

PURIFICATION OF POTATO VIRUS Y AND THE PRODUCTION OF ANTISERUM

S. M. PAUL, KHURANA, M. N. SINGH and SHIV KUMAR

Division of Plant Pathology, Central Potato Research Institute, Solan 171 001, India.

Potato virus Y (PVY) is an important virus. It is the most important virus causing potato mosaic under tropical conditions. A number of PVY strains with different field symptoms, occur in our country, having a high degree of serological relationship^{1,2}. It is difficult to diagnose the virus under field conditions unless detected serologically or electron microscopically. Viruses of the poty-group tend to aggregate, both end-to-end and laterally, posing problems during purification³⁻⁵. Most of the earlier efforts to purify PVY either yielded low quantity of the virus or the resultant antiserum had low titre⁶⁻⁸. The present attempt was successful to purify the type strain of PVY by a simple (partial) purification schedule.

The stock culture of PVY^o was maintained in *Datura metel* and multiplied in tobacco var Havana-425/White Burley. The inoculated leaves of tobacco Havana-425 were harvested 10 days after inoculation but for White Burley, 4-week-old infection i.e. leaves showing mosaic, served as the virus source. Infectivity of the leaf extract was determined on 6 half leaves of *Chenopodium amaranticolor*. Freshly harvested infected leaves were chilled for an hour in ice water. The chilled leaves were ground manually with 1:1 (W/V) phosphate buffer (0.1 M, pH 7.0), supplemented with 0.2%, 2-mercaptoethanol and 15 mM each of DIECA and EDTA. The slurry obtained was strained through 2-4 layers of cheese cloth. The filtrate was clarified by centrifugation at 10,000 rpm for 15 min in a refrigerated centrifuge (Remi C-24). The supernatant was clarified by mixing with 1:1 (V/V) mixture of chloroform + carbon tetrachloride and vigorous shaking for 15-20 min. N-butanol (8%) was also found effective for clarification. The aqueous phase was collected and recentrifuged at 2,500 rpm for 10 min. Triton X-100 (3%) was then added to the supernatant and stirred on a magnetic stirrer for 15 min. The virus was precipitated by the addition of 4% PEG-6,000 + 0.2 M NaCl, initially by stirring for 15 min and then incubating it at 4°C for 75-90 min. The precipitate was pelleted (10,000 rpm for 30 min), resuspended in 0.01 M, phosphate buffer (pH

7.0) containing 5 mM EDTA, 0.5 M urea and 0.1% of 2-mercaptoethanol and clarified by centrifugation (2,500-5,000 rpm for 5 min). The final preparation was dialyzed overnight against 1000 c.c. of 0.01 M phosphate buffer at 4°C. The product after dialysis, was creamish/pinkish and opalescent. It was highly infective at a dilution of 1:10,000. A very large number of flexuous virus particles were observed in negatively stained preparation under an electron microscope (JEM-100S) measuring between 670 and 730 nm in length (figures 1 and 2) without much aggregation. A number of particles, however, varied in their length because of fragmentation or end-to-end union.

New Zealand white rabbits (9-12 months old) were used for production of antiserum. They were immunized by four intramuscular injections. First time, the above antigen (1 ml/rabbit) was emulsified with 1:1 Freund's complete adjuvant (Difco) for injection. But for the remaining three injections, the incomplete adjuvant was used. The rabbits were bled through marginal ear vein 10 days after the last

