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MORPHOLOGICAL CHANGES IN THE FLEXUOUS POTATO VIRUSES UPON DECORATION IN IMMUNOSORBENT ELECTRON MICROSCOPY

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Summary. – Effect of titer and pH of decorating antiserum, and the virus-source host species on the virion morphology upon decoration of potato viruses X, S and Y was studied. There was good decoration without any apparent adverse effect in the case of PVX and PVS with exception of pH 6.0 and antiserum titer 1:0.5, which caused decoration of only a small proportion of the virions. On the other hand, the PVY° virion morphology showed only slight to extensive disorganization depending on the pH and titer of the antiserum and the virus-source host species. Virion structure was, however, preserved when either PVY° (o strain) and its antiserum were made to react in liquid phase, or virions were fixed with 3 % glutaraldehyde before decoration.

Key words: potato virus X; potato virus S; potato virus Y; immunosorbent electron microscopy

Introduction

Immunosorbent electron microscopy (ISEM) is a very useful technique for the diagnosis of plant viruses (Torrance and Jones, 1981). It is also valuable in ascertaining the identity of a virus and its serological relationship to others (Roberts *et al.*, 1980; Shukla and Gough, 1984), provided the virion is not disfigured or disintegrated upon decoration. The disorganization of virions of barley yellow mosaic virus (BYMV) upon decoration was already reported (Huth *et al.*, 1984; Langenberg, 1986). Use of immunogold labelled antibodies has been recommended for the electron microscopic identification of such viruses (Langenberg, 1986), but it is not feasible for many laboratories. We, therefore attempted a study of different factors, like pH and titer of the decorating antisera, prior fixation of the virus, and source-host of the virus, which might influence the virion morphology during decoration. Present paper deals with results of such studies conducted with three flexuous potato viruses, PVX, PVS and PVY.

Materials and Methods

Viruses. Pure cultures of PVY° and common strains of PVX and PVS, maintained at Central Potato Research Institute, Shimla in tobacco (*Nicotiana tabacum* cv. Havana), *N. glutinosa* and potato, respectively, were used. Besides *N. tabacum* cv. Havana, potato cv. Kufri Chandramukhi and *Datura metel* were also used as hosts of PVY°. Infected leaf tissue (0.5 g) was ground finely with 2 ml of 0.1 mol/l phosphate buffer pH 7.2 (PB) in the case of PVX and PVS, and with PB with 0.1 mol/l EDTA in the case of PVY°. The macerate was diluted with the respective buffer before use, to 1:30 (w/v) for PVS and PVY°, and to 1:1000 for PVX.

Antisera specific to PVX, PVS and PVY° had microprecipitin titers of 1:2000, 512 and 512, respectively. Firstly, titer of all the antisera was adjusted to 1:512 with saline and then they were further diluted to 1:0.5, 1:2.5, 1:10 and 1:50 in the case of PVX and PVS, and to 1:1, 1:2.5, 1:10 and 1:50 in the case of PVY°.

Trapping and decoration. Grids coated with individual antisera (pH 7, dil. 1:0.5 for PVX and PVS, but 1:1 for PVY°) were used to trap virions from respective crude extracts. Trapped virions were then decorated (Khurana and Garg, 1989) with homologous antisera at different pH and dilutions required for the experiment.

Antigen-antibody reaction in liquid phase. PVY° extract and its homologous antiserum (pH 7, dil. 1:50) were made to react by mixing 0.1 ml of each and incubating at 37 °C for 1 hr. The antigen-antibody clumps thus formed were trapped on PVY° antiserum coated grids and stained with 2 % aqueous uranyl acetate.

Fixation of PVY° with glutaraldehyde. Either virus extract was pre-fixed with 3 % glutaraldehyde solution for 2 hrs at room temperature followed by trapping of the virions on antiserum-coated grids and then decorated (pH 7, dil. 1:10 of the decorating antiserum), or virions were first trapped on antiserum-coated grids followed by their fixation with glutaraldehyde and then decorated. At least two grids were prepared in each case and three squares per grid were screened with JEOL-100S electron microscope at 80 kV. The studies were repeated twice to ascertain the consistency of results (Pares and Whitecross, 1985).

