Partial Characterization of Zucchini Yellow Mosaic Virus Isolated from Squash in Turkey

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ABSTRACT

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A virus identified as zucchini yellow mosaic virus (ZYMV-TS2) isolated from diseased squash in Turkey was partially characterized and compared with other ZYMV isolates from Egypt, Italy, and the United States. ZYMV-TS2 was also compared with isolates of watermelon mosaic virus strain 2 (WMV-2) and the WMV-1 strain of papaya ringspot virus (PRSV-W). ZYMV isolates from Turkey and Egypt were similar to the Connecticut strain but different from the Florida strain in timing and severity of symptoms in squash. All ZYMV isolates were distinguished from WMV-2 by giving only local symptoms on Black Turtle 2 bean and from PRSV-W by giving only local reactions on *Chenopodium quinoa*. Purification of ZYMV-TS2 yielded 5–22 mg of virus per kilogram of tissue, with a 260/280 nm ratio of 1.23–1.32. Virus-specific polyclonal antibodies capable of detecting ZYMV were produced to ZYMV-TS2 in mice. Unfractionated ascites fluid reached a maximum titer of 1:1,600,000 in indirect ELISA. Serological comparisons using various antisera to ZYMV, PRSV-W, and WMV-2 revealed that ZYMV and WMV-2 cross-reacted strongly with some antisera and not at all with others. No cross-reactions were observed between PRSV-W and WMV-2 or between PRSV-W and ZYMV.

A new disease of zucchini crops was first observed in Northern Italy in 1973 (7). Zucchini yellow mosaic virus (ZYMV) was reported as the causal agent of this disease in 1981 in France (as muskmelon yellow stunt virus) and in Italy (5,7,17). Since then, ZYMV has been detected in other countries of Europe, Northern Africa, and the Middle East (4,6). Within the continental United States, this virus has been reported in New York, Connecticut, Florida, and California (11,12).

ZYMV is a recently recognized member of the potyvirus group and causes a severe disease in many cucurbit species (8). Watermelon mosaic virus 2 (WMV-2) and papaya ringspot virus, type W (PRSV-W, formerly named WMV-1) are also members of the potyvirus group and cause diseases in cucurbits (14,15). ZYMV appears similar to PRSV-W in host range and symptomatology. Serologically, however, ZYMV is closely related to WMV-2, although detection of this relationship depends on the antiserum used (12). This study was initiated to characterize a ZYMV isolate from Turkey and to examine its relationships with other isolates of ZYMV, WMV-2, and PRSV-W. Portions of this work were presented previously (2,3).

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MATERIALS AND METHODS

Viruses. ZYMV-TS2 was isolated from diseased squash (*Cucurbita pepo* L.) collected from fields in Adana, Turkey, by M. Yilmaz (3). ZYMV-Fl and ZYMV-CT from Florida and Connecticut, respectively, and WMV-2-NY from New York were kindly provided by R. Provvidenti. ZYMV-E from Egypt, formerly reported as WMV-E (9), PRSV-W-ATCC (American Type Culture Collection), and WMV-2-ATCC were kindly provided by H. A. Scott. WMV-2-Fl was a gift from D. Purcifull. All viruses were maintained in squash cultivars Early Prolific Straightneck or Cocozelle and as desiccated samples for controls in serological assays.

Antisera. Antibodies were produced against ZYMV-TS2 in mice by methods described later. Antiserum to cylindrical inclusion bodies of ZYMV-CT was kindly provided by D. Gonsalves. H. A. Scott provided antisera to ZYMV-E, WMV-2-ATCC, and to PRSV-W-ATCC. Antisera to the Italian isolates ZYMV-It and WMV-2-It were provided by V. Lisa (Torino, Italy), and antisera to WMV-2-Fl and PRSV-W-Fl were gifts from D. Purcifull.

Host range. Desiccated tissue of each isolate was resuspended and triturated in neutral 0.01 M phosphate buffer and rub-inoculated onto Carborundum-dusted cotyledons of newly emerged Cocozelle seedlings. After 7–14 days, leaves with typical symptoms were used as inoculum for the host range studies.

