IMMUNOSORBENT ELECTRON MICROSCOPIC STUDIES ON A TOBAMOVIRUS CAUSING BRINJAL NECROTIC MOSAIC

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Summary. – Serological relationships of brinjal necrotic mosaic virus (BNMV), a strain of tobacco mosaic virus (TMV) causing necrotic mosaic disease of brinjal in India to other TMV strains was investigated by immunosorbent electron microscopy (ISEM). The intensity of trapping and decoration revealed a close relationship of BNMV to TMV-D, TMV-U1 and TMV-WU1 strains, and a distant relationship to TMV-A1 and TMV-P11 strains. There was a negligible relationship to TMV-P14, tomato mosaic virus (ToMV) and cucumber green mottle mosaic virus (CGMMV). Therefore, BNMV is proposed to be distinct from the previously reported TMV-A1 strain of brinjal.

Key words: brinjal necrotic mosaic virus; tobacco mosaic virus strains; immunosorbent electron microscopy; India

The virus causing necrotic mosaic disease of brinjal in India was isolated from brinjal (*Solanum melongena* L. cv. BR-112) and named BNMV. On the basis of host reactions, mode of transmission, biophysical properties, particle morphology, ultrastructural studies of infected cells and positive serological reactions with different strains of TMV, BNMV was identified as a strain of TMV (Gupta *et al.*, 1992). Present paper reports the results of ISEM investigations on the relationship of BNMV to other TMV strains.

Antiserum to BNMV was produced by immunizing a rabbit by giving one intravenous injection of virus emulsified with Freund's incomplete adjuvant followed by two intramuscular injections in one week intervals.

ISEM was performed with purified virus preparations (0.1 mg virus/ml) diluted 1:1000 with 0.1 mol/l phosphate buffer pH 7.2 using the antisera to following viruses. Titers of antisera and donors are given in parentheses:

TMV-A1, aubergine strain (1024); TMV-D, dahlemense strain (256); TMV-P11 and TMV-P14, pepper strains (1024; all from Dr. D. Z. Maat, The Netherlands); ToMV (256; Dr. S. E. Albrechtsen, Denmark); TMV-U1, vulgare strain (128; Dr. S. Sarkar, FRG); TMV-WU1, tobacco strain (256; Dr. D. Z. Maat); CGMMV (256; Dr. D. Z. Maat); BNMV (256).

Antisera to TMV-A1, TMV-P11 and TMV-P14 were diluted with phosphate buffer (0.1 mol/l, pH 7.2) 1:400, homologous antiserum and antisera to TMV-D, ToMV, TMV-WU1 and CGMMV 1:100, and antiserum to TMV-U1 1:50 in order to make their final titer equal.

The procedure used for trapping and decoration was a slightly modified version of Milne and Luisoni (1977).

Trapping was done by floating collodion-film coated and carbon backed grids on the microdrops of various diluted antisera at room temperature for 5 mins. The grids were then washed with 30 drops of distilled water, drained briefly by touching their edges with filter paper, again floated on microdrops of diluted virus and incubated in a humid chamber at 37 °C for 1 hr. The grids were again washed and drained as above, and then immediately stained with 2 % uranyl acetate.

In each experiment, virus particles were counted at a displayed magnification of 21 000 × on fluorescent screen (5 × 4 cm). Ten counts from each grid square were made (Cohen *et al.*, 1982) and their mean was calculated. Fifteen such means were determined from 15 squares of three grids per treatment. The grand mean did not differ significantly from the individual means.

Decoration was done by floating the grids with trapped and unstained virions on the drops of diluted antisera in a humid chamber at 37 °C for 1 hr. The grids were then washed and stained as described above.

