chromatography (Whatam No. 4) and thin layer chromatography (0.5 mm thick Silica gel G, Kieselgel G, Chemica 60760). Aliquots of 10 µl plant extract was used for paper and thin layer chromatography. Chromatograms were developed with butanol: acetic acid: water (60:15:25 by volume).

The dry chromatograms were sprayed with ninhydrin (0.2% in butanol) and heated in an oven at 80^0C for about 5 minutes. The amino acids were identified by comparing with authentic amino acid standards.

The amino acids in extracts were also analysed by HPLC technique, using a method based on the pre-column derivatisation with phenylisothiocyanate (PITC).

3. Results

According to our results of paper and thin layer chromatography, only seven amino acids: alanine, threonine, serine, arginine, glycine, glutamic and aspartic acids were detected in the leaves of both healthy and naturally infected plants with PRSV. A visual increase in the concentrations of all amino acids mentioned above except for glutamic acid was clearly observed in the infected leaves when compared to healthy leaves. Same pattern of amino acid distribution was found in the infected leaf samples collected from papaya plants of different age and in those collected from different climatic regions: wet, dry and intermediate zones.

Similar results were obtained for mechanically inoculated plants too and these changes in concentrations of amino acids were first detected only after three hours but was not after $1^1/_2$ hours of inoculation.

The HPLC analysis detected 15 amino acids including those mentioned above (Table 1). According to these results, a two fold increase in the concentrations of alanine, serine, threonine, glycine and aspartic acid was

observed after three hours of inoculation (Table 1 & Figure 1). The contents of lysine and arginine were also higher but to a lesser extent. This has also confirmed the results of paper and thin layer chromatography. A decrease in the contents of valine, histidine, methionine and tyrosine was observed (Table 1 & Figure 1) while leucine, iso-leucine, phenylalanine and glutamic acid contents remained unchanged (Table 1). The quantitative difference observed in amino acids was consistent throughout the period of disease development.

Table 1. The concentrations (p. mols/μl) of free amino acids in papaya leaves as affected by PRSVs infection.

Amino Acid	Time after inoculation					
	3 hours		4 weeks		8 weeks	
	Healthy	PRSVs	Healthy	PRSVs	Healthy	PRSVs
Arginine	76.41	98.01	122.00	208.06	182.70	270.30
Serine	31.39	79.87	46.22	94.98	67.61	121.80
Aspartic acid	40.96	80.06	62.90	127.50	69.81	150.90
Glutamic acid	62.96	63.29	72.25	73.43	124.50	124.02
Threonine	32.91	72.22	54.32	90.85	56.30	93.91
Alanine	23.75	44.58	24.42	55.07	25.92	57.73
Lysine	15.32	20.38	28.30	36.04	30.65	39.14
Glycine	12.79	23.59	16.55	32.62	33.07	61.71
Valine	11.64	8.47	13.12	10.21	16.32	13.31
Histidine	4.70	2.83	6.93	4.26	9.73	6.32
Methionine	6.98	3.98	7.52	5.76	7.75	6.31
Tyrosine	3.65	2.44	9.85	6.54	21.67	19.03
Leucine	5.79	6.23	8.58	9.86	8.96	10.01
Isoleucine	7.05	8.29	7.23	8.69	7.50	8.75
Phenylalanine	8.17	9.33	8.70	9.70	8.90	9.90

