

## Characterization of a Potyvirus That Causes Zucchini Yellow Mosaic

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### ABSTRACT

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A potyvirus, for which the provisional name of zucchini yellow mosaic virus (ZYMV) is proposed, was isolated from diseased zucchini squash (*Cucurbita pepo*) in northern Italy. It was sap transmitted to 15 herbaceous species belonging to seven different botanical families, and caused severe symptoms in zucchini, cantaloupe, and watermelon. The virus was transmitted nonpersistently by *Myzus persicae*. It was purified from *C. pepo* by extraction in 0.5 M  $K_2HPO_4$  containing 0.02 M  $Na_2SO_3$ , 0.01 M DIECA, and 0.005 M EDTA, clarification with Freon 113, sedimentation by ultracentrifugation, and rate-zonal centrifugation in 10–50% sucrose gradients in 0.05 M sodium citrate containing 0.02 M sodium sulfite. The purified virus had an ultraviolet absorption spectrum typical of a nucleoprotein with low nucleic acid content and a buoyant density in CsCl

of 1.323 g/ml. Homologous antisera had titers of 1:1,024 and 1:2,048, as determined by slide precipitin tests. The virus reacted with two sera to Italian isolates of watermelon mosaic virus 2, but not with a serum to an isolate from Florida. Antisera to ZYMV also reacted slightly against bean yellow mosaic virus. The virus was not serologically related to 11 other potyviruses. Its modal length was 750 nm. Pinwheels and scrolls were observed in the cytoplasm of infected *C. pepo* cells. Phenol-extracted RNA of ZYMV was infectious and migrated as a single homogeneous species in 2.4% polyacrylamide gels; its molecular weight was estimated at  $\sim 3 \times 10^6$ . Upon electrophoresis of viral protein in sodium dodecylsulfate-polyacrylamide gels, a single band of protein (estimated molecular weight  $3.6 \times 10^4$ ) was detected.

*Additional key words:* purification, serology, electron microscopy.

In the Mediterranean region, 11 viruses have so far been recorded naturally infecting cucurbits, of which only four, cucumber mosaic, squash mosaic, and watermelon mosaic viruses WMV-1 and WMV-2 are known to cause important crop losses (14). In Italy, in addition to the above viruses, a necrotic strain of clover yellow vein virus (CIYVV) also has occasionally been isolated from zucchini squash (*Cucurbita pepo* L.) (13,14,25,28).

Since 1973, a new disease syndrome has been observed in zucchini crops of northern Italy. It is characterized by pronounced reduction in growth, yellowing, mosaic and blistering of the leaves, and fruit distortion and malformation. Extractions from symptomatic plants yielded a potyvirus that differed from all the other viruses known to infect cucurbits. This paper describes some properties of this potyvirus, which is provisionally named zucchini yellow mosaic virus (ZYMV).

### MATERIALS AND METHODS

**Viruses.** ZYMV was isolated in *Chenopodium quinoa* Willd., following sap inoculation from a zucchini fruit with severe malformation (Fig. 1) and then propagated and maintained in *C. pepo* 'Genovese.' Italian isolates of WMV-2 from zucchini and BYMV from bean (13) were cultured in *C. pepo* and *Nicotiana clevelandii* Gray, respectively.

**Host range.** Groups of at least 10 plants of each species were tested by sap inoculation with ZYMV from zucchini tissues extracted in 0.01 M phosphate buffer, pH 7.0. The plants were maintained under controlled glasshouse conditions and later checked by back inoculation to *C. quinoa*.

**Aphid transmission.** Apterous viviparous *Myzus persicae* Sulz. subjected to a 2-hr fast, allowed a 60-sec acquisition feeding, and 12-hr inoculation periods were used to transmit ZYMV. Zucchini was used as both donor and test plant.