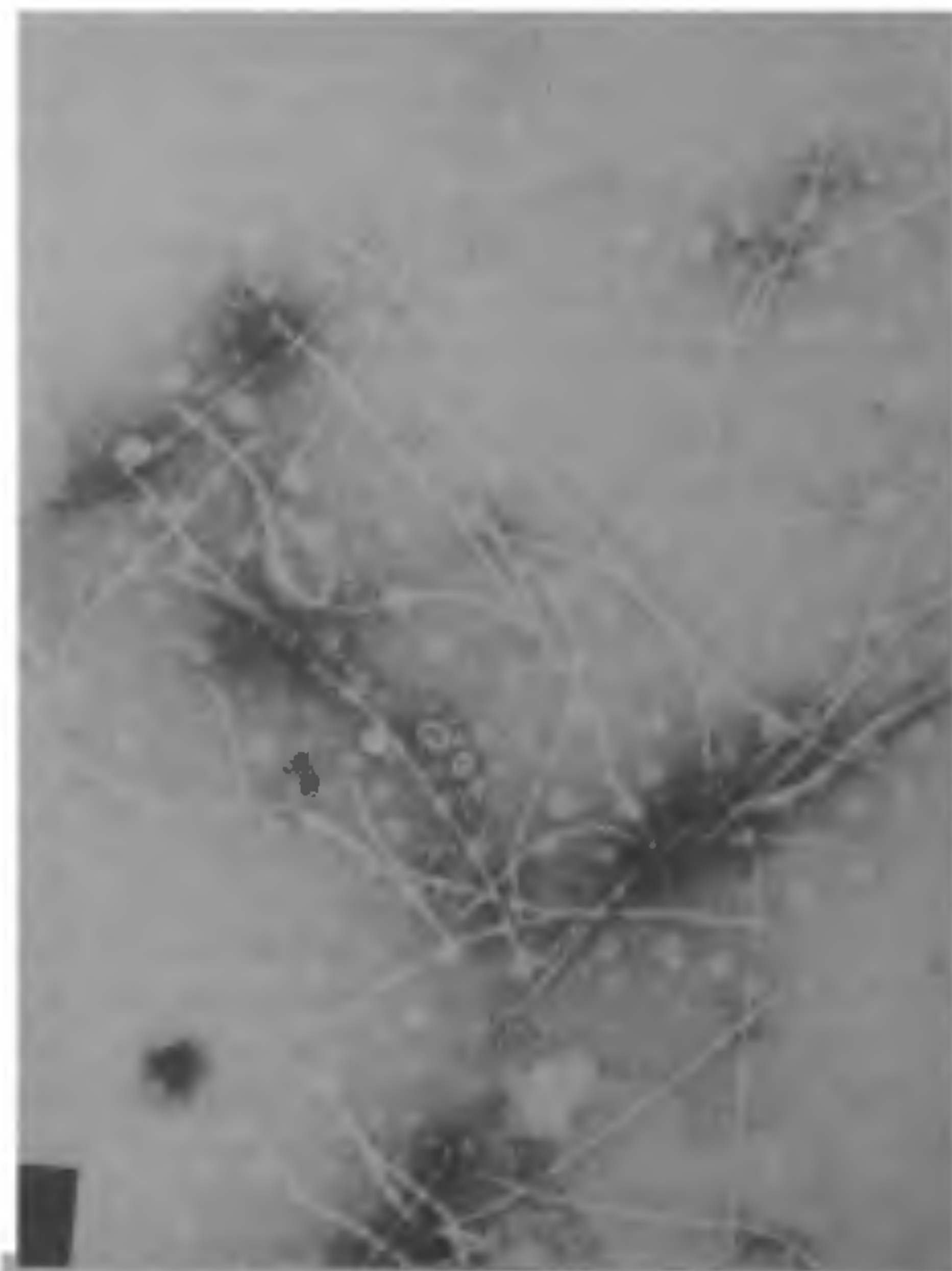


Figure 1. Electron micrograph of a negatively stained preparation showing PVY particles ($\times 41,500$).

