A Comovirus Affecting Tabasco Pepper in Central America

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ABSTRACT

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A virus having isometric particle morphology with an average diameter of 30 nm was isolated from tabasco pepper (*Capsicum frutescens*) in Honduras. The virus was mechanically inoculated to several cultivars of *C. frutescens*, *C. chinense*, and *C. annuum*. Symptoms consisted of mosaic and yellow mottle in *C. frutescens* and *C. chinense* and a very mild mottle in *C. annuum*. The virus was purified, and an antiserum was prepared. Viral ssRNA, dsRNA, and coat protein were analyzed by gel electrophoresis. Light and electron microscopy studies indicated that the virus has properties similar to members of the comovirus group. Serological tests with several comoviruses revealed this virus is closely related to Andean potato mottle virus (APMV), and therefore, the virus was designated as a pepper strain of APMV (APMV-P). Moreover, it was distantly related to the cowpea severe mosaic and bean pod mottle viruses. The banded cucumber beetle (*Diabrotica balteata*) was determined to be a vector of APMV-P.

Two cultivars of *Capsicum frutescens* L., Tabasco and Greenleaf Tabasco, are commonly grown in some regions of the United States, Mexico, Central America, and the Caribbean (1). Viral diseases are one of the major limiting factors in the production of this crop in Central America. During surveys conducted in tabasco pepper fields in 1990, 1991, and 1992, flexuous, rod-shaped potyviruses, such as pepper mottle (PepMoV) and tobacco etch (TEV) viruses, were commonly found. However, electron microscopy indicated that a virus with isometric morphology also was present in about 25% of the plants with virus symptoms. Preliminary studies suggested that this virus was different from viruses previously described in *Capsicum* spp. This virus induced mosaic symptoms on tabasco peppers and had properties characteristic of the comovirus group and was serologically related to Andean potato mottle comovirus (APMV). Therefore, it was tentatively designated as a pepper isolate of APMV (APMV-P).

The purpose of this investigation was to determine the properties of this comovirus from tabasco pepper and to compare it with several other comoviruses.

MATERIALS AND METHODS

Virus isolates. An isolate of APMV-P from El Negrito, Honduras, was used throughout this study. Other isolates of the same virus were obtained from different locations in Honduras and Nicaragua, and most of them were isolated in mixed infections with PepMoV. All other viruses used for comparison with APMV-P, including tobacco mosaic tobamovirus (TMV), potato virus X potexvirus (PVX), bean pod mottle comovirus (BPMV), cowpea severe mosaic comovirus (CPSMV), and broad bean wilt fabavirus (BBWV), were isolates from Louisiana. Mechanical inoculations to several plant species with APMV-P were conducted with 0.02 M potassium phosphate buffer, pH 7.2.

Beetle transmission. The banded cucumber beetle (*Diabrotica balteata* LeConte) a common insect pest of pepper in Central America was collected from sweet potato (*Ipomoea batatas* (L.) Lam.) fields near Baton Rouge, LA. Beetles were kept for 2 wk on healthy pepper plants before being used in transmission experiments. Individual beetles were placed in 15-ml glass tubes containing Tabasco pepper leaves infected with APMV-P to acquire the virus. After feeding for 24 h, beetles were transferred onto caged, healthy Tabasco seedlings for a 24-h transmission period. Beetles were discarded, and plants were placed in a growth chamber with a photoperiod of 8–10 h at 25 C. The plants were examined periodically for symptom development. Three weeks after the transmission period, plants were tested for APMV-P by immunodiffusion tests.

Virus purification. The pepper isolate of APMV was purified from infected Tabasco leaves. Tissue was ground in a blender with 1 ml of 0.2 M potassium phosphate buffer, pH 7.0, per gram of tissue. After filtering through cheesecloth, the extract was mixed with 1 volume of chloroform/butanol (1:1), shaken for 30 min at room temperature, and clarified by low-speed centrifugation (8,000 *g* for 15 min). Virions were precipitated with 8% polyethylene glycol (molecular weight 6,000) followed by alternate low-and high-speed centrifugation (90,000 *g* for 120 min). This was followed by two cycles of sucrose density gradient centrifugation (25,000 rpm for 180 min) in 10–40% linear gradients prepared in 0.02 M potassium phosphate buffer, pH 7.0. The absorbance of centrifuged gradients was recorded at 254 nm, and fractions corresponding to absorbance peaks were collected.

