A Severe Strain of Tobacco Mosaic Virus from Cactus

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ABSTRACT

A previously unreported strain of tobacco mosaic virus was found in the Beavertail cactus, *Opuntia basilaris*, growing in the wild. Host reactions were most like those induced by the yellow-mottling distorting-type and J14D1 strains in *Nicotiana sylvestris* and Turkish tobacco, respectively. In crude sap, the thermal inactivation temperature was 85-90 C, and the dilution end point $10^{-8} - 10^{-9}$. Long needles were observed in polyethylene glycol precipitates of infected sap. Electron microscopy of purified virus revealed rigid rods typical of TMV with an NML of 302 ± 5 nm. A sedimentation coefficient of 183S was determined. The virus was serologically related to common TMV.

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Additional key words: needles, premature collapse.

The following viruses occur naturally in the Cactaceae; Sammons' Opuntia Virus (SOV) (12); Cactus Virus X (1); Cactus Virus II (2); Zygocactus Virus (4); Zygocactus Virus X (5); and the isometric Saguaro Virus (10). Among the six, SOV is the only TMV-like virus, although with an average length significantly greater than that of common TMV or any other strain (3). We have isolated a TMV-like virus from *Opuntia basilaris* collected in the wild near Kingman, Arizona. The goal of this study was to determine its relationship to SOV and other strains of TMV.

MATERIALS AND METHODS.—A 15 × 15-cm piece of cactus pad with much of the wax removed was ground in a small amount of 0.1 M pH 7 phosphate buffer and inoculated with the forefinger on leaves of *Chenopodium quinoa* previously dusted with Carborundum powder. Several single lesion transfers were made from *C. quinoa* and the virus was increased in the systemic host *N. tabacum* 'Turkish'. The plants were grown in a greenhouse at a minimum temperature of 20-25 C; maximum temperature seldom exceeded 30 C.

The virus was purified by polyethylene glycol precipitation (6) and sucrose density-gradient centrifugation. The extinction coefficient was determined by measuring the absorbance of several dilutions of purified virus at a wavelength of 260 nm in a Shimadzu MPS-5d recording spectrophotometer. The absorbance was plotted against concentration of the virus. The extinction coefficient ($E^{0.1}_{260}\%$) was calculated using Beers' Law.

The sedimentation coefficients ($s^{o}_{20,w}$) of the virus were determined in a Spinco Model E analytical ultracentrifuge. The speed was maintained at 26,000 rpm with a Beckman An-D rotor at 4 C.

Virus was examined in a Siemens Elmiskop I microscope. Negative staining with 2% phosphotungstic acid and palladium shadow-casting were employed. Size measurements of virus particles were made on infected crude sap using a preparation of common TMV as a standard at the Biologische Bundesanstalt, Braunschweig, Germany.

Antiserum was prepared in albino rabbits by giving four intramuscular injections one week apart of 1 mg/ml

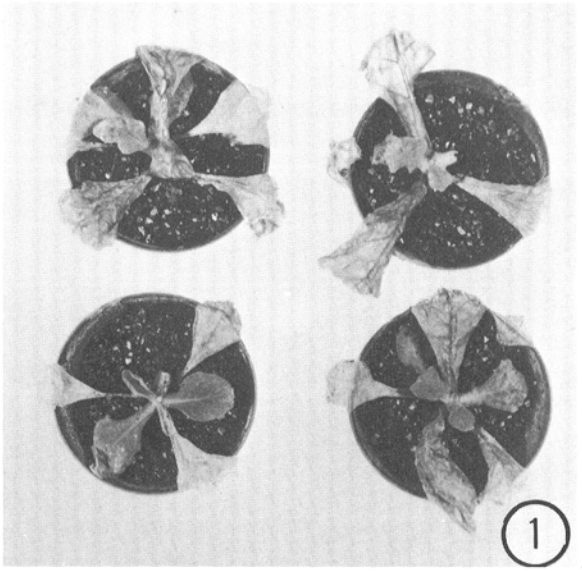

Fig. 1. Beavertail cactus virus (tentative name for a new strain of TMV) infection on *Nicotiana sylvestris* plants, showing complete and premature collapse.

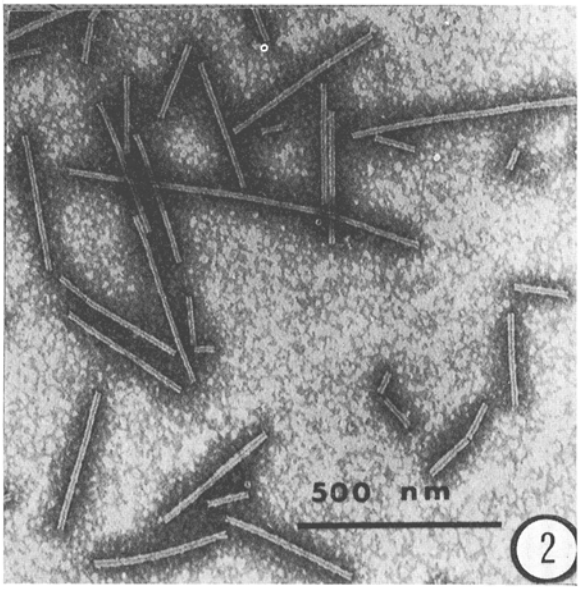

Fig. 2. Electron micrograph of purified beavertail cactus virus (tentative name for a new strain of TMV) stained in 2% phosphotungstic acid at pH 7.2.