Results

There was no adverse effect on the morphology of PVX and PVS virions during decoration. Heavy decoration was observed under all conditions except at antiserum pH 6 and dil. 1:0.5 when only a small proportion of virions showed decoration and the majority remained undecorated (Fig. 1a, Fig. 1b).

There was fragmentation of PVY° virions upon decoration with all titers and pH of decorating antiserum. Moderate to high degree of decoration without any apparent change in virion morphology was observed with certain combinations of pH and titer of the antiserum (pH 6, 7 and 8, dil. 1:1; pH 6, dil. 1:50; pH 8, dil. 1:10 and 1:50). The antiserum at pH 6, dil. 1:10, and at pH 7, dil. 1:10 and 1:50 caused varying degrees of degeneration of virions from all the three virus sources. The antiserum at pH 6 and dil. 1:10 caused disorganization of virions which became highly flattened (Fig. 1c). The antiserum at pH 7, dil. 1:50 caused complete disintegration of virions from tobacco (Fig. 1f) and loosening of virions allowing stain entry, which gave to virions electron dense rope-like appearance in the case of potato and *D. metel* (Fig. 1e). The antiserum at pH 7, dil. 1:10 caused loosening of virions and stain entry irrespective of virus source employed (Fig. 1d).

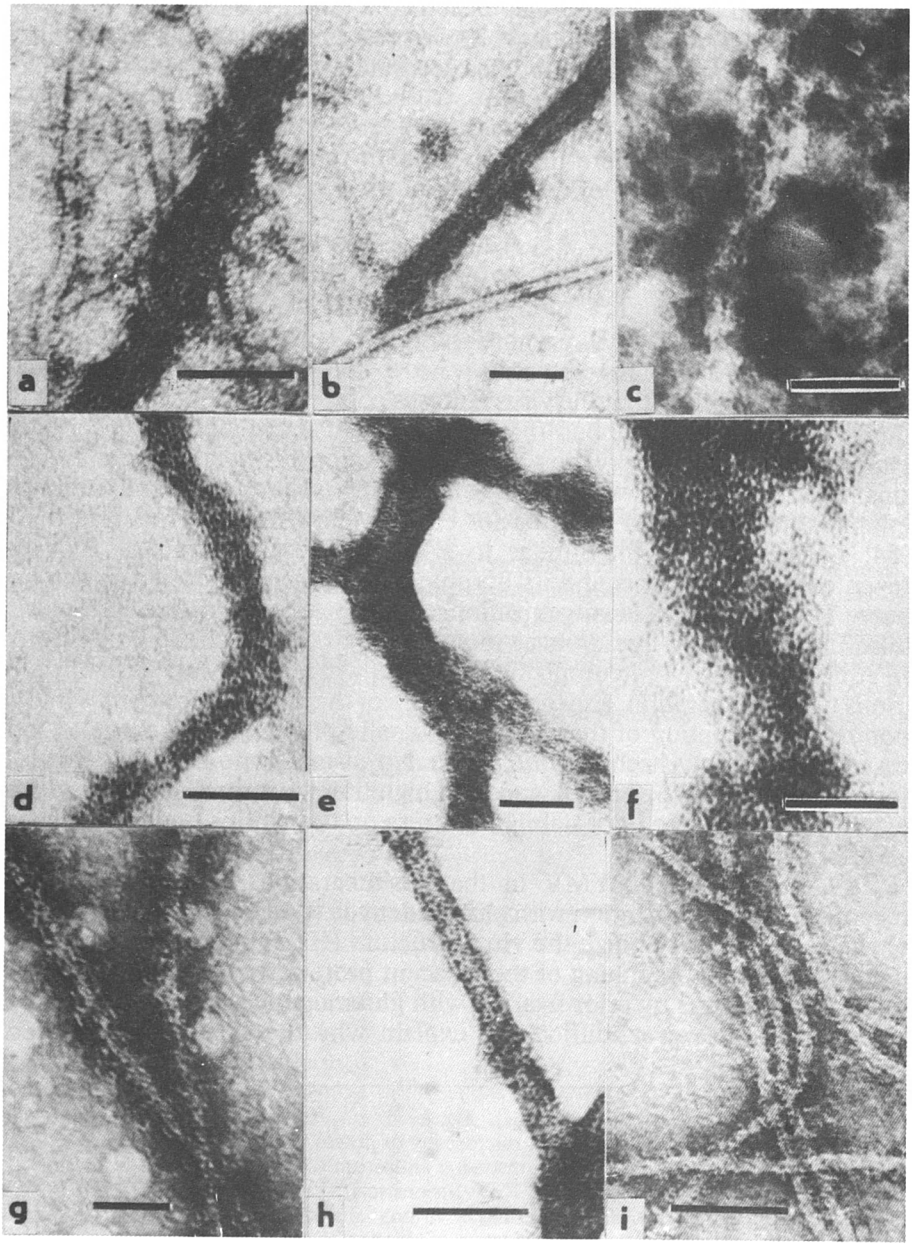

Fig. 1
For legend see page 410

Disintegration of virions was also observed if either the infected tissue or the virions were fixed with 3 % glutaraldehyde prior to decoration (Fig. 1g, Fig. 1h). Nonetheless, the amount of antibodies decorating the virions was comparatively lesser.

Degeneration of PVY° virions was also prevented when the virus and its homologous antiserum were made to react in the liquid phase (Fig. 1i) even when the pH and dilution of the antiserum used were 7 and 1:25, respectively.

Discussion

Decoration of only a small proportion of PVX and PVS virions when pH and dilution of the decorating antisera were 6 and 1:0.5, respectively, may be explained by assuming that the virus cultures comprised virions differing in their affinity to the antisera, that became evident only at above mentioned conditions. Another interesting observation was deterioration/disintegration of PVY° virions upon decoration with antiserum having specific pH and titer. Such a phenomenon has been reported for barley yellow mosaic virus (Huth *et al.