Protein analysis and serology. Capsid protein subunits of APMV-P were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (7). Molecular weight marker proteins used included bovine serum albumin, 66,000 Da; egg albumin, 45,000 Da; glyceraldehyde-3-phosphate dehydrogenase, 36,000 Da; bovine carbonic anhydrase, 29,000 Da; trypsinogen, 24,000 Da; soybean trypsin inhibitor, 20,000 Da; and lysozyme 14,300 Da. Antiserum to APMV-P was obtained from a rabbit after four weekly subcutaneous injections containing 2.5 mg of purified virus in 0.5 ml of 0.02 M potassium phosphate buffer emulsified in 0.5 ml of Freund's incomplete adjuvant (Sigma Chemical Co., St. Louis). Antisera to CPSMV, quail pea mosaic comovirus (QPMV), BBWV, BPMV, and squash mosaic comovirus (SMV) were supplied by R. Gergerich (University of Arkansas, Fayetteville). Antiserum to the type strain of APMV was supplied by L. Salazar (International Potato Center, Lima, Peru). The Ouchterlony double-diffusion test in 1% agarose with 0.85% NaCl was used to determine serological relationships among viruses. Comparisons among APMV-P isolates were conducted with sap extracts from infected Tabasco plants. All other serological tests were conducted with purified virus preparations (0.5 mg/ml). Antisera were diluted 1:10 for Ouchterlony tests.

Nucleic acid analysis. Nucleic acid (ssRNA) was isolated from purified preparations of APMV-P using the sodium perchlorate method (10). Samples of ssRNA were denatured with formamide-formaldehyde and analyzed in 1.5% agarose gels containing 20 mM-HEPES, pH 7.8, and 1 mM-EDTA and 6% formaldehyde. Extraction and purification of dsRNA was performed using the cellulose chromatography method described by Valverde et al (9). DsRNA samples were analyzed in 6% polyacrylamide gels.

Light and electron microscopy. Epidermal strips were obtained from Tabasco infected with APMV-P and stained with Azure A as described by Christie and Edwardson (3). Mounted

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tissues were examined with a light microscope for viral inclusions. Purified preparations of APMV-P were negatively stained with 2% uranyl acetate and viewed with an electron microscope.

RESULTS

Host range and virus distribution. Tabasco pepper samples from two farms at two locations in Honduras (El Negrito and La Flecha) and three locations in Nicaragua (Palo Verde, Managua, and Tipitape) were infected with APMV-P. Symptoms consisted of foliar mosaic and yellow mottle on Tabasco and Greenleaf Tabasco. Symptoms on other *Capsicum* species included yellow mottle on *C. chinense* Jacq., cvs. Jacquin PI159236 and PI152225 (Fig. 1.), and very mild mottle on five *C. annuum* L. cultivars, Yolo Wonder, Yolo Y, Jalapeño M, Long Red Cayenne, and Hungarian Wax. Symptoms on other plant species included mild mosaic on *Nicotiana clevelandii*, *N. benthamiana*, and *N. debneyi*. Nonhosts, as determined by immunodiffusion tests, included *Lycopersicon esculentum*, cvs. Peto 95, UC 82, and Rutgers; *Datura stramonium*; *D. metel*; *Chenopodium quinoa*; *N. tabacum*, cvs. NC-95 and Havana 425; *Physalis ixocarpa*; *P. floridana*; *Pisum sativum*; *Phaseolus vulgaris*, cv. Pinto; *Solanum tuberosum*, cv. Pontiac; and *Vigna unguiculata*, cv. Blackeye.

Fig. 1. Mottle symptoms on *Capsicum chinense* PI152225 induced by a pepper strain of Andean potato mottle virus.

