

Biochemical Analysis of Healthy and Mosaic Virus Infected Papaya Leaves.

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Ethanollic extracts of healthy and mosaic virus infected papaya leaves were examined by ion exchange and paper chromatography methods for the presence of ninhydrin positive compounds and UV active compounds. Aspartic acid, asparagin, alanine, gamma amino-butyric acid, cysteic acid, glutamic acid, leucine, and valine were detected. The qualitative compositions of amino acids in both healthy and virus infected leaves were similar but relative concentrations for some nitrogenous compounds were lower in the diseased tissue.

Carica papaya L. commonly known as "papaw" or "papaya" is widely grown in Sri Lanka. Papaya mosaic first reported from the Kurunegala district in 1952 in Sri Lanka, is prevalent in mid country papaya plantations. Although some work is available regarding the nature and spread of the disease (Adsuar, 1974, Kawanishi et al, 1966). Very little information is available on metabolic changes brought about by the presence of the virus in the host tissue. In this investigation we have examined the free amino acids and UV active compounds found in the healthy and diseased leaves of papaya.

MATERIAL AND METHODS

Ten grams of fresh leaves of both healthy and infected plants were separately ground in a mortar with 50 ml of 90 per cent ethanol. This was then centrifuged. The supernatant was evaporated to dryness and the residue

was stirred with 25 ml of distilled water and recentrifuged. The supernatant which contained free amino acids and other water soluble compounds was passed through an ion-exchange resin column. A glass column having a diameter of 6 mm was packed with washed cationic exchange resin to a height of 8 cm. Before use the resin column was converted to the H^+ ion form by passing 25 ml of 2N HCL through it, followed by distilled water to wash away the unabsorbed chloride ions. The excess acid and the unabsorbed chloride ions were washed with distilled water, until the washings were free of chloride ions. The aqueous plant extract was then allowed to run slowly through this column. Flow rate was regulated to bring about better adsorption of the amino acids. This was followed by washing of the column with distilled water. The absorbed amino acids were then eluted with 25 ml

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of 3N NH_4OH . The elute was collected in a clean vessel and evaporated to dryness. The residue was taken up in 2.5 ml of distilled water and 10 ml aliquots were used for two dimensional paper chromatography. After developing the paper it was sprayed with ninhydrin (0.25% in butanol). The colour, the size, the relative position and intensity of the ninhydrin positive compounds were recorded.

Concentrated ethanolic extracts of healthy and diseased leaves were applied on to Whatman No: 4 chromatographic paper and descending one directional chromatography was carried out with butanol-acetic acid-water as the solvent system. Developed chromatograms were examined under UV light for UV active compounds. The experiment was replicated three times.

RESULTS AND DISCUSSION

1. Ninhydrin positive compounds

There was no difference in the qualitative composition of the ninhydrin positive compounds in the leaves of the healthy and infected plants. Asparagin, glutamin, gamma amino butyric acid, aspartic acid, alanine, cysteic acid, glutamic acid, leucine and valine were detected in all leaf samples. The relative concentrations of the amino acids in healthy and infected leaves were different and these results are shown in Table I. Relative concentrations of cysteic acid, leucine, and alanine were lower

in the diseased plant compared to the healthy plants.

TABLE I. Amino acid composition of healthy and virus infected papaya leaves.

Amino acid	Healthy	Diseased
Aspartic acid	M	M
Asparagine	M	M
Alanine	M	W
Gamma amino butyric acid	M	M
Cysteic acid	W	T
Glutamic acid	S	S
Leucine	W	T
Valine	S	S

S - strong M - moderate W - weak T - trace

There were 3 UV active substances in the extracts of diseased leaves while the extracts of healthy leaves had only two UV active compounds. (Table II). The additional compound found in the diseased leaves appeared pale blue under UV light and did not change colour on exposure to ammonia vapour.

Two amides, five amino acids and the non protein amino acid gamma amino butyric acid were present in the ethanolic extracts of healthy and virus infected leaves of papaya. Several researchers have found large numbers of amino acids in other fruit

TABLE II UV active compounds found in healthy and diseased leaves of papaya.

Appearance under UV-light	Healthy	Infected	Treatment with ammonia
Dark purplish blue	+	+	—
Pale blue	—	+	—
Ivory	+	+	Yellow

crops (Buckley *et al.*, 1964). The lower level of cysteic acid, leucine, and alanine, found in the infected leaves may be attributed to the utilization by the pathogen, to produce viral protein coat. The additional spot observed in the diseased plant is probably a phytoalexin with a coumarin ring structure according to the UV data obtained. This investigation

showed that the mosaic virus affects the amino acid metabolism of papaya leaves and bring about a reduction in the level of some amino acids in the host tissue which will eventually lead to a reduction in growth and yield of susceptible papaya cultivars.

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