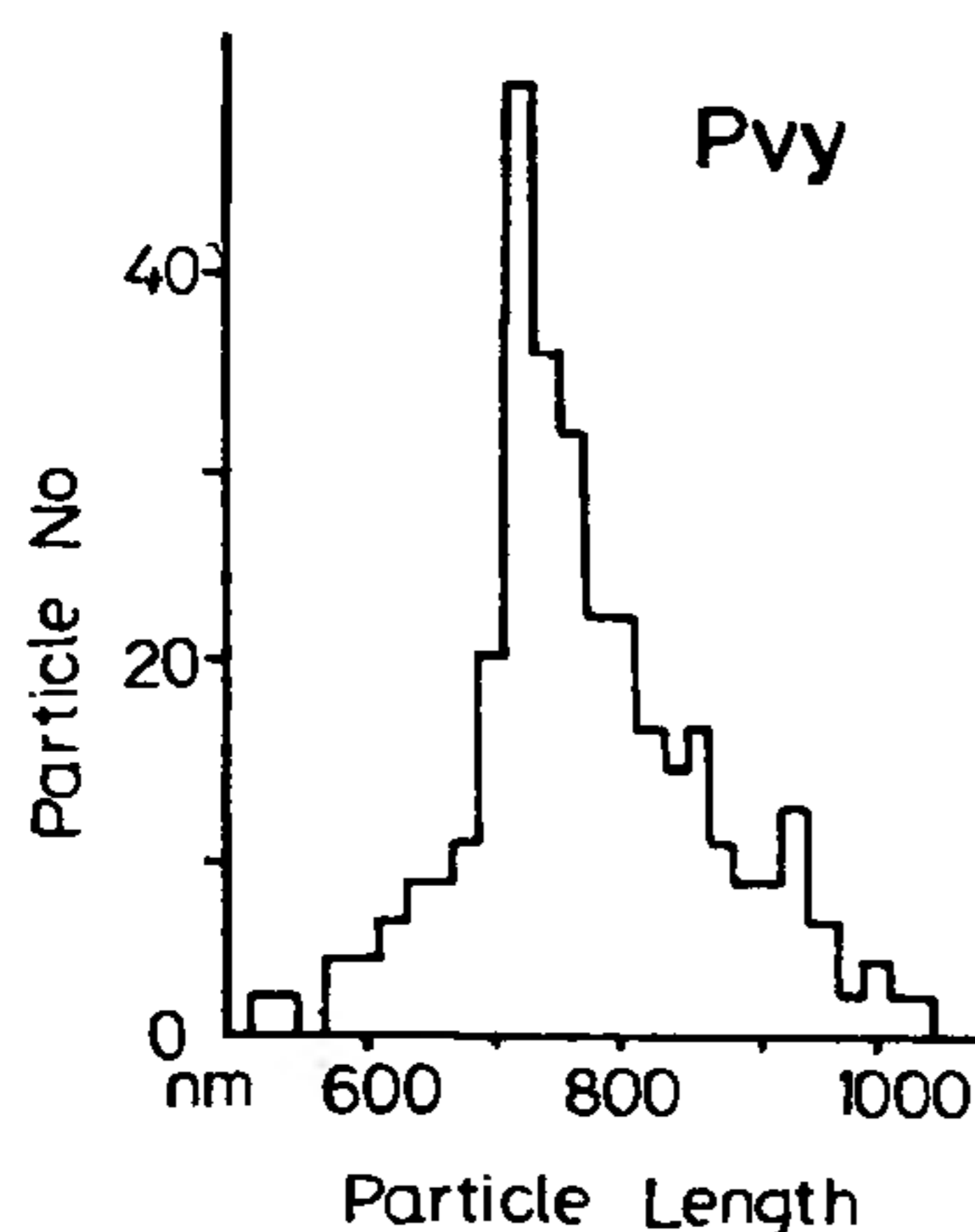


Figure 2. Length distribution curve of PVY° (purified from tobacco cv Havana-425).

injection. The antiserum obtained was absorbed for 2 hr at 37°C with an equal volume of similarly purified host (healthy tobacco) protein. The absorbed antiserum was clarified by low speed centrifugation. The antiserum produced had a titre of 1:1024 (through microprecipitin tests), showed strong specificity against many isolates/strains-Y°, Y^c and Y^N of PVY and also field samples of potato mosaic. It was comparable in reactions with the reference antisera against PVY from Netherlands and Canada. In double diffusion gel tests⁹, the virus gave sharp continuous bands against PVY° antisera both from Canada and Netherlands (figure 3). Non-specific reaction was not observed against the sap of healthy potato, *D. metel*, *Physalis floridana* or potato viruses X, S and M. A 260/A 280 ratio