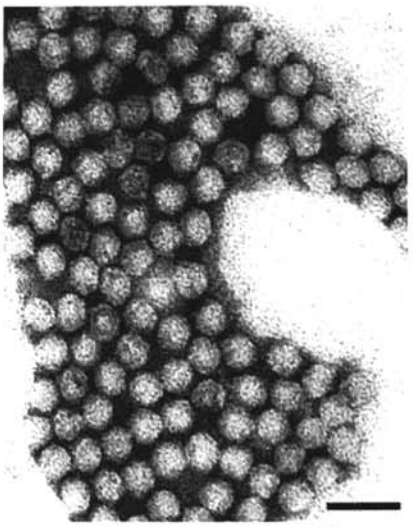

Fig. 2. Electron micrograph of a purified preparation of a pepper strain of Andean potato mottle virus negatively stained with 2% uranyl acetate. Bar represents 50 nm.

Purification, light, and electron microscopy. Yields of approximately 5 mg of virus per 100 g of tissue were obtained from Tabasco pepper infected with APMV-P. Electron microscopy of purified virus preparations revealed isometric particles about 30 nm in diameter (Fig. 2). Vacuolate inclusions, characteristic of comovirus infections (3), were readily observed in epidermal strips from infected plants. Inclusions stained purple with Azure A.

Beetle transmission. Seven of 27 (26%) of the *D. balteata* tested in transmission experiments transmitted APMV-P to individual Tabasco test plants. Plants developed symptoms approximately 10 days after transmission tests. Immunodiffusion tests confirmed APMV-P infections in symptomatic plants.

Electrophoresis of viral protein and ssRNA. Two polypeptides, molecular weight 21 and 41 $\times$ 10^3, were detected by electrophoresis of SDS-soluble protein of APMV-P. Two RNAs, molecular weight 1.4 and 2.0 $\times$ 10^6, were obtained from purified preparations of APMV-P by electrophoresis under denaturing conditions.

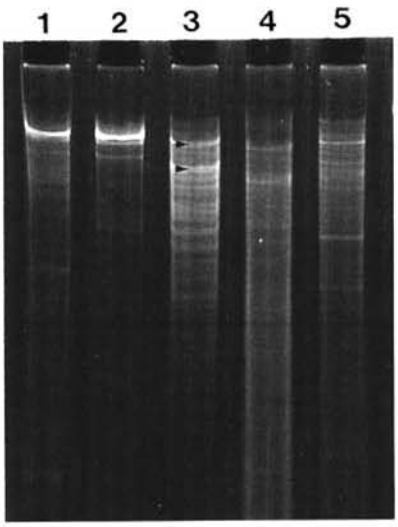

Fig. 3. Polyacrylamide gel (6%) electrophoresis of dsRNAs extracted from virus-infected plants. Lanes 1–3, *Nicotiana benthamiana* infected with tobacco mosaic tobamovirus, potato virus X, and a pepper strain of Andean potato mottle comovirus (APMV-P), respectively. Lane 4, *Chenopodium quinoa* infected with broad bean wilt fabavirus. Lane 5, *Phaseolus vulgaris* cv. Pinto infected with bean pod mottle comovirus. Two major dsRNAs of APMV-P with molecular weights of 3.9 and 2.6 $\times$ 10^6 are shown with arrows in lane 3.

DsRNA. DsRNA profiles of APMV-P and other comoviruses are shown in Figure 3. Two major dsRNA bands, molecular weight 3.9 and 2.6 $\times$ 10^6, were consistently obtained in dsRNA extracts from APMV-P-infected plants. Minor dsRNAs (stained with less intensity than the major ones) also were obtained. No dsRNA was obtained in extracts from healthy plants.

Serology. An antiserum prepared to APMV-P had a titer of 1/256 in immunodiffusion tests. Different APMV-P isolates could not be differentiated in