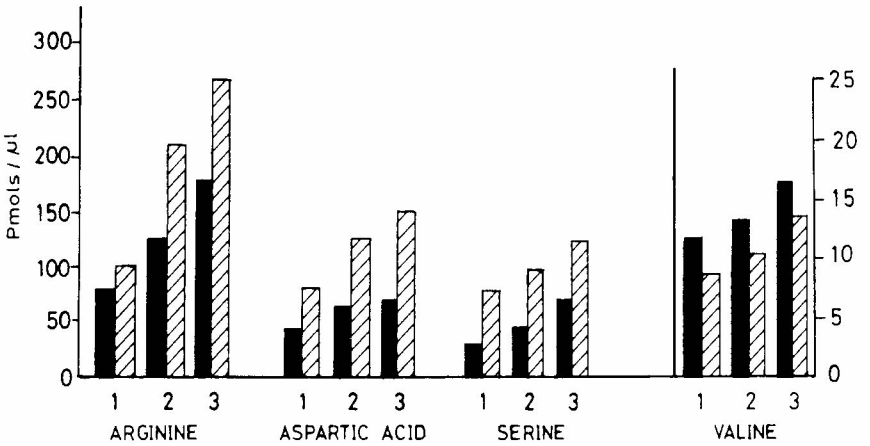

Figure 1 Free amino acids contents in leaves of *Carica papaya* as affected by PRSVs (1-three hours after inocultion, 2-four weeks after inoculation ▨ - Healthy, ■ - Infected).

4. Discussion

Present investigation reveals that PRSV infection did not alter the composition of free amino acids but affected the contents in the leaves of papaya plants.

Our studies indicated that contents of seven amino acids, alanine, serine, glycine arginine, aspartic acid, threonine and lysine were increased up to two fold in the PRSV infected leaves. This is more or less in agreement with the findings of Mathur and Shukla (1976). They too reported an increase in the contents of first five amino acids we reported above. In addition to these, they have observed that proline, glutamic acid and methionine levels also have been increased in the PRSV infected papaya leaves.

Further, we found the contents of valine, histidine, methionine and tyrosine were decreased while leucine, isoleucine, phenylalanine and glutamic acid remained unchanged in both naturally infected and mechanically inoculated plants with PRSVs. This is in contrast with the findings of Mathur and Shukla (1970). They found a decrease in the contents of leucine, lysine, threonine and hydroxyproline while cysteine and tyrosine levels have remained unchanged. Moreover, they have detected proline, hydroxyproline

and cysteine in both healthy and virus infected samples whereas we have not detected these in ours. This difference perhaps may have caused by the variations in the PRSV strains. The PRSV isolates used in this study, seemed to be biologically different to the isolates reported by them (Salim and Wijendra, 1995).

The increase in the contents of free amino acids due to the infection, possibly may have resulted from the hydrolysis (breakdown) of proteins induced by the virus. This can be confirmed by our previous study, in which we reported the decrease in protein nitrogen and the increase in non-protein nitrogen contents, with an unchanged total nitrogen content in the PRSV infected leaves (Wijendra et al., 1995). It has also been reported that normal proteins are hydrolysed in the infected tissues and the resulting amino acids are apparently used for the synthesis of viral capsid protein (Diener, 1963).

The decrease observed in the concentrations of some amino acids in the PRSV infected leaves may have caused due to their rapid utilization by the virus for the synthesis of capsid protein or/and due to the interference of the virus on the synthesis of these amino acids by the host.

It has been reported that there is no general pattern in changes of free amino acids in leaves infected with virus and the effect is reported to be specific for a given host-virus combination (Saksena, 1983)

For the papaya-PRSV combination reported here, we have found that the pattern of the distribution of free amino acids in the infected leaves remained consistent irrespective of the age of the infected plants and the climatic zones in Sri Lanka, where they were growing.

Although, the paper chromatography detected only seven amino acids it provided a sufficient contrast of the quantitative difference of amino acids in the healthy and infected leaves as confirmed by HPLC analysis. Therefore, the analysis of free amino acids in the inoculated leaves even by paper chromatography provides a satisfactory method to assay the PRSV infection in papaya as early as three hours of inoculation, without waiting for symptom expression which takes about 4-5 weeks during cooler weather conditions but heavily masked in warmer periods. Though the ELISA test too, confirmed the infection after three hours of inoculation, its adoption widely in developing countries is impractical as this technique requires expensive chemicals, equipments and also competent staff. However, the ELISA test is important in order to confirm the identity of the virus. Further, it was reported by us previously that the gel-immunodiffusion test too was not successful for the identification of PRSV isolates we describe here (Salim and Wijendra, 1995)

Therefore, we conclude, that analysis of amino acids in the papaya leaves, by paper chromatography can be used as a simple, rapid and highly economic method for screening of these PRSV isolates in about 3 hours of infection. This technique is particularly useful in Sri Lanka, as PRSV is apparently the only virus reported so far to cause disease in papaya.

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6. Refernces

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