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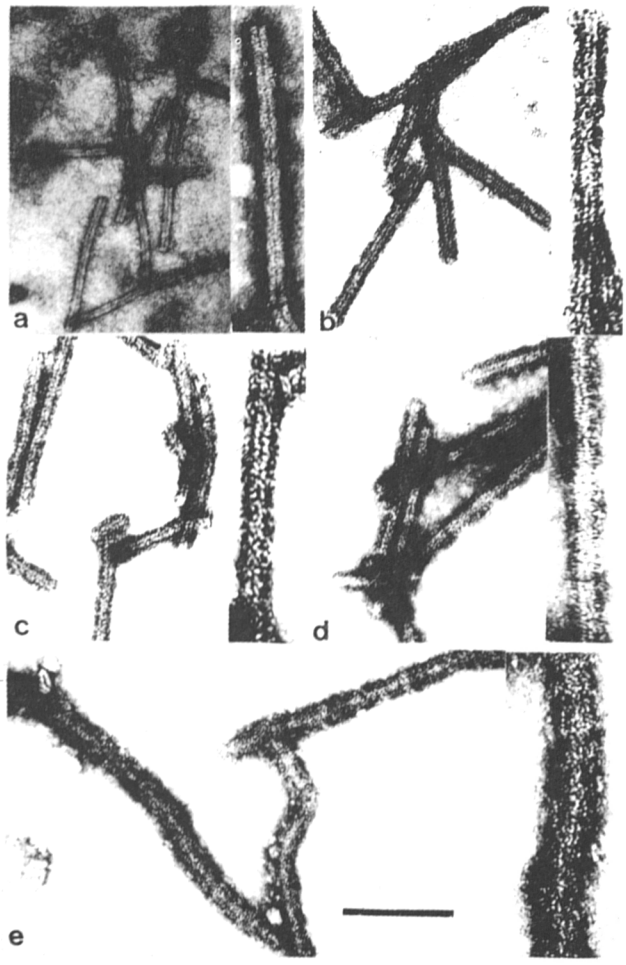

Fig. 1
Decoration of BNMV particles with antisera
(a) Undecorated; Decorated with: (b) TMV-WU1 antiserum, (c) TMV-U1 antiserum, (d) TMV-D antiserum, and (e) homologous antiserum. Barr = 100 nm. (Enlargement ratio of the insets to the respective micrographs is 1.88)

In trapping tests, trapping of BNMV was greatly enhanced by its homologous antiserum, and antisera to TMV-D, TMV-U1 and TMV-WU1. Antiserum to TMV-A1 had a moderate effect, whereas antisera to TMV-P11, ToMV and TMV-P14 had only a little one (Table 1).

In decoration tests, heaviest decoration of BNMV particles was observed with its homologous antiserum followed by TMV-D, TMV-U1 and TMV-WU1 antisera (Fig. 1a–e). There was a nonperceptible decoration with antisera to TMV-A1, TMV-P11, ToMV and TMV-P14. Antiserum to CGMMV failed to trap or decorate BNMV particles at all.

These studies showed that BNMV had close relationship to TMV-D, TMV-U1 and TMV-WU1, only a distant relationship to TMV-A1 and TMV-P11, and no relation to CGMMV. Nonetheless, BNMV failed to produce local

Table 1. Trapping of BNMV particles with antisera to different TMV strains

Antiserum to	No. of virus particles trapped (a)	Trapping intensity (b)
BNMV	77.8 ± 2.5	97
TMV-A1	8.6 ± 0.5	11
TMV-D	52.1 ± 2.0	65
TMV-P11	4.2 ± 0.3	5
TMV-P14	0.8 ± 0.2	1
ToMV	2.4 ± 0.2	3
TMV-U1	43.3 ± 2.1	54
TMV-WU1	36.2 ± 2.3	45
Preimmune serum	0.8 ± 0.2	1

a: Grand mean (see its definition in the text).
b: Ratio of particles trapped with the antiserum to the particles trapped with preimmune serum.

lesions on *Nicotiana sylvestris* Speg. & Comes, a characteristic feature to TMV-D (Melchers *et al.*, 1940; Melchers, 1942). Tobias *et al.* (1982) investigated the interrelationships among tobamoviruses of eggplant, pepper and tobacco on the basis of host response and serology. They found the eggplant strain (TMV-A1) to be serologically closely related to TMV strains F0, P8, P14 and SL, and quite different from WU1. In contrast, our eggplant/brinjal strain was closely related to strains D, U1 and WU1, but markedly different from A1 and P14. Based on these differences we consider BNMV as a new strain of TMV infecting brinjal.

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