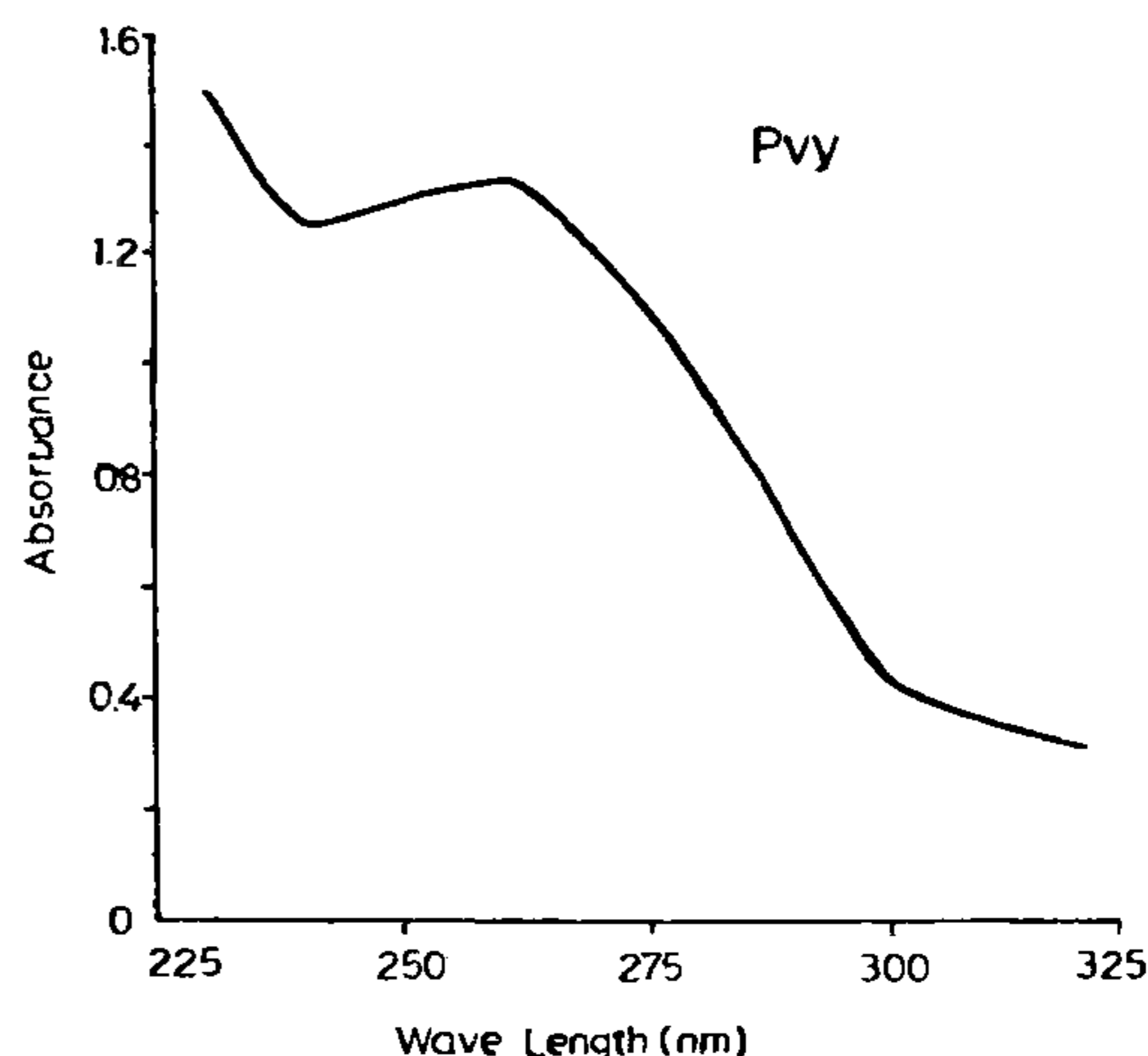


Figure 4. UV absorption spectrum of partially purified PVY° (from tobacco cv Havana-425).

was 1.29 and A max/A min ratio was 1.06—characteristic of the poty-viruses¹⁰. The virus yield ranged from 2.25–2.5 mg/100 g of leaves.

Purification of PVY is reported to be difficult because the virus gets lost during clarification. After freezing the leaves were found to yield very little of the virus. Chilling of virus-infected leaves in ice cold-water was enough. Tris-HCl buffer (0.05 M, pH 8.0) was highly deleterious for extraction of the virus. The borate (0.01M, pH 7.5) and citrate (0.5 M, pH 7.5) buffers for extraction resulted in the loss of virus infectivity. Such a loss occurs commonly due to aggregation of virus particles but without loss of serological activity^{7-9,11}. A number of prolonged, sophisticated purification methods are known for poty-viruses¹². Damirdagh and Shepherd⁴; and Delgado-Sanchez and Grogan¹³ prevented the aggregation by using urea and 2-mercaptoethanol. This may either be due to their hydrophobic response or the action of EDTA/DIECA as surface active agents. PEG + NaCl have been effective for the precipitation of other poty-viruses¹⁴⁻¹⁷.

