Identification of Two Distinct Strains of Watermelon Mosaic Virus 2 Affecting Cucurbits in Texas

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ABSTRACT

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Two distinct strains of watermelon mosaic virus 2 (WMV-2) were isolated in Texas. One strain, designated isolate M, was obtained from the wild cucurbit *Melothria pendula*, and the other, isolate S, was isolated from zucchini squash (*Cucurbita pepo*). Neither isolate caused symptomatic infections of the differential hosts garden pea *Pisum sativum* (cultivar Alaska) or *Nicotiana benthamiana*, thus distinguishing them both from WMV-2 and zucchini yellow mosaic virus (ZYMV). Isolate S caused a systemic infection on *Phaseolus vulgaris* (cultivar Black Turtle 2), whereas isolate M did not, thus distinguishing them from each other. Isolates S and M were characteristic of WMV-2 in that they both produced typical cytoplasmic inclusions, had modal lengths of 755 and 775 nm, respectively, and protein capsid subunits of 3.2×10^4 daltons. Antisera produced to S and M reacted in sodium dodecyl sulfate (SDS) immunodiffusion tests with WMV-2, S, and M antigens but not with ZYMV antigens.

About 35,000 ha of cucurbitaceous crops are planted in Texas each year, and 8–10% are not harvested because of diseases. Viral diseases are among the most serious affecting squash, pumpkin, and melons. Each year since 1981, watermelon (*Citrullus lanatus*) plants have been severely affected with diseases that suggest a virus etiology. *Melothria pendula* L., a wild cucurbit, is usually abundant in the areas near the cultivated crops and quite often shows symptoms of virus infection. Watermelon mosaic virus 1 (WMV-1) was reported to infect watermelons in the Rio Grande Valley of Texas in 1965 (9) and more recently was reported in the areas of San Antonio and Houston (3).

This paper presents evidence for two distinct strains of WMV-2 in Texas based on differences in differential host reaction, serology, particle morphology, and biophysical characteristics.

MATERIALS AND METHODS

Virus transmission. Specimens of a wild cucurbit (*M. pendula*) and a zucchini squash (*Cucurbita pepo* L. cv. Zucco) showing symptoms suggesting virus infection were collected from Brazos County, Texas. Leaves from each specimen were macerated separately in a 1:1 (w/v) 0.05 M phosphate buffer, pH 7.2, containing 0.1% 2-mercaptoethanol. Extracted juice was mixed with a small amount of 600-mesh Carborundum and rubbed on cotyledons of Zucco squash seedlings grown in a sand-peat (1:1) mix. Infected squash plants were maintained in the greenhouse as a source of inoculum.

The differential hosts, *Pisum sativum* L. (cultivars Alaska and Little Marvel), *Phaseolus vulgaris* L. (Black Turtle 2), *Luffa acutangula* Roxb., and *Nicotiana benthamiana*, were inoculated separately with virus isolates S and M and compared with WMV-2 and zucchini yellow mosaic virus (ZYMV-FL) host reactions (5,6).

Virus characterization. Zucco squash leaf tissue inoculated respectively with isolate M and isolate S was fixed in 3% glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated, and embedded in a graded Epon 12 plastic. Tissue sections were mounted on Formvar-coated grids and stained with 2% uranyl acetate for 10 min, rinsed with distilled water, stained with 0.5% lead citrate for 5 min, and rinsed again. The tissue sections were examined with a Hitachi Hs7s electron microscope for cytoplasmic inclusions.

Viral particle length was calculated from photomicrographs by immunospecific electron microscopy (ISEM) (1). Anti-WMV-2 antiserum was diluted with tris buffer (0.05 M, pH 7.2) and bound to Parlodion-coated electron microscope grids, which were floated on drops of crude plant extract for 4 hr, then washed and stained with uranyl acetate. Particle length was determined with electron micrographs whose magnification was standardized with carbon gratings. Measurements were taken with a Hitachi Hicomscan 1111 digitizer, and the data were analyzed with a Tandy model 2000 computer.

Purified S and M virus used for capsid protein molecular weight determinations and for production of homologous antiserum was prepared following the procedure outlined by Purcifull et al (8). The molecular weight of the capsid subunit was determined by polyacrylamide gel electrophoresis (PAGE), using a 10–20% logarithmic polyacrylamide gel after virus degradation with SDS. β-Galactosidase, phosphorylase A, bovine serum albumin, catalase, ovalbumin, and carbonic anhydrase were used as standards. The PAGE gels were run at a constant 200V. The gels were stained with Coomassie Brilliant Blue-R 250/trichloracetic acid and photographed with a yellow filter (4).

