PURIFICATION AND SEROLOGY OF COWPEA (*VIGNA SINENSIS* SAVI) MOSAIC VIRUS

An aphid and seed borne mosaic disease of cowpea (*Vigna sinensis* Savi) was reported by Nariani and Kandaswami[3] (Fig. 1). Haque and Chenulu[2] studied the virus vector relationship with respect to the vector, *Aphis craccivora*. The present paper deals with the purification, electron microscopy and serology of the causal virus.

FIG. 1. Cowpea mosaic

The virus was multiplied in cowpea var. Pusa Phalguni in the insect-proof glass house. For purification, mosaic affected leaves were minced in a waring blender with 1·5 × wt./Vol. M/20 phosphate buffer (pH 7·5) containing 0·1% thioglycollic acid. The juice was expressed through a double layer of muslin cloth and *n*-butanol added, drop by drop to 8·5% concentration. The mixture was stored in a frigidaire at 4° C for 2 hours and centrifuged at 12,500 rpm for 25 minutes. The supernatant was passed through Whatman filter paper No. 1 and centrifuged at 30,000 rpm for two hours to pellet the virus. The pellets were suspended in M/30 phosphate buffer (pH 7·5) and centrifuged at 7,000 rpm for 10 minutes. The supernatant was clear and highly infective and contained numerous rod shaped particles about 300 nm long (Fig. 2) when examined under the electron microscope Philips EM 300. The material for electron microscopy was prepared by mounting on formvar coated copper grids and staining with 1% phosphotungstic acid or 0·5% uranyl acetate.

The antiserum of the CpMV was prepared by injecting white albino rabbits intramuscularly with purified virus preparations emulsified with Freund's

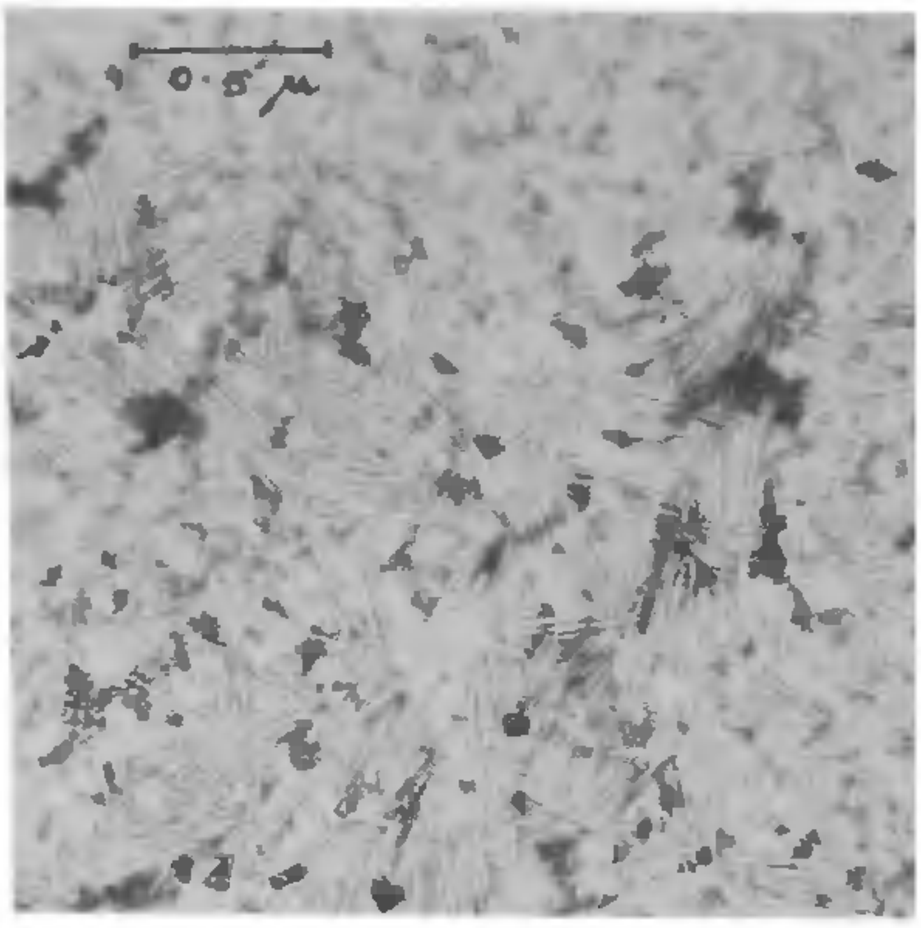

FIG. 2. Electron micrograph of the virus PTA stained

adjuvant (1 : 1) thrice at weekly i .ervals followed by an intravenous injection with purified virus alone after a week. The blood was collected after 10 days following the last injection, stored overnight and serum separated from the red blood cells by centrifugation at 7,000 rpm for 15 minutes. It was stored with 50% glycerine. The antiserum was found to be specific and reacted in precipitin tube tests with the diseased plant sap and the purified virus preparation but not with healthy plant sap. It had a titre of 1 : 1024.

The antiserum gave a positive precipitin reaction in precipitin tube tests with a TMV strain showing relationship with the latter. Thus the CpMV belongs to the tobamovirus group and is distinct from the spherical cowpea mosaic virus reported by Chenulu *et al.*[1] but resembles the cowpea chlorotic spot virus reported by Sharma and Varma[4] in particle morphology.

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