

PURIFICATION AND PARTIAL CHARACTERIZATION OF SESBANIA MOSAIC VIRUS

P. SREENIVASULU AND M. V. NAYUDU

Department of Botany, S. V. University, Tirupati 517 502, India

ABSTRACT

A virus inciting mosaic disease on *Sesbania grandiflora* has been characterized. It was purified by differential centrifugation and by density gradient centrifugation. The virus particles were spherical and 28 nm in diameter. The molecular weight of the virus protein was found to be 32000 daltons.

INTRODUCTION

SESBANIA grandiflora Pers. is grown extensively as shade and standard in betel vine and pepper gardens. It has good fodder, food and medicinal value. A preliminary investigation has been made on mosaic disease of *S. grandiflora* occurring naturally in fields around Tirupati¹, and herein the authors describe the successful purification and partial characterization of the virus.

MATERIAL AND METHODS

The sesbania mosaic virus was maintained on *Sesbania* by mechanical inoculation. The local lesion assay host for this virus is *Cymopsis tetragonoloba* Taub. (cluster bean).

The virus infected *Sesbania* leaves were collected and washed with tap water and then extracted with 0.1 M potassium phosphate buffer (pH 8.0) containing 0.5% 2-mercaptoethanol and 0.01 M sodium diethyldithiocarbamate. The leaf tissue was macerated in a pre-cooled mortar and pestle using cold extracting buffer (3 ml/g leaf) and squeezed through two layers of muslin cloth. The sap was treated with cold chloroform (10%) for 10–15 min with intermittent shaking and centrifuged at 5000 rpm for 10 min. The virus in the aqueous phase was pelleted in SW-27 tubes at 24,000 rpm for 80 min. The pellet was suspended in 0.01 M phosphate buffer, pH 8.0, clarified at 8000 rpm for 10 min and the supernatant layered on sucrose gradients (10–40%) in SW-27 tubes. After rate zonal density gradient centrifugation at 24,000 rpm for 80 min., the virus from the single light scattering zone was diluted with buffer and pelleted at 36,000 rpm for 1 hr in a Beckman R-40 rotor. The virus pellet, suspended in 0.01 M phosphate buffer (pH 8.0), was used for further studies.

Infectivity of the purified virus was checked on both *Sesbania* and cluster bean. The ultraviolet absorption spectrum of the virus was taken in Gilford recording spectrophotometer. Purified virus preparations stained in neutral 2% sodium phosphotungstate were

observed in Philips 201 C model electron microscope. The virus protein molecular weight was determined by polyacrylamide gel electrophoresis^{2,3}. Purified virus pellets were solubilized in 6 M urea in 0.0625 M Tris-HCl buffer (pH 8.0) containing 2% sodium dodecyl sulphate (SDS) and 1% (v/v) 2-mercaptoethanol. The resolving gel contained 10% acrylamide and 0.27% bis acrylamide prepared in 0.375 M Tris-HCl buffer (pH 8.8) with 1% SDS and 0.5 M urea. A spacer gel of 3.6% acrylamide and 0.09% bis acrylamide in 0.125 M Tris-HCl buffer (pH 6.8) containing 0.1% SDS and 0.5 M urea, was layered on the resolving gel. The solubilized virus samples were co-electrophoresed with markers at 30 V for 6 h. The gels were stained and scanned at 540 nm in a Gilford recording spectrophotometer⁴. The markers used to estimate the molecular weight of virus polypeptide were phosphorylase B, bovine serum albumin, egg albumin, chymotrypsinogen, soybean trypsin inhibitor and lysozyme (Bio-Rad Laboratories). Orcinol test was performed to know the nature of the purified virus nucleic acids^{5,6}.

RESULTS AND DISCUSSION

A single light scattering zone, about 5.5 cm from the bottom of the tube, was observed after density gradient centrifugation. Samples from this zone diluted in phosphate buffer produced typical symptoms of disease on both *Sesbania* and cluster bean. The ultraviolet absorption spectrum of the purified virus (figure 1) showed $A_{260/280}$ of 1.50 indicating that virus contained about 18% nucleic acid⁷. The $A_{260/242}$ ratio was 1.33 indicating that the sample is almost free from the protein contaminants, as also known from gel electrophoresis. Assuming an extinction co-efficient of 7.0, nearly 1 mg of virus was obtained from 2 g of leaf. Orcinol test indicated that the virus contained ribonucleic acid. Purified virus preparations contained spherical particles of 28 nm diameter (figure 2). When disrupted with SDS, urea and 2-mercaptoethanol, the virus formed one band following electrophoresis in 10% acrylamide gels. The molecular weight of the virus polypeptide was