Serology. Purified virus (2 mg/ml) was emulsified with an equal volume of Freund's complete adjuvant. One milliliter of virus emulsion was injected into virgin white New Zealand rabbits (half intramuscularly and half subcutaneously) every week for 4 wk and every other week for 6 wk. After the fourth injection, the rabbits were bled on alternate weeks.

Intragel cross-absorption immunodiffusion tests were performed with antisera prepared against isolates M and S and antisera prepared and furnished by D. E. Purcifull (Department of Plant

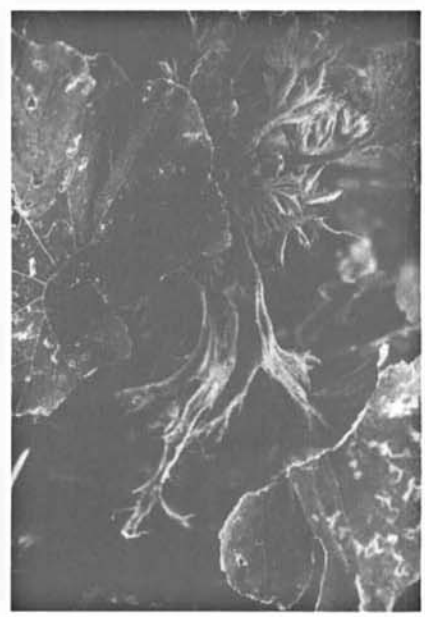

Fig. 1. Symptoms of severe leaf distortion on squash naturally infected with isolate M of watermelon mosaic virus 2.

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Table 1. Host reactions of diagnostic species to virus isolates S, M, watermelon mosaic virus 2 (WMV-2), and zucchini yellow mosaic virus (ZYMV)

Plant	Virus isolates[a]			
	S	M	WMV-2	ZYMV-FL
Pisum sativum				
Alaska	–[b]	–	SM	LL
Little Marvel	–	–	–	–
Phaseolus vulgaris				
Black Turtle 1	–	–	–	–
Black Turtle 2	SM	–	SM	LL
Luffa acutangula	SM	–	–	SM
Nicotiana benthamina	–	–	SM	–

[a]S was isolated from zucchini squash, M was isolated from *M. pendula*, and WMV-2 and ZYMV-FL were provided by D. E. Purcifull, University of Florida, Gainesville.
[b]SM = systemic mottle or mosaic, LL = local lesion, and – = no reaction.

Pathology, Plant Virus Laboratory, University of Florida, Gainesville) against WMV-2 and the Florida strain of ZYMV. Immunodiffusion tests were performed using antigens degraded by SDS (7). Fresh leaves were ground in distilled water (1:1) containing 3% SDS and filtered through cheesecloth. The test was performed in petri dishes containing 0.8% agarose, 0.5% SDS, and 1% sodium azide.

RESULTS AND DISCUSSION

Virus transmission and host reactions. The original symptoms observed in naturally infected *M. pendula* included a mild mosaic and veinbanding. The virus isolated from *M. pendula* was designated M. Symptoms observed in artificially inoculated Zucco squash included severe distortion of leaf contours with shoestringlike extensions of the leaf lamina and a chlorotic mottle (Fig. 1). Younger leaves on shortened petioles were drastically reduced in size. Flower abnormalities included petal distortions, faded color, and a proliferation of flower buds. Green blisters and stripes were common on the fruit. Plants infected at an early stage of development were severely stunted.

The isolate from Zucco squash, designated S, produced severe stunting and tip necrosis in leaves and flower buds. Necrosis occasionally progressed down the stem, causing death of young plants. Each of the symptoms was observed in all squash cultivars inoculated; however, necrosis was not observed in watermelon, cantaloupe, or cucumber.

Neither isolate caused systemic infections of *P. sativum* (cultivars Alaska and Little Marvel) or *N. benthamiana*, thus distinguishing both isolates from type WMV-2 and ZYMV (Table 1). Isolate S caused a systemic infection on bean (*P. vulgaris* cv. Black Turtle 2), whereas isolate M did not, thus distinguishing the isolates from each other. Isolate S, however, resembled ZYMV in its reaction on *L. acutangula*.

Photomicrographs of cells of squash leaves infected with isolate M or S contained cytoplasmic pinwheel inclusions typical of members of the potyvirus group (2) (Fig. 2).