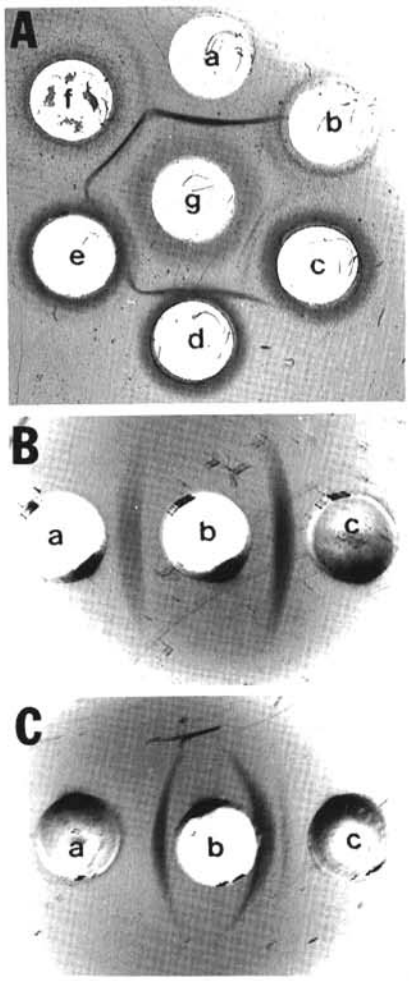

Fig. 4. Ouchterlony double-diffusion tests. **(A)** Center well (g) contains antiserum to an El Negrito isolate of Andean potato mottle virus-P (APMV-P). Peripheral wells (a, d, e, and f) contain sap extracts from Tabasco pepper plants infected with four isolates of APMV-P (El Negrito, La Flecha, Managua, and Tipitape respectively); b contains phosphate buffer; and c contains sap extract from healthy Tabasco pepper. **(B)** Center well (b) contains a purified sample of an El Negrito isolate of APMV-P. Other wells contain: (a) bean pod mottle comovirus (BPMV), and (c) APMV-type strain antisera. **(C)** Center well (b) contains a purified sample of BPMV. Other wells contain: (a) APMV-type and (c) BPMV antisera.

serological tests. In all cases, adjacent precipitin bands coalesced without spur formation (Fig. 4A). All homologous antigen and antiserum combinations produced strong reactions. Antiserum to APMV-P reacted weakly with CPSMV and BPMV. Antiserum to APMV type reacted strongly with APMV-P and BPMV (Fig. 4B) and weakly with CPSMV. Antiserum to BPMV reacted with APMV-P (Fig. 4C). Antiserum to CPSMV reacted weakly with APMV-P and BPMV. Antisera to SMV, QPMV, and BBWV did not react with APMV-P.

DISCUSSION

Several important viral diseases of *Capsicum* species are caused by members of the poty- and tobamovirus groups (6). Natural infections of pepper by comoviruses have not been reported. Moreover, the only comovirus known to naturally infect members of the Solanaceae is APMV (2,4,8), which occurs naturally only in the Andean region of South America (4).

Properties of viral nucleic acid and protein, particle morphology and size, cytopathic inclusion bodies, beetle transmissibility, and serological properties of the Tabasco pepper virus appear typical for a comovirus. Serological relationships among comoviruses are not uncommon. Several legume-infecting comoviruses have a close serological relationship (5). Based on the intensity of the precipitin bands in Ouchterlony tests, we conclude that the Tabasco comovirus isolate is more closely related to APMV than to BPMV or CPSMV. Serological and host-reaction studies conducted here suggest that this comovirus isolate from pepper is a new strain of APMV. Unlike this isolate, three strains (B, C, and H) of APMV infect *Physalis floridana* and potato (2,4,8). Strain P is more similar to strain H because both failed to infect *N. tabacum* and *D. stramonium* (2). Differences in host reaction and geographic distribution suggest that APMV-P may be a different virus. Moreover, beetle transmission of APMV has not been reported.

The banded cucumber beetle is a polyphagous pest commonly found on pepper, corn, sweet potato, and legume crops throughout Central America, the Caribbean, Mexico, and some regions of the southeastern United States. The percentage of APMV-P transmissions obtained using *D. balteata* was similar to that reported for other comoviruses. Other beetles, such as flea beetles (*Epitrix* sp.), also occur in pepper fields and should be tested as possible vectors of APMV-P.

The pepper strain of APMV-P may be widely distributed among *C. frutescens* cultivars and may occur in *C. annuum* cultivars as well. APMV-P infections of *C. annuum* could have been overlooked due to the mild symptoms. Preliminary experiments indicate that double infections of *C. annuum* and *C. frutescens* with APMV-P and PepMoV or TEV induce synergistic reactions.

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