*, 1984; Langenberg, 1986). Similar to BYMV, disorganization of PVY° was prevented when the virus and its homologous antiserum reacted in the liquid phase. The absence of disorganization of PVY° virions in the case of virus-antiserum reaction in liquid phase may be due to (a) crosslinking of virions by the antibodies, thereby neutralizing the stress which otherwise destabilizes the virions during decoration, and/or (b) absence of the stress which comes into play upon direct adsorption of the virions onto solid phase causing partial degradation of the virions (Altschuh *et al.*, 1985). No disintegration observed with low titer antiserum at pH 6, 7 and 8, and with high titer antiserum at pH 6 and 8 may be due to a low degree of destabilizing stress on the virions under such specific conditions.

PVY° differed from BYMV in that disintegration of the latter was not prevented using prior fixation with glutaraldehyde (Langenberg, 1986). Glutaraldehyde is known to stabilize the virus structure (Hajimorad and Francki, 1991) obviously due to crosslinking of the adjacent protein subunits. Non-disintegration of PVY° virions by prior fixation with glutaraldehyde may be attributed to this fact. It is, however, difficult to explain why the same process failed to

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Fig. 1

Immunosorbent electron microscopy of potato viruses X, S and Y

Decoration of PVX (a) and PVS (b) virions using antiserum dil. 1:0.5 and pH 6 when only a few virions showed decoration. Decoration of PVY° virions (c–i) using antiserum dil. 1:10, pH 6 (c) and pH 7 (d); dil. 1:50, pH 7 (e, f). Note flattening of virions (c) and loosening of virions (from potato) with stain entry (d, e) to complete disorganization of virions (from tobacco) (f). There was decoration without disorganization of virions when either virions (g) or the virus extract was fixed with 3 % glutaraldehyde prior to decoration at pH 7, dil. 1:10 or 1:50. Normal appearance and decoration of virions (i) when the virus and antiserum reacted in liquid phase. Bar = 100 nm.

stabilize the BYMV virions. Reduction of the amount of decoration of glutaraldehyde-fixed virions may be due to reduction of the number of surface epitopes available for the binding of antibodies.

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