

## An Improved Method for Purification of Sweet Potato Feathery Mottle Virus Directly from Sweet Potato

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### ABSTRACT

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Sweet potato feathery mottle virus was purified from infected sweet potato leaves using short CsCl step gradient centrifugations with a minimum of low-speed centrifugations. Virus yields of 50–100 mg/kg from sweet potato leaves were obtained, compared with 5–10 mg/kg from

*Ipomoea nil* leaves. The virus thus obtained was highly infectious, with little healthy material in it. Antiserum produced to purified virus had a titer of 1:4,096 in ring precipitin tests and was used in enzyme-linked immunosorbent assays and in immunosorbent electron microscopy.

*Additional keyword:* serology.

Sweet potato feathery mottle virus (SPFMV), an aphid-transmitted potyvirus (9), has been observed in different regions of the world where sweet potato, *Ipomoea batatas* (L.) Lam., is grown (7,10,12). In Israel, the virus is widespread, as determined by surveys using serological tests with antisera obtained from J. W. Moyer and R. N. Campbell. Under laboratory conditions, the SPFMV present in Israel was readily transmitted by aphids from infected sweet potatoes to virus-tested sweet potato plants obtained from meristems. SPFMV was mechanically transmitted to *Ipomoea nil* (L.) Roth., *I. purpurea* (L.) Lam., *I. lacunosa* L., *I. trichocarpa* Ell., *I. tricolor* Cav., *I. hederacea* Jacq., *I. wrightii* (Well.), *Nicotiana benthamiana* Domin., *Chenopodium amaranticolor* Coste & Reyn., and *C. quinoa* Willd.; SPFMV was transmitted by grafting to *I. setosa* Ker. All of these are known as indicator plants for SPFMV (4,10).

Purification of SPFMV has been reported (2,3,11), but only when *I. nil* leaf material served as a source, whereas purification directly from sweet potatoes was not successful. Therefore, an additional step involving transfer of virus from sweet potatoes to *I. nil* was required. Virus yields obtained from *I. nil* ranged between 5–20 mg of virus per kilogram of infected tissue and required complex purification protocols (2,3,11).

Here we report a procedure for purifying SPFMV directly from sweet potatoes that requires a relatively short time and yields five–10 times more virus than reported previously. The purified preparation was used to prepare an antiserum.

### MATERIALS AND METHODS

Leaves of sweet potato cv. Georgia Jet, showing SPFMV symptoms and giving a positive reaction in immunosorbent electron microscopy (ISEM) with an SPFMV-RC antiserum obtained from Moyer, served as source material. Plants were grown in a greenhouse, inoculated by grafting, and sampled 4–6 wk later. In some experiments, field-grown infected plants were also used. Only young, upper leaves (up to the third fully developed leaf) with symptoms were used for purification, either fresh or after several days at 4 C (but not frozen).

**Electron microscopy.** During purification, fractions were examined in a Jeol 100 CX III electron microscope after negative staining with 2% uranyl acetate, pH 4.3. For ISEM, the procedure of Milne and Luisoni (8) was followed by using antisera obtained from Moyer or Campbell or an antiserum produced from our purified preparations. Particle length was measured on enlarged

photographs, and the normal length was calculated from the measurements of 200 particles. For calibration of magnification, a cross-line grating replica with spacings of 2,160 lines per millimeter was used.

**Infectivity.** Local lesion assays were performed on *C. quinoa*. Dilution end points also were determined on *I. nil*.

**Purification.** Symptomatic leaves (100 g) were homogenized at 4 C for 3–5 min in an Omni mixer in a solution composed of: 2 ml of 0.5 M borate buffer, pH 8.0, containing 0.01 M sodium ethylenediaminetetraacetic acid (EDTA), 1 ml of chloroform, 1 ml of carbon tetrachloride, and 1  $\mu$ l of thioglycolic acid (extraction buffer) per gram of leaf tissue. The homogenate was not filtered, but was centrifuged for 10 min at 10,400 g. The aqueous phase was collected, and 50 ml was layered on a 20-ml 25% sucrose cushion made in 0.05 M borate buffer, pH 8.0, containing 0.001 M EDTA. These were then centrifuged for 2 hr at 95,000 g (Beckman Type 35 rotor). Each pellet was resuspended in 2 ml of 0.05 M borate buffer, pH 8.0, containing 0.001 M EDTA and left overnight at 4 C. The suspension was then brought to 5.5 ml, again agitated gently with the aid of a pipette, and layered (without low-speed centrifugation) on a CsCl step gradient (1). Clarification by low-speed centrifugation at this step resulted in a marked loss of virus in the low-speed pellet.