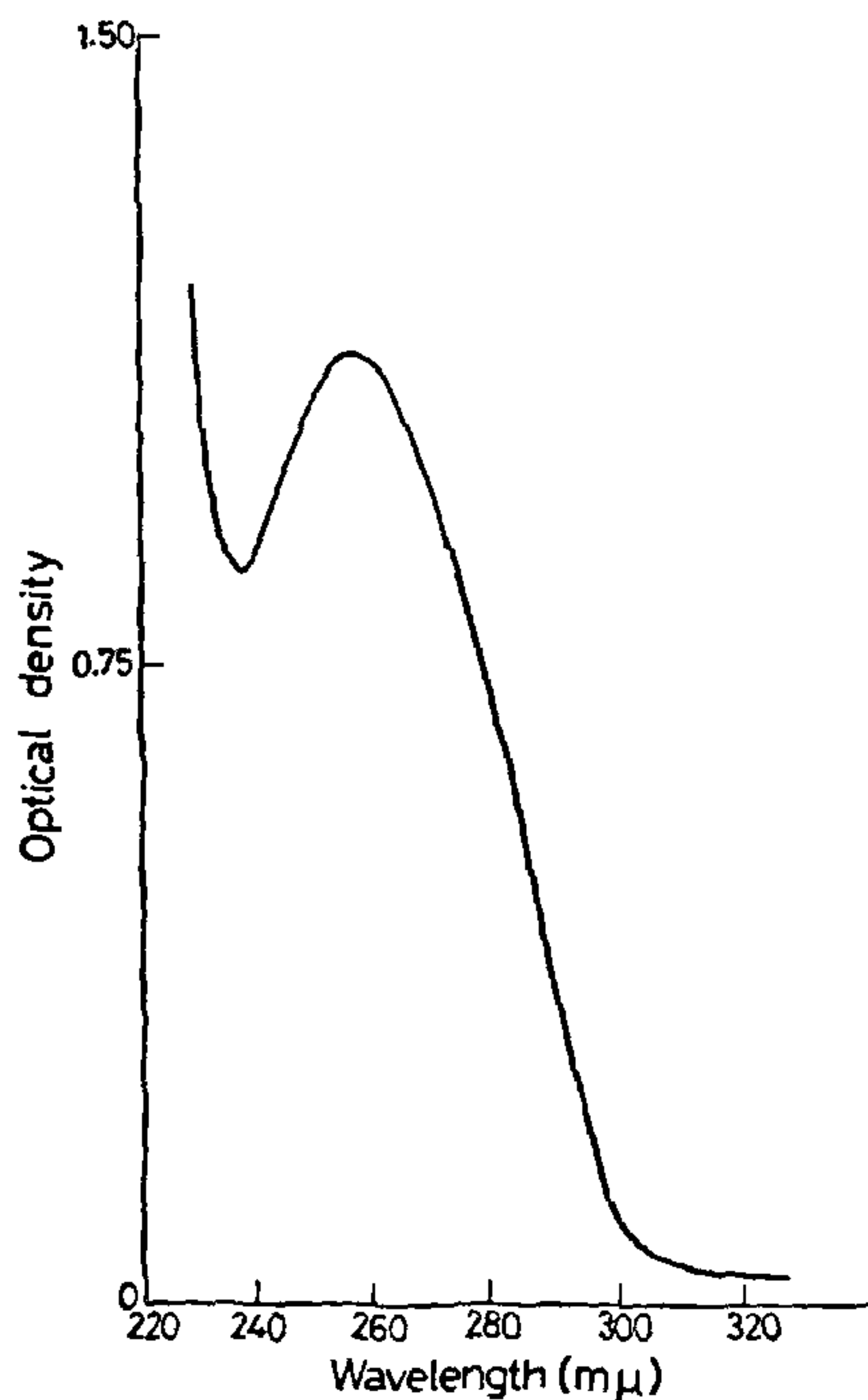


Figure 1. UV spectrum of purified virus.

estimated to be 32,000 daltons. Thus the sesbania mosaic virus is a single component spherical particle virus with RNA. Its physical properties of TIP: 80-85; DEP; 1:300,000 - 500,000; LIV: 21 days at 28-30° C and host range limited to legumes (systemic in bean, broad bean besides *Sesbania*, and chlorotic local lesions in cluster bean) indicate its probable affinity to cowpea mosaic virus group, but differs in having a single component protein¹. Serological and RNA characterization will clarify the affinities of this virus.

ACKNOWLEDGEMENT

The authors are thankful to Dr. D. V. R. Reddy,



Figure 2. Negatively stained spherical virus particles purified from *Sesbania* leaves.

Principal Virologist, ICRI SAT, Hyderabad for facilities.

1. Solunke, B. S., *Studies on Sesbania Mosaic Virus Disease*, M. Phil. Thesis, S. V. University, Tirupati, 1979.
2. Laemmli, U. K., *Nature (London)*, 1970, **227**, 680.
3. Reddy, D. V. R. and Black, L. M., *Virology*, 1977, **80**, 336.
4. Reddy, D. V. R. and Macleod, R., *Virology*, 1976, **70**, 274.
5. Kirby, K. S., *Biochem. J.*, 1965, **96**, 266.
6. Shatkin, A. J. In *Fundamental techniques in virology* (eds.) Habel, K and Salzman, N. P., 1969, pp. 231-237, Academic Press, New York.
7. Gibbs, A. and Harrison, B., *Plant virology: The principles*, 1976 Edward Arnold, London. 292 pp.