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EFFECT OF VARIOUS STAINS ON THE STABILITY AND PARTICLE LENGTH DISTRIBUTION OF THREE POTY VIRUSES*

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SUMMARY

Four negative stains were tested on Peanut mottle virus (PMV), two isolates of Peanut green mosaic virus-systemic necrosis (PGMV-SN) and systemic mosaic (PGM V-SM), to determine their effects on the stability and particle length distribution.

When phosphotungstate (PTA) at pH 7.0 was used, particle length and width of PMV and PGMV-SM were considerably affected resulting in variation, whereas PGMV-SN was less affected. When uranyl acetate (UA) and uranyl formate (UF) were used, the particles of PGMV-SM and PMV were better preserved, in contrast to PGMV-SN which was unstable in both the uranyl stains. PGMV-SN was well preserved in ammonium molybdate (AM) of pHs 3.5 and 6.5 but was disrupted in pH 8.0. Although PMV could be stained with AM of pH 3.5, particles were poorly stained in AM of pHs 6.5 and 8.0. Particles of PGMV-SM were poorly stained in AM of all the three pH values.

Three viruses belonging to the potato virus Y group were isolated from field infected groundnut plants. Peanut mottle virus (PMV) produced mottling and dark green patches on systemically infected leaves (Reddy *et al.*, 1978). The other two viruses which were serologically related to Peanut green mosaic virus (PGMV) either produced systemic necrosis (PGMV-SN) or systemic mosaic (PGMV-SM) in groundnut plants (Sreenivasulu *et al.*, 1981). We report here the effect of four commonly employed negative stains viz., phosphotungstic acid, ammonium molybdate, uranyl acetate and uranyl formate, on the stability and particle length distribution of PMV, PGMV-SN and PGMV-SM.

MATERIALS AND METHODS

Peanut mottle virus isolated from the Punjab state of India and PGMV-SN and PGMV-SM isolated from farmers field near Tirupati, Andhra Pradesh state, were maintained on groundnut (cv. TMV-2) plants by mechanical sap inoculation.

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Immunosorbent Electron Microscopy

PMV and PGMV antisera produced at ICRISAT, having ring precipitin titres of 1/2560 and 1/256, respectively were used to trap the viruses by Immunosorbent Electron Microscopy (ISEM).

Negative Stains

A 2% solution of phosphotungstic acid (PTA) was prepared in distilled water and pH was adjusted to 7.0 with 1N NaOH.

Aqueous 2% ammonium molybdate (AM) was prepared and adjusted to pHs 3.5, 6.5 and 8.0 with 1N HCl or 1N NaOH.

Uranyl acetate (UA) at 2% ooncenirations was prepared in distilled water and employed without adjusting the pH. A saturated aqueous solution of uranyl formate (UF) was prepared, filtered and few drops of 1N NaOH were added gradually until an insoluble precipitate appeared. The insoluble precipitate was removed by filtration and the stain was diluted in equal volume of water (Barnett and Murant, 1970).

Sample Extract for ISEM

Small portions of young leaflets mixed with corbulundum powder (600 mesh) were ground in a mortar with 0.5 ml of 0.07 M phosphate buffer pH 6.0 (PB). The extract was centrifuged at 3000 rpm for 10 min and the supernatant was used for trapping the virus particles. Formvar carbon coated electron microscope grids were floated for 15 min on 10 µl drops of antisera prepared in PB and washed with 20 drops of PB. Excess buffer was drained by the edge of a filter paper and the grids were then floated on 20 µl of sample extract and incubated for 60 min at room temperature. The grids were stained by washing with 5–10 drops of the stain (Lesemann *et al.*, 1980). The above procedure was used for PTA and AM stains. In the case of uranyl stains, the grids were washed with 20-30 drops of distilled water prior to staining.

The grids were observed in a Philips 201 C transmission electronmicroscope. Randomly selected areas on grid were photographed at magnifications of 10,000X, 28,500X and above. The particle measurements were done on

micrographs of calibrated magnifications using a micrometer lens. The magnification calibration of the microscope was done using a germanium shadowed carbon replica of a ruled diffraction grating having 28, 800 lines per inch.

RESULTS AND DISCUSSION

Phosphotungstate at pH 7.0 considerably affected the PMV (Fig. 1) and PGMV-SM particles, whereas it preserved the PGMV-SN particles (Fig. 4). PTA stained densely and distorted the virus particles leading to variations in the particle length and width. A similar effect of PTA on another poty virus Iris Fulva mosaic virus, has been reported (Barnett and Alper, 1977). The particle length distribution of PTA stained PMV ranged from 660-740 nm. A mean value of 740 nm for particle length of a severe strain of PMV was given by Sun and Herbert, 1972. The particle length distribution of PTA stained PGMV-SM and PGMV-SN ranged from 690-750 nm and 690-710 nm, respectively (Fig. 5).