Step gradients were prepared by layering 1 ml each of 0, 10, 20, 30, and 40% (w/v) CsCl dissolved in 0.05 M borate buffer, pH 8.0, containing 20% sucrose and 0.001 M EDTA in 89  $\times$  14 mm cellulose nitrate tubes of a Beckman SW 41 rotor. The virus suspension (5.5 ml) was layered on each gradient and centrifuged at 38,000 rpm for 2.5 hr at 8 C. Two bands were observed (Fig. 1a); the opaque lower band contained high concentrations of virus. The gradient columns were fractionated into 0.3 ml fractions with the aid of a peristaltic pump, and the virus-containing fractions were determined by electron microscopy. Fractions containing the highest concentrations of virus were pooled.

This partially purified preparation was diluted with an equal volume of 0.05 M borate buffer, pH 8.0, containing 0.001 M EDTA, and was centrifuged again on a CsCl step gradient as described. In most cases, only the "virus" band was observed (Fig. 1b). The gradient was fractionated, and the pooled virus-containing fractions were dialysed against 0.05 M borate buffer, pH 8.0.

Virus concentration was estimated spectrophotometrically with an extinction coefficient of 2.5 (mg/ml)<sup>-1</sup>cm<sup>-1</sup> at 260 nm, uncorrected for light scattering (3,6).

**Serology.** Rabbits were injected intramuscularly at weekly intervals. Four injections, each consisting of 1–2 mg of virus in 0.5 ml of 0.05 M borate buffer, pH 8.0, emulsified in an equal volume

of Freund's incomplete adjuvant, were administered. Two additional injections were given at a monthly interval. Blood was collected periodically starting before the fifth injection. Antisera were used nonabsorbed. For ring precipitin tests, the serum was diluted with phosphate-buffered saline (0.02 M phosphate plus 0.15 M NaCl and 0.0026 M KCl at pH 7.4) (PBS) in 30% glycerol.

For decoration, antisera were diluted 1:20 with 0.1 M phosphate buffer, pH 7.0. Grids were not precoated with antiserum.

Enzyme-linked immunosorbent assay (ELISA) was done according to Clark and Adams (5), with immunoglobulins and conjugate used at dilutions of 1:1,000. Leaf samples for ELISA were diluted 1:10 (w/v) in PBS containing 0.05% Tween and 2% polyvinylpyrrolidone. A Bio-Tek Instruments ELISA reader model EL 310 was used.

## RESULTS

**Purification.** In preliminary experiments using phosphate or citrate buffers for extraction, virus was lost in the pellet even at low-speed centrifugation. Precipitation with polyethylene glycol

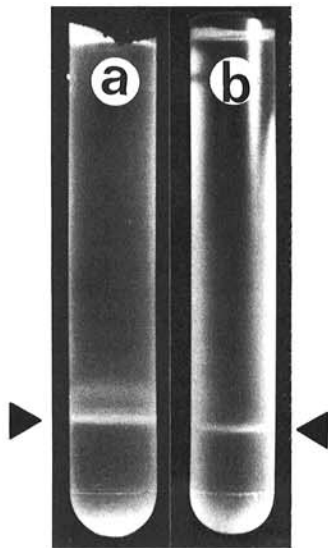


Fig. 1. Band of purified sweet potato feathery mottle virus (arrow) after one (a) or two (b) centrifugations on step gradients of CsCl.

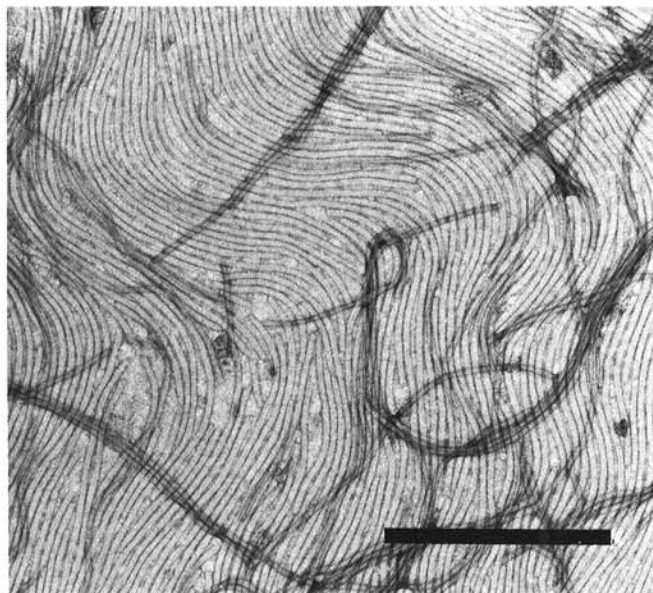


Fig. 2. Electron microscopy of CsCl step gradient purified sweet potato feathery mottle virus, negatively stained with 2% uranyl acetate. Bar = 0.5  $\mu$ .

and subsequent sucrose gradient centrifugation also did not give satisfactory results, as most of the virus aggregated and sedimented to the bottom of the tube.

