

DETECTION OF FREE AMINO ACIDS IN THE ROOT EXUDATES OF HEALTHY AND MOSAIC AFFECTED TOBACCO PLANTS

The root exudates of plants are known to contain amino acids which greatly influence the number and type of microorganisms present in the rhizosphere region (Lochhead and Thexton 1947, Katznelson *et al* 1954, Rovira 1956 a, b). When the metabolism of the plant is altered as a result of virus infection or otherwise, changes can be expected in the root exudates which in turn will be reflected in the microbial population of the rhizosphere.

A marked stimulation of the microbial population was noted in the rhizosphere of tobacco plants which were inoculated with TMV. This beneficial effect on the rhizosphere microflora was considered likely, to a large extent, to be due to changes in the amino acids present in the root exudates. An attempt was therefore made to determine whether infection of tobacco plants by TMV could bring about any detectable change in the amino acids present in the root exudates.

Nine glass jars, 22x 10 cm in size were filled with acid washed river sand and the mouth of the jar covered with a thick wad of cotton. These were sterilized in an autoclave at 15 lb pressure for 20 minutes. Forty five days old tobacco plants grown in earthen pots containing soil and sand mixed in equal proportions were carefully uprooted and their roots washed in distilled water. The roots were then dipped in 1: 1000 mercuric chloride solution for three minutes and washed with sterile distilled water after which they were planted in six of the previously sterilized glass jars, one plant in each. Sterilized Hoagland's solution was poured into the jars at the rate

of 50 ml in each and the mouth of the jar covered with sterilized cotton. After 15 days, three plants were inoculated with TMV. Twenty five days after transplanting, the sand in the jars was analysed for amino acids. The inoculated plants had by this time developed clear symptoms of mosaic.

Alcohol extract of the sand was prepared as per procedure described by Gilbert and Altman (1966). The free amino acids were separated by circular paper chromatography adopting the method described by Giri and Rao (1952), using Whatman No. 1 filter paper and n-butanolacetic acid water (4:1:1 v/v) as developing solvent. The chromatogram was sprayed with 0.3 per cent ninhydrin in acetone and dried in a hot air oven at 65°C for 15 minutes. The Rf values of different coloured spots developed were calculated. Chromatograms of known amino acids were run simultaneously and their Rf values were also calculated. The amino acids in the sand were identified by comparing their Rf values with those of the standards.

The sand in which healthy tobacco plants were grown showed the presence of proline and glutamine while that in which the inoculated plants were grown, showed the presence of lysine in addition to the above two amino acids. No amino acid could be detected in the sand kept as control.

The results go to show that infection of tobacco plants by the TMV can bring about changes in the amino acids of the root exudate. To have a better understanding of these changes, quantitative estimation of the amino acids present in the root exudates will also be necessary.

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Agricultural College & Research
Institute, Vellayani, Trivandrum

C. Balagopal
J. Sam Raj
K. I. Wilson.

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