The authors are grateful to Dr N. M. Nayar and Dr G. S. Shekhawat for encouragement, and to Dr I. D. Garg for electron microscopy. Sincere thanks are also due to Dr J. A. de Bokx, IPO, Netherlands and Dr R. P. Singh, Agric. Canada, Fredericton for gift of antisera.

14 July 1986; Revised 28 November 1986

1. Khurana, S. M. Paul, Singh, M. N. and Nagaich, B. B., *J. Indian Potato Assoc.*, 1979, **6**, 228.



Figure 3. Ouchterlony double diffusion gel test of PVY° with the indigenous and imported antisera. Wells contained 1) healthy leafsap; 2, 4, 6) indigenous antiserum; 3, 5) antisera to PVY from Canada/Netherlands, and 7) PVY° antigen.

2. Singh, Sarjeet, Khurana, S. M. Paul and Lal, S. B., *J. Indian Potato Assoc.*, 1983, **10**, 42.
3. Shepherd, R. J. and Pound, G. S., *Phytopathology*, 1960, **50**, 797.
4. Damirdagh, I. S. and Shepherd, R. J., *Phytopathology*, 1970, **60**, 132.
5. Rishi, N. and Rishi, S., *Indian J. Virol.*, 1985, **1**, 79.
6. Bawden, F. C. and Pirie, N. W., *Br. J. Exp. Pathol.*, 1939, **20**, 322.
7. Cremer, M. C., *Proc. 1st Conf. Potato Virus Diseases*, Lisse-Wageningen, 1951, p. 85.
8. Purcifull, D. E. and Shepherd, R. J., *Phytopathology*, 1964, **54**, 1102.
9. Purcifull, D. E., Batchelor, D. C., *Fla. Agric. Exp. Stn. Techn.*, 1977, No. 788, 1-39.
10. Hollings, M., Brunt, A. A., *Descriptions of plant viruses*, 1981, No. 245, 1.
11. Bartels, R., *Phytopathol. Z.*, 1957, **30**, 1.
12. Tosic, M., Ford, R. E., Moline, H. E. and Mayhew, D. E., *Phytopathology*, 1974, **64**, 439.
13. Delgado-Sanchez, S. and Grogan, R. G., *Phytopathology*, 1966, **56**, 1397.
14. Brakke, M. K., *Virology*, 1959, **9**, 506.
15. Hebert, T. T., *Phytopathology*, 1963, **53**, 362.
16. Rajyalakshmi, R., Nayudu, M. V. and Sreenivasulu, P., *Curr. Sci.*, 1984, **53**, 332.
17. Mali, V. R., Nirmal, D. D., Mundhe, G. E., Vyanjane, N. T. and Raut, K. G., *Indian Phytopathol.*, 1985, **38**, 282.

CYTOMIXIS AND CHROMOSOMAL VARIATION IN POLLEN MOTHER CELLS OF *SESBANIA AEGYPTIACA* (POIR.) PERS.