Using the procedure outlined, yields of purified SPFMV from sweet potato leaves ranged from 50 to 100 mg/kg of infected tissue. The predominant virus band in the CsCl gradient was located approximately 20–21 mm from the bottom of the gradient (Fig. 1). Preparations for electron microscopy from this band contained large numbers of virus particles, with relatively little cellular contamination (Fig. 2). Almost no SPFMV particles were found outside this band, which was recovered within two 0.3-ml fractions. The  $A_{260}/A_{280}$  was 1.19, uncorrected for light scattering.

In general, yields of SPFMV from sweet potato leaves during March and April were higher than during August–October, and those from greenhouse-grown plants were higher than those from field-grown plants. The yield of SPFMV from *I. nil* leaves by this procedure was about one-tenth of that obtained from sweet potato foliage. Relative virus titers in sweet potato leaves, as determined by ELISA, were 3–5 times higher than those in *I. nil* leaves. For these tests, sweet potato and *I. nil* leaves were homogenated in extraction buffer as described, centrifuged for 10 min at 10,400 g, and the water phase tested by ELISA.

Infectivity of purified preparations, comprising 2 mg/ml SPFMV and diluted 1:10 and 1:100 with 0.05 M borate buffer, containing 0.1 M diethyldithiocarbamic acid, pH 8.0, gave 35 and 3 lesions per leaf of *C. quinoa*, respectively (averages from two experiments using 10–15 leaves each). When such preparations were diluted 1:100 and 1:1,000 and inoculated to *I. nil*, 17 out of 20 and 7 out of 20 plants became infected, respectively.

**Particle morphology.** Normal particle length from the purified preparations was 830–850 nm. A main maximum of 800–900 nm (3), which contained more than 70% of the particles, was used to calculate the normal length.

**Serology.** The antiserum obtained from Moyer and our antiserum gave a distinct decoration of SPFMV particles using either purified (Fig. 3) or dip preparations from sweet potato and *I. nil* leaves.

The titer of our antiserum in ring precipitin tests reached 1:4,096, and ELISA values obtained with infected sweet potato leaves ranged between 1.2–2.5, compared with 0.025–0.05 from uninfected control leaves. ELISA values of 10 and 1  $\mu$ g/ml gave 1.5–1.6 and 0.57, respectively. Comparable control preparation gave ELISA values of 0.07, 0.095, and 0.08, respectively.

## DISCUSSION

It has been shown that SPFMV can be purified directly from sweet potato leaves without transferring the virus to *I. nil* before

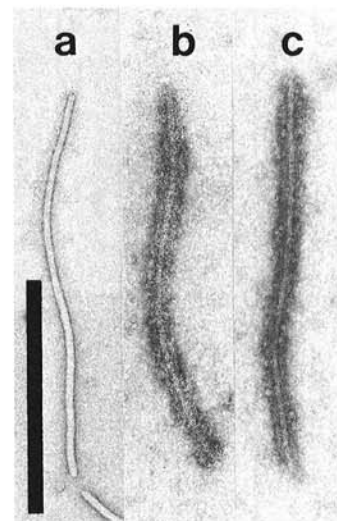


Fig. 3. Sweet potato feathery mottle virus particles: (a), without decoration; (b), decorated with antiserum obtained from J. W. Moyer (SPFMV-RC); (c), decorated with antiserum produced in this study. Bar = 0.5  $\mu$ .

purification (2,3,11). In addition, virus yields from sweet potatoes were substantially higher (eight–10 times) than those obtained from an equal amount of *I. nil* leaves. This seems to be because of a higher relative virus titer in sweet potato leaves than in *I. nil* and adaptation of the purification procedure specifically to sweet potato.

It seemed essential not to freeze the leaves before extraction. After resuspension of the pellet obtained by ultracentrifugation, low-speed centrifugation was avoided, because during this step more than half of the SPFMV particles were lost in the pellet even at low-centrifugal forces.

CsCl gradients were found to be an efficient step during purification. Sucrose gradient centrifugation gave only a small virus-containing peak; most of the virus particles aggregated and sedimented to the bottom of the tube. This was also true when SPFMV fractions after two CsCl gradients were subjected to sucrose gradient centrifugation.

SPMV purified by this procedure was infectious, with little healthy material in it. This was deduced from the low background values obtained in ELISA of healthy plant extracts, using the nonabsorbed SPFMV antiserum. Serological reactions were easily obtained with antisera using CsCl-purified virus in ring tests, ELISA, and decoration.

The ease of this purification procedure, savings in centrifugation time, and the yields of SPFMV obtained directly from sweet potatoes should be helpful for comparing different isolates of SPFMV and perhaps other viruses infecting sweet potatoes.

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