Purification. ZYMV-TS2 was purified from systemically infected leaves of Senator zucchini 2–3 wk after inoculation by the methods of Sako et al (18) with slight modifications. After clarification and Triton X-100 treatment, the virus solution was layered onto an 8-ml pad of 20% sucrose in 0.02 M phosphate buffer, pH 8.5, and centrifuged at 73,500 × *g* for 90 min in a Beckman Type 30 rotor. Pellets were resuspended, layered onto 10–40% sucrose density gradients, and centrifuged at 104,000 × *g* for 2 hr in a Beckman SW 28 rotor.

Antibody production. Antibodies to ZYMV-TS2 were prepared in three female Swiss Webster mice (16,19). Each

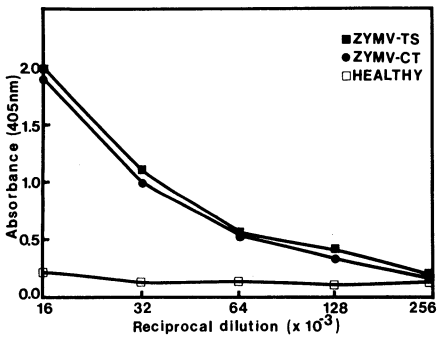

Fig. 1. Titer of anti-ZYMV-TS2 (zucchini yellow mosaic virus) unfractionated ascites fluid in indirect ELISA against extracts of healthy or ZYMV-Ct- or ZYMV-TS2-infected squash plants at various dilutions.

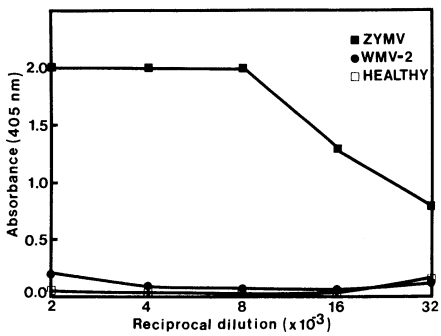

Fig. 2. Titer of anti-ZYMV-TS2 (zucchini yellow mosaic virus) unfractionated ascites fluid in indirect ELISA against extract of healthy or ZYMV-TS2- or WMV-2-infected squash plants at various dilutions.

mouse was immunized by intraperitoneal (IP) injection of 20 μg of purified virus in 0.1 ml of phosphate buffer emulsified with an equal volume of Freund's complete adjuvant. Four similar injections of 20 μg of purified virus mixed with Freund's incomplete adjuvant were given at weekly intervals. These were followed by IP injection of 0.2 ml of crude ascites cells taken from a mouse infected with tumor-inducing *Rous sarcoma* virus. Two days later, a final IP injection of 20 μg of purified virus mixed with Freund's incomplete adjuvant was administered. Four days after the last injection, ascites fluid collection started and continued every 2 or 3 days for up to 1 mo. Ascites fluid was clarified by low-speed centrifugation, and sodium azide was added to 0.02% before storage at 4 C.

Serology. Ouchterlony gel double-diffusion tests were performed in 0.5% agar gel (Oxoid, L-28 agar) containing 0.25% sodium dodecyl sulfate (SDS), 1% sodium chloride, and 0.1% sodium azide (13). Indirect enzyme-linked immunosorbent assay (ELISA) was performed using the buffers described by Clark and Adams (1).

Microtiter plates were coated for 4 hr at 37 C with crude plant sap diluted 1:100 (w/v) in coating buffer. Unfractionated antivirus ascites fluid at various dilutions was incubated overnight at 4 C. Goat antimouse IgG-alkaline phosphatase conjugate (Sigma) diluted 1:1,000 (v/v) was incubated at 37 C for 3–4 hr. The absorbance at 405 nm was measured on a Gilford PR-50 EIA apparatus 10–30 min after addition of substrate. An ELISA reaction was considered positive if it exceeded the mean plus two standard deviations of the healthy tissue controls at the same dilution.