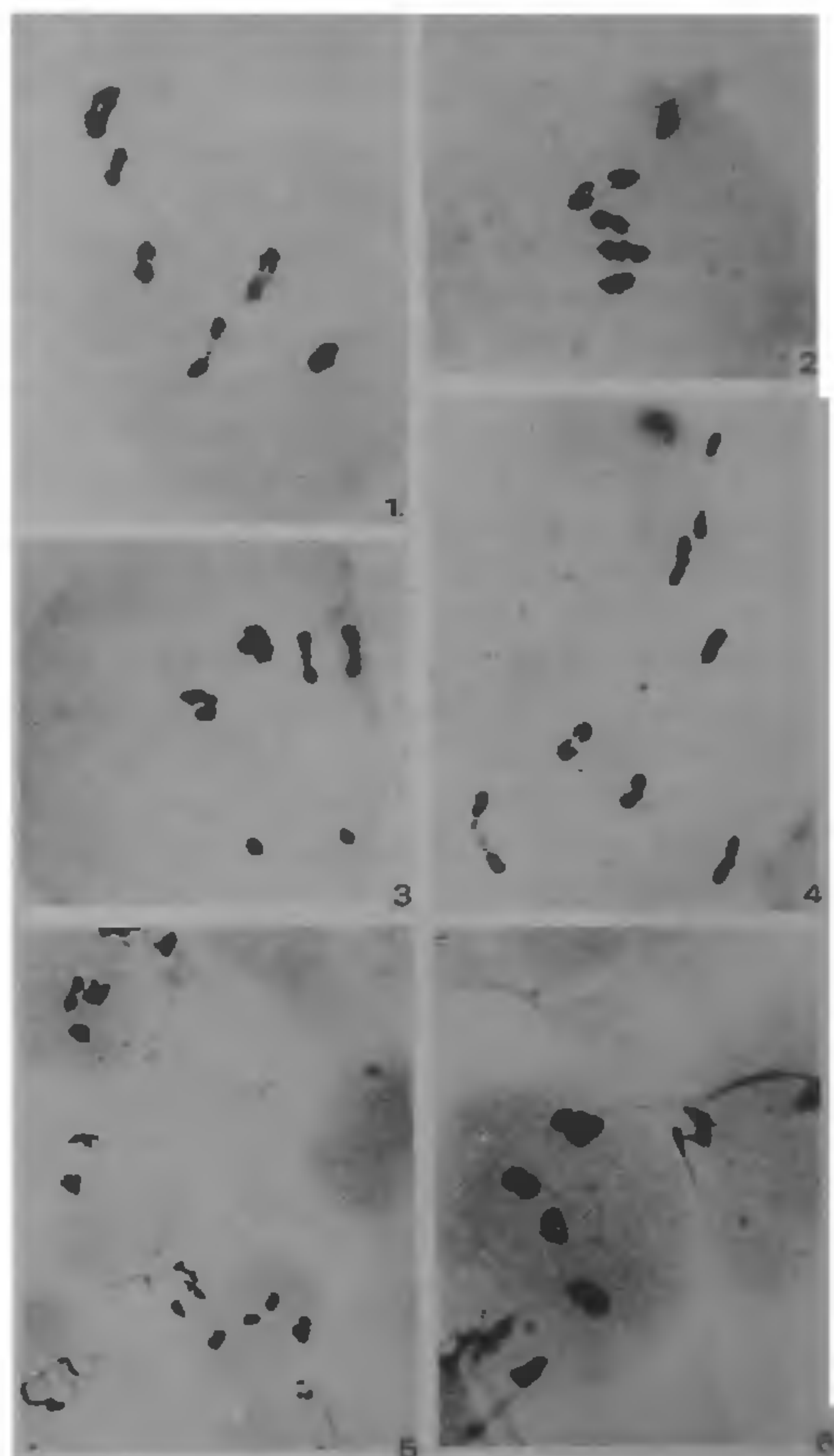
R. S. PARIHAR and S. N. ZADOO

*Division of Plant Improvement,
Indian Grassland and Fodder Research Institute,
Jhansi 284 003, India.*

SINCE its first observation in the genus *Crocus*¹ the phenomenon of cytomixis has been found to occur in many genera and has been reviewed from time to time²⁻⁶. Besides the pollen mother cells, cytomixis has been observed in mitotic cells of root tips⁷⁻⁹.

While carrying out meiotic analysis of some species of the genus *Sesbania* Scop, cytomixis was observed in pollen mother cells of *S. aegyptiaca* (Poir.) Pers. var. *bicolor* [= *S. sesban* (L.) Merr. var. *bicolor*] after anthers were squashed in 1% aceto-orcein solution following the usual methods.

Cytological analysis of var. *bicolor* revealed it to be a diploid with $2n = 12$ chromosomes based on $x = 6$ (figure 1). Six bivalents were more or less a regular feature observed at metaphase-I followed by normal course of meiosis. However, a few cells (7.2%) with lower i.e. $2n = 10$ (figures 2 and 3) or higher number i.e. $2n = 14$ (figure 4) were also observed in the material. The cells with lower chromosome numbers showed the occurrence of 5 bivalents (figure 2) or 4 bivalents + 2 univalents (figure 3), whereas the cells with higher chromosome numbers showed the occurrence of 7 bivalents at meiotic metaphase-I (figure 4). In the same



Figures 1-6. Meiosis in *S. aegyptiaca* var. *bicolor*; 1. Metaphase-I showing 6 II; 2. Metaphase-I showing 5 II; 3. Metaphase-I showing 4 II + 2I; 4. Metaphase-I showing 7 II; 5 and 6. Groups of cells showing transfer of chromatin material.