of purified virus mixed with an equal amount of Difco incomplete Freund's adjuvant. This was followed by an intravenous injection. One or two weeks after a booster injection, the rabbits were bled and serum prepared in the standard fashion. The possible serological relationship to common TMV was determined by the Ouchterlony gel diffusion method in 0.75% agar dissolved in 0.2 M ammonium phosphate and 0.83% NaCl.

RESULTS.—The virus produced local lesions in the inoculated leaves of *Nicotiana glutinosa* L., *N. tabacum* L. '*Xanthi-nc*', and *Datura stramonium*, similar to those produced by the U-1 strain of TMV. On Turkish tobacco, both local necrotic lesions on inoculated leaves and systemic mottling, some necrosis, and severe leaf distortion were produced. Symptoms on *N. sylvestris* were extreme, with water-soaked primary lesions on inoculated leaves 3-4 days after inoculation, followed by a systemic necrosis and severe deformation, blistering, and massive glandular hairs on younger uninoculated leaves, with premature death of all inoculated plants (Fig. 1).

The virus showed a thermal inactivation point of 85-90 C at 10 minutes, a dilution end point between 10^{-8} and 10^{-9}, and a longevity in vitro of several weeks when assayed on *N. glutinosa* and *C. quinoa*.

Virus treated with PEG and dissolved in 0.05 M pH 7.5 phosphate buffer produced a milky-appearing suspension with a pH of 5.5. The suspension exhibited long, slender needles when examined in a light microscope. These needles appeared slightly longer than the "crystals" obtained in the classical work of Stanley after ammonium sulfate precipitation of TMV (13). When we raised the pH of the suspension to 7.5 with NaOH, the needles disappeared. No needles could be observed in healthy sap treated with PEG.

Purified virus gave an ultraviolet absorption spectrum typical of nucleoprotein with a minumum at 244-246 nm, and a maximum at 260-262 nm, corrected for scattering. An extinction coefficient of 3.10 at 1 mg/ml was calculated. The sedimentation coefficient corrected for water at 20 C ($s_{20,w}$) was 183S. Elongated rigid rods were observed in negatively stained and palladium shadowed preparations. The normal length of the virus was determined to be 302 ± 5 nm (Fig. 2).

The antiserum prepared against the virus showed a positive reaction with common TMV antigen, as did TMV antiserum with the test virus. No reaction occurred with healthy plant extract. Test antiserum absorbed with an excess of common TMV antigen showed a weaker precipitation reaction with the homologous antigen than did unabsorbed antiserum in an Ouchterlony double-diffusion plate. Also, antiserum against common TMV absorbed with an excess of the present virus showed a similarly weak precipitation reaction when compared to unabsorbed TMV antiserum. These results indicate that both viruses contain some antigenic determinants which are not present in the other.

DISCUSSION.—The results clearly indicate that our virus is a strain of TMV. However, our isolate differs from most strains, which give either local lesions or systemic mottling in *N. sylvestris*, in that both types of reaction are produced. Jensen (7) reported a strain of TMV, yellow-mottling distorting type (YMD) which caused local lesions, systemic necrosis and premature death in *N. sylvestris* and bright-yellow mottling in Turkish tobacco. Another strain of TMV, J14D1, gave local lesions in *N. sylvestris* and local lesions, systemic necrosis and premature death in Turkish tobacco (8).

The reaction of *N. sylvestris* is somewhat similar to that induced by YMD strain in that host; but the response of Turkish tobacco to the two strains differs. On the other hand, our virus produces a similar reaction to that produced by JI4D1 in Turkish tobacco although differing in *N. sylvestris*. The host reaction induced by this virus in all the other plant species tested was similar to that induced by common TMV but differs from SOV which fails to infect tobacco plants (3). The wide host range characteristic of our virus also clearly distinguishes it from that typical of SOV.

The intact rods of TMV have been shown to have a range of sedimentation coefficients between 187S to 212S (11, 13). The $s_{20,w}$ of 183S for this virus is close to the value previously reported by Lauffer for common TMV (9). No significant difference in particle size was found between common TMV and the present virus, unlike that observed with SOV.

The virus exhibited a serological relationship to common TMV with the cross-absorption test indicating that our virus, although serologically similar to common TMV, is not identical to it.

On the basis of the above results, we conclude that this virus is a new strain of TMV, and we tentatively name it the Beavertail Cactus strain.

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