RESULTS

Purification. Squash leaves infected with ZYMV-TS2 yielded a range of 5–22 mg of virus per kilogram of tissue for different preparations based on an extinction coefficient of 2.4 for WMV (18). The virus sedimented as a single component in sucrose density gradients. A dilution of the virus preparation used in antibody production measured 0.22, 0.17, and 0.02 absorbance units at 260, 280, and 320 nm, respectively, for a 260/280 ratio of 1.34 (corrected for light scattering).

Antibody production. Ascites fluid was collected at 2- to 3-day intervals from development of the tumors until death. Total yields of fluid from the three mice were 5, 27, and 202 ml, respectively, varying with the longevity of the mice.

ELISA. The reciprocal dilution end point of ascites fluid preparations ranged between 128,000 and 1,600,000, depending on collection date of fluid, when tested in indirect ELISA using plates coated with a 1:100 dilution (w/v) of ZYMV-infected sap. The curves of absorbance value versus dilution were not significantly different for ZYMV-TS2 and ZYMV-CT using ascites fluid from the first draining, indicating that these viruses are closely

Table 1. Host reactions resulting from mechanical inoculation with papaya ringspot virus (PRSV-W), watermelon mosaic virus 2 (WMV-2), and zucchini yellow mosaic virus (ZYMV-Ct, Fl, TS2, and E)

Species and cultivar	PRSV-W	WMV-2 (NY, Fl, ATCC)	ZYMV CT, E, TS2	ZYMV Fl
Capsicum annuum				
Yolo Wonder	−/−[a]	−/−	−/−	−/−
Chenopodium quinoa	−/−	LLc/−	LLc/−	LLc/−
Citrullus vulgaris				
Charleston Grey	−/Mo,Ma	−/Mo	−/ChlS,Mo,Ma	−/ChlS,Mo,Ma
Cucumis sativus				
National Pickling	−/VChl,Mo	−/VChl	−/VChl,Mo	−VChl,Mo
Straight 8				
Cucurbita maxima				
Great Northern	−/VChl,Mo	−/Mo	−/ChlS,Mo,Ma	−/ChlS,Mo,Ma
Cucurbita pepo				
Early Prolific Straightneck, Multipik, Elite, Senator, Eskandavi	−/VChl,LR(7)[b]	−/VChl(7),Vb,Mo	−/VChl,LR(5),Mo,Ma,Stu	−/VChl(10),Mo,Ma
Cucumis melo				
B63-3	−/Mo,Stu	−/Mo,Ma	−/ChlS,Mo,Ma	−/ChlS,Mo,Ma
Lycopersicon esculentum				
Campbells 147	−/−	−/−	−/−	−/−
Phaseolus lunatus				
Henderson Bush	−/−	−/−	−/−	−/−
P. vulgaris				
Black Turtle 2	−/−	−/Mo	Vb,Mo/−	Vb,Mo/−
Pinto	−/−	−/−	−/−	−/−
Physalis floridana	−/−	−/−	−/−	−/−
Nicotiana tabacum	−/−	−/−	−/−	−/−
Vigna unguiculata	−/−	−/−	−/−	−/−

[a] Local/systemic symptoms: − = no symptom, ChlS = chlorotic spots, LLc = chlorotic local lesions, LR = leafroll, Ma = malformation, Mo = mosaic, Stu = stunt, Vb = veinbanding, and VChl = veinclearing.

[b] Number of days after inoculation when symptoms first appeared.