When uranyl stains were used, the particles of PMV and PGMV-SM were better preserved although there was a slight increase in their overall length and width; this is probably due to 'swelling' of the capsomeres. UF was preferred over UA for staining PMV since no apparent damage to the particles was visible (Fig. 2). The intact PMV particles measured 720 nm in UF and 690-720 nm in UA. However, UA preserved PGMV-SM better with particle length ranging from 710-720 nm, while with UF the lengths ranged from 700-750 nm.

PGMV-SN was unstable in both UA and UF with lengths varying between 750-850 nm and 650-750 nm, respectively.

AM stained PGMV-SN particles were well preserved at pHs 3.5 and 6.5 with length distribution of 650-700 nm. At pH 8.0 AM caused fragmentation of particles. PMV stained well and was fairly stable in AM of pH 3.5 with particle length of 750 nm. However, at pH 8.0 the staining was poor (Fig.3). PGMV-SM particles were stained poorly in all the three pH values of AM.

In the absence of EDTA or magnesium chloride which have been shown to distort poty viruses (Barnett and Alper, 1977), and by using the ISEM technique, which renders the preparation free of most of the host material, we conclude that staining with UF is preferable for determining the particle length distribution,

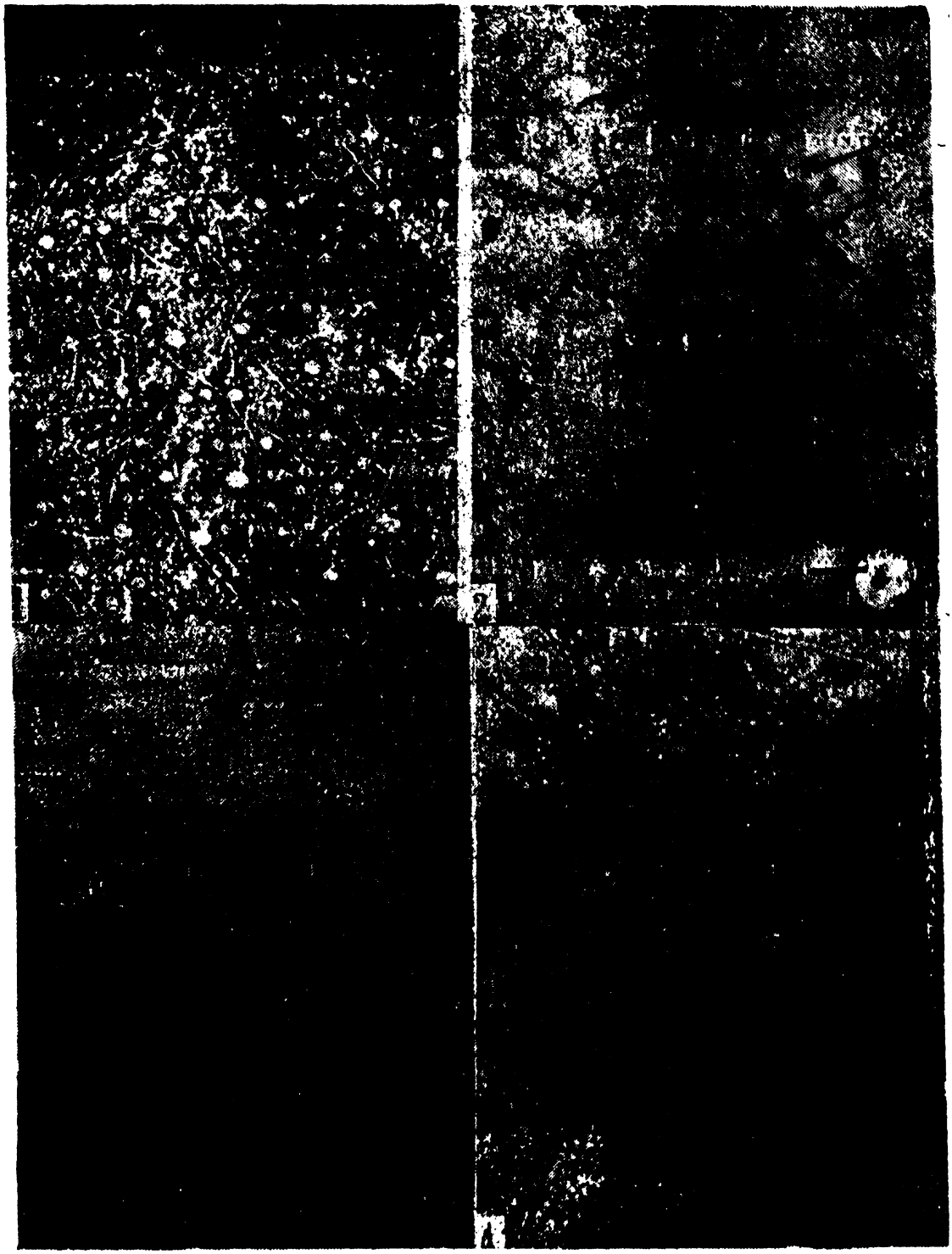

Fig. 1. Peanut mottle virus stained with uranyl acetate. Bar represents 330 nm.
Fig. 2. Peanut mottle virus stained with uranyl formate Bar represents 500 nm.
Fig. 3. Peanut mottle virus stained with ammonium molybdate at pH 6.5. Bar represents 260 nm.
Fig. 4. Peanut green mosaic virus-systemic necrosis stained with phosphotungstate pH 7. Bar represents 175 nm.

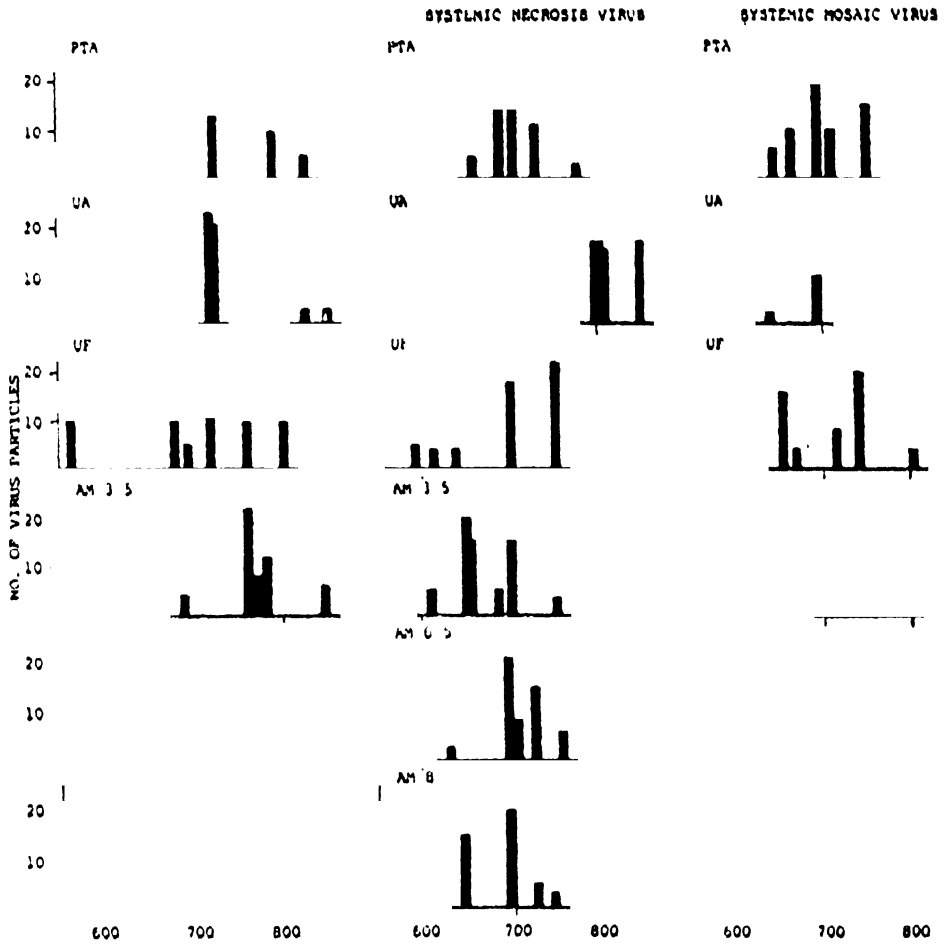

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Fig. 5. Histogram of particle length distribution of three potyviruses Peanut mottle virus (PMV), Peanut green mosaic virus (Systemic necrosis) and Peanut green mosaic virus (Systemic mosaic), when stained with phosphotungstate (PTA), uranyl acetate (UA), uranyl formate (UF) and ammonium molybdate (AM).

since in general it does not distort the particles. PGMV-SN is stable in AM of pHs 3.5 or 6.5 and therefore it is a reliable stain for determining particle length distribution. UA is preferred for discerning the sub unit structure of the particles.

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