Serological Studies on Tomato Mosaic Virus

The tomato, *Lycopersicon esculantum* Mill. is very susceptible to many fungal, bacterial and viral infections. Hitherto, seventeen viruses which cause considerable loss in yield have been reported[1]. BROADBENT[2] found that different strains of tobacco mosaic virus (TMV) occur on tomato. Even though the tomato mosaic disease is very common and severe in India under natural conditions, there has not been much work done on the relationship between tobacco mosaic virus from tobacco and tomato. To determine their relationships, symptomatology, host range, mode of transmission, physical and chemical properties were investigated in a previous study[3]. The present study was made to determine the serological relationships of the above mentioned viruses.

For the study, 15 tomato isolates (No. 1 to 15) exhibiting distinct symptoms of various degree were collected from different parts of Madras State, India. All the isolates were purified by mechanically inoculating them on *N. glutinosa* leaves. The inoculum was prepared by grinding single local lesion with phosphate buffer (pH 7.0) and inoculating young healthy tomato plants. Stock cultures were maintained by this method. For comparative studies, tobacco mosaic virus from tobacco was obtained from the University Botany Laboratory, Madras. Since the previous study indicated that isolate No. 10 from tomato was highly virulent, it was used as antigen. Infected tissues were homogenized with acid washed sand and an equal quantity of buffered (pH 7.0) physiological saline in a cold mortar and pestle. The homogenate was centrifuged at $1930 \times g$ for 10 min. The supernatant was collected, stored at 4 °C and used as stock solution for immunizing the rabbits. Healthy albino rabbits (weighing 2 kg) were selected for immunization. They were immunized by administering 5 i.v. injections and 3 i.m. injections of 2 ml of antigen during a 4 day period. To obtain serum, the rabbits were bled by vein puncturing 10 days after the last injection. The blood was collected in a centrifuge tube and left to clot at room temperature. The serum was decanted and centrifuged at $480 \times g$ for 15 min to remove the blood cells. An equal volume of purified healthy tomato sap was mixed with the antiserum and centrifuged at $480 \times g$ for 10 min to remove the antibodies of the host protein. The serum was preserved by adding 0.1% phenol and kept at 0 °C.

Slide agglutination and tube precipitin tests were used to determine the serological relationships of the virus isolates. Crude infective sap of various isolates were prepared by crushing the young infected tomato leaves. A drop of crude sap was placed on a clean microscope slide and adjacent to this a drop of healthy sap was placed. A drop of diluted antiserum (1:2) was added to both crude infected and healthy saps and stirred for a while and agglutination was observed under the microscope. For the tube precipitin test, infective sap of various tomato isolates including TMV was prepared by grinding the leaf tissues with phosphate buffer pH 7.0 and centrifuged at $12{,}100 \times g$ for 15 min. The supernatants were passed through celite to remove all the coloring matter. This partially purified virus (0.5 ml) was mixed with equal volume of antiserum diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 with phosphate buffer. They were kept in a thermostatically controlled water bath at 37 °C. The tubes were observed at intervals of 15, 30, 45 min, 1, 2 and 3 h.

The slide agglutination tests revealed that all the tomato virus isolates were serologically related as well as being related to TMV.

There was no agglutination found with the healthy sap. The concentration of the precipitate formed was recorded at each dilution and shown in the Table. The results indicate that maximum amount of cloudy precipitate was formed by the isolate No. 12 in 1:8 dilution while in the case of TMV the cloudy precipitate was maximum in 1:16 dilution. However, in the case of isolate No. 10 (antigen) maximum precipitation was in 1:32 dilution. In all other isolates maximum precipitation was recorded in 1:16 dilution which is similar to TMV. These variations in antigen-antibodies precipitation may be due to the virus titer in the host tissues.

Tube precipitin tests of different virus isolates

	Antiserum dilutions						
Isolates	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Antigen	++[a]	+++	++	+++	++++	+	−
Isolate No. 12	++	+++	++++	+	−	−	−
TMV	+++	++	++	++++	+	−	−

[a] Relative amount of precipitation indicated approximately by multiple symbols (+).

Though tomato isolates were differentiated into typical TMV and its strains based on their physical, chemical and other properties[3], serological tests indicated that all the tomato isolates were serologically related to each other and also with tobacco mosaic virus from tobacco[4].

Résumé. La souche du virus en mosaique de la tomate et celle du virus en mosaique du tabac se sont trouvées alliées comme l'ont montré les résultats des expériences de précipitation et d'aglutination.

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