D-33: The effect of papaya ringspot virus infection on the composition of free amino acids in leaves

N Salim, S S Ranaweera, W A S Wijendra
(Dept. of Botany, Univ. of Sri Jayewardenepura, Nugegoda)

A mosaic disease caused by papaya ringspot virus (PRSV) is one of the most destructive disease of papaya (*Carica papaya* L.) in many countries including Sri Lanka. Although the symptomatology, spread of the disease and biochemical properties of the virus have been described, only a few reports are available on the metabolic changes brought about by the virus in the host. In this work the effect of PRSV infection on the free amino acid composition of the host under naturally infected and artificially inoculated conditions, was examined.

Infected leaf samples were taken from 1-2½ years old papaya plants which were growing in different climatic zones of Sri Lanka: wet zone (Colombo), intermediate zone (Kurunegala) and dry zone (Dambulla). These virus isolates have caused mosaic symptoms and blisters in leaves, distortion of leaves and the reduction of leaf lamina to various degrees in infected plants. As controls, leaf samples were collected from healthy plants apparently of similar age.

Papaya seedlings were mechanically inoculated with buffered (potassium phosphate, pH 7.5) extracts of PRSV infected leaves. The inoculated plants were maintained in insect-protected cages, kept in a greenhouse at $32 \pm 2^{\circ}C$. Leaf samples for analysis were collected 1½ h, 3 h, 24 h, 2 weeks, 4 weeks and 8 weeks after inoculation. The presence of virus in field samples and in those inoculated mechanically were confirmed by an indirect enzyme-linked immunosorbent assay (ELISA) test (Lommel *et al.*, 1982) Fresh leaves were extracted following Draper procedure (1976). The amino acids in extracts were analysed by ascending paper, thin layer chromatography (TLC), HPLC and also by an automatic amino acid analyser. For the identification of amino acids authentic amino acid standards were used.

Only 7 amino acids: alanine, serine, threonine, glycine, arginine, aspartic and glutamic acid were detected by paper, TLC and HPLC, 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. The pattern of amino acid distribution was similar in infected host plants despits their age and climatic zones where they were growing. Similar results were obtained for mechanically inoculated plants, and the difference in concentrations of amino acids was detected only after 3 h but was not detected after 1½ h of inoculation.

The amino acid analyser detected 15 amino acids including those mentioned above. According to these results there was a 2 fold increase in the concentrations of alanine, serine, threonine, glycine and aspartic acid after 3 h of inoculation. The contents of lysine and arginine were also higher but to a lesser extent. This has confirmed the results of paper and thin layer chromatography. A decrease in the contents of valine, histidine, methionine and tyrosine was observed but leucine, iso-leucine, phenylalanine and glutamic acid contents remained unchanged. The quantitative difference in amino acids was consistent throughout the period of disease development.

Although, paper chromatography detected only 7 amino acids it provided a sufficient contrast of the quantitative difference of amino acids in the healthy and infected leaves. Therefore, the analysis of amino acid contents in leaves provides a satisfactory method to assay the PRSV infection in papaya as early as 3 h of inoculation, without waiting for symptom expression which takes about 4-5 weeks. The ELISA test too, was positive at 3 h incoclation but, as it employs very expensive chemicals and equipment, frequent use of this test to assay the infection is uneconomic when compared to paper chromatography. However, the ELISA test is important in order to confirm the identity of the virus.

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