Modal lengths of the two isolates, S and M, were 750–760 and 770–780 nm, respectively, and the protein subunits of each had a molecular weight of 3.2×10^4 daltons (Fig. 3), which compares favorably with measurements reported for WMV-2 (8).

When cross-absorbed against the Florida isolate of ZYMV, anti-S and anti-M antiserum reacted in SDS immunodiffusion tests with WMV-2, S, and M antigens but not with ZYMV antigens (Fig. 4). Anti-S and anti-M antiserum cross-absorbed with ZYMV antigen did not produce any perceivable reaction. Antiserum to S and M cross-absorbed with WMV-2 antigen reacted only faintly with isolate S and M antigens but not at all with ZYMV or WMV-2 antigens.

Based on symptoms, host range, biophysical characteristics, and serology, the viral isolates S and M appear to be distinct strains of WMV-2.

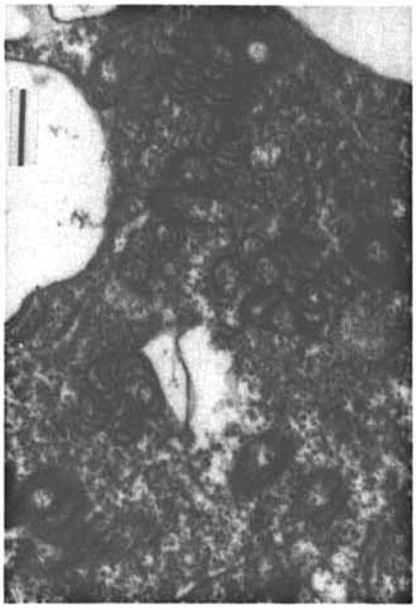

Fig. 2. Ultrastructure of Zucco squash leaf tissue infected with isolate M of watermelon mosaic virus 2 showing cytoplasmic inclusions characteristic of the potyvirus group. Scale bar = 300 nm.

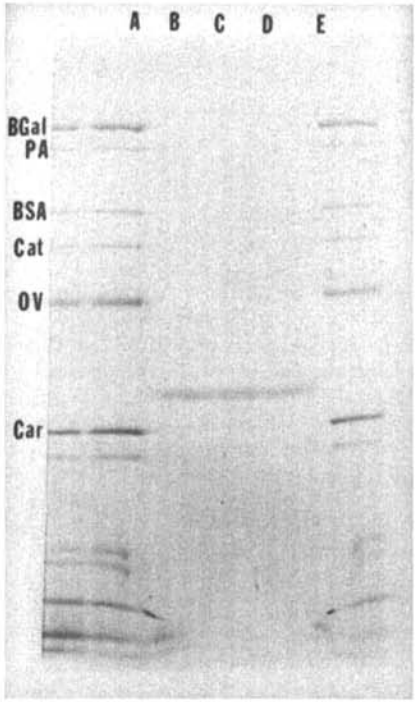

Fig. 3. Electrophoresis of coat protein subunits of isolates S and M and watermelon mosaic virus 2 (WMV-2) in 10–20% logarithmic polyacrylamide gel. BGal = β-galactosidase, PA = phosphorylase A, BSA = bovine serum albumin, Cat = catalase, OV = ovalbumin, and Car = carbonic anhydrase. Migration from top to bottom: lanes A and E = standards, lane B = isolate S, lane C = WMV-2, and lane D = isolate M.

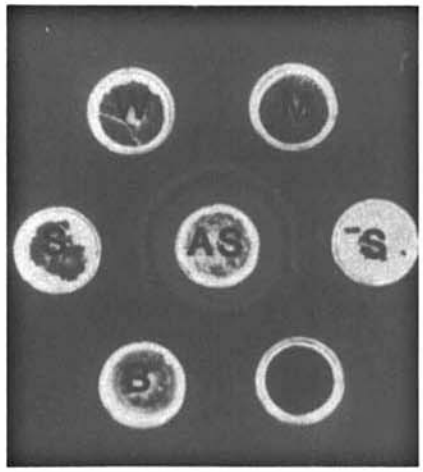

Fig. 4. Serological distinction of watermelon mosaic virus 2 (WMV-2), zucchini yellow mosaic virus (ZYMV), and isolates S and M. S = isolate S, M = isolate M, W = WMV-2, Z = ZYMV-FL, B = blank, and AS = anti-S serum. Antiserum in the center well was cross-absorbed in the gel against ZYMV. Reactions of the same antigens to anti-M serum were indistinguishable from those shown.

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