Table 2. Serological relationships among isolates of zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus 2 (WMV-2), and papaya ringspot virus (PRSV-W) as determined by agar gel double-diffusion

Antigens	Antisera								
	ZYMV-TS2	ZYMV-CT	ZYMV-It	ZYMV-E	WMV-2-ATCC	WMV-2-It	WMV-2-Fl	PRSV-W-ATCC	PRSV-W-Fl
ZYMV-CT	+[a]	+	+	+	−	−	S	−	−
ZYMV-E	+	+	+	+	−	−	S	−	−
ZYMV-TS2	+	+	+	+	−	−	S	−	−
ZYMV-Fl	+	+	+	+	−	−	S	−	−
WMV-2-ATCC	−	NT	NT	S	+	NT	NT	NT	−
WMV-2-Fl	−	−	−	S	+	+	+	−	−
WMV-2-NY	−	−	NT	S	+	+	+	−	−
WMV-2-NJ	−	−	−	S	+	+	+	−	−
PRSV-W-ATCC	−	−	NT	−	−	NT	NT	+	+

[a] + = Virus-specific positive reaction, − = no reaction, S = heterologous reaction with spur formation to homologous antigen, and NT = not tested.

related if not identical (Fig. 1). ELISA values were not significantly different between healthy sap and WMV-2-infected sap at dilutions greater than 1:4,000 of the first draining of ZYMV-TS2 ascites fluid (Fig. 2).

Host range. The isolates of ZYMV from Turkey, Egypt, and Connecticut were identical in host reactions and differed slightly from the Florida isolate (Table 1). With the Connecticut group, the veinclearing symptoms on squash appeared within 5 days and subsequent symptoms were severe. However, veinclearing on squash did not develop until 10 days after inoculation of the Florida isolate, and subsequent symptoms were milder than those of the Connecticut group for about 1 mo. All ZYMV isolates tested under these conditions were distinguishable from WMV-2 and PRSV-W isolates by localized infections of Black Turtle 2 bean, a host that is systemically infected by WMV-2 and resistant to PRSV-W (10). *Chenopodium quinoa* Willd. reacted with local lesions to ZYMV and WMV-2 but not to PRSV-W.

Serological comparisons. Most of the ascites fluids collected did not react in Ouchterlony gel double-diffusion plates. One of the ascites fluid preparations that had a high titer in indirect ELISA reacted weakly in Ouchterlony plates and had a titer of 1:64 in microprecipitin tests against purified virus at 0.1 mg/ml. Isolates of ZYMV, PRSV-W, and WMV-2 were compared for serological relationships in Ouchterlony tests using antisera donated by colleagues as previously noted, and results are shown in Table 2. No serological differences were detected among the ZYMV isolates from Turkey, Egypt, or the United States against any of the ZYMV antisera tested. Cross-reactions were observed in the form of spur lines between WMV-2 and ZYMV using antiserum to ZYMV-Egypt but not with other ZYMV antisera. Conversely, cross-reactions occurred between WMV-2 and ZYMV using antiserum to WMV-2-Florida but not other WMV-2 antisera. No reactions occurred between PRSV-W and ZYMV or between PRSV-W and WMV-2.

DISCUSSION

Host range results obtained under uniform conditions are consistent with host range reactions previously reported for these and other isolates (5–8,11,12). This indicates that there is little variation in this property among ZYMV isolates from Turkey, Egypt, and the United States and that these reactions are useful in differentiating among the potyviruses that infect cucurbits.

ZYMV isolates have been reported (11) to fall into two biotypes, those represented by the Florida isolate that produce milder symptoms on squash that are delayed by 3–5 days and those represented by the Connecticut isolate that produce severe yellowing symptoms. It is interesting to note that ZYMV-TS2 and ZYMV-E were similar to the Connecticut biotype.

This study has shown that all isolates of ZYMV tested (from Turkey, Egypt, and the United States) are serologically identical by Ouchterlony gel double-diffusion tests. In addition, the serological relationship between ZYMV and WMV-2 found by others (6,11,12) has been confirmed as well as the observation that cross-reactions between these viruses depend on the antisera used (12).

ZYMV is one of the most aggressive and destructive viruses of squash in the world. Since the first report in 1981, it has spread rapidly throughout Europe, the Middle East, and the United States. From the limited comparisons on host range and serological properties presented here, it appears that this spread could be due to one or two strains that have become rapidly disseminated. Control of this disease will probably be most effectively obtained by the development of resistant cultivars.

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