**Purification.** All operations were done at 0–4 C. Infected zucchini leaves were harvested about 2 wk after inoculation with ZYMV and homogenized with three volumes (w/v) of an extraction solution consisting of 0.5 M  $K_2HPO_4$ , 0.02 M  $Na_2SO_3$ ,

0.01 M Na-diethyldithiocarbamate (DIECA), and 0.005 M ethylenediaminetetraacetate sodium salt (EDTA), pH 8.5. The slurry was emulsified with an equal volume of Freon 113 (a fluorocarbon: 1,1,2-trifluoro-1,2,2-trichloroethane) and, after centrifugation at 25,000 g for 10 min, the virus was recovered from the aqueous phase by ultracentrifugation at 21,000 rpm for 240 min in a Beckman R21 rotor. The sediment was suspended in 0.05 M sodium citrate containing 0.02 M  $Na_2SO_3$ , adjusted to pH 7.5 with a few drops of 1.0 M citric acid (= citrate solution), and centrifuged at 25,000 g for 5 min. The supernatant was then layered onto a preformed linear 10–50% sucrose gradient in the citrate solution and centrifuged at 22,000 rpm for 120 min in a Beckman SW25.2 rotor. The light-scattering band was withdrawn, the material was pelleted by centrifugation at 50,000 rpm in a Beckman R50.2 rotor for 90 min, and resuspended in appropriate media as specified below. At various purification steps the virus was checked by infectivity testing, electron microscopy, and by serology when an antiserum became available.

**Spectrophotometry.** Virus and nucleic acid preparations were scanned in a Pye Unicam SP 1800 Spectrophotometer (Pye Unicam Ltd., Cambridge, UK), equipped with a constant-temperature cell, a temperature controller, and Pye Scalamp thermocouple for thermal denaturation studies.

**Isopycnic centrifugation.** Purified ZYMV in 0.01 M phosphate buffer, pH 7.0, was mixed with 5 ml of a CsCl solution in the same buffer ( $\rho = 1.31$  g/ml) and centrifuged in the Beckman SW39L rotor at 33,000 rpm for 40–50 hr at 10 C. Centrifuge tubes were then punctured at the bottom and the contents were collected dropwise into 24–27 fractions. These were monitored in the spectrophotometer at 260 nm, and the densities of selected fractions were determined by the refractive index measured with an Abbé refractometer.

**Serology.** Two rabbits were injected intramuscularly with purified virus emulsified with Freund's complete adjuvant. The first received two injections, each one containing  $\sim 1.0$  mg of virus in 1.0 ml of 0.05 M phosphate buffer, pH 7.5; the second one received 3.2 mg of virus in 1.3 ml of the same buffer. Bleedings were taken at monthly intervals. Normal sera were obtained from uninoculated rabbits. Antibody-antigen reactions were tested in slide precipitin, agar gel, or sodium dodecylsulfate (SDS)-agar gel

double diffusion tests, as specified in the text. When necessary, antisera were absorbed with antigens from healthy plants as described by Luisoni and Conti (15). Some serological reactions were checked by immune electron microscopy by using the 'decoration' technique (19).

Preparations of ZYMV were tested against sera to bean common mosaic virus, bean yellow mosaic virus (BYMV), celery mosaic virus, henbane mosaic virus, potato virus Y (PVY), turnip mosaic virus, *Primula obconica* potyvirus (12), WMV-1 and -2 (Italian isolates), wisteria vein mosaic virus, potyvirus Z-1 from zucchini (13) of this laboratory collection, CIYVV (an English isolate from M. Hollings, and a pea necrosis strain from D. Z. Maat), lettuce mosaic virus (from J. A. Tomlinson), and to Florida isolates of WMV-1 and -2 (from D. E. Purcifull).

**Electron microscopy.** The modal length of the particles was determined from crude preparations deposited on carbon-Formvar films on 38- $\mu$ m (400-mesh) grids negatively stained with 1% uranyl acetate (UA). Fields were photographed at  $\times 24,000$  in a Philips EM 300 (Philips, Eindhoven, The Netherlands) or AEI Corinth (Kratos, formerly Associated Electrical Int., Manchester, UK) electron microscope by using similarly treated particles of tobacco mosaic virus (TMV) as length standards (modal length taken as 300 nm). Purified preparations also were negatively stained in UA. For thin sectioning, systemically infected leaves of *C. pepo* (13 days after inoculation) were fixed in 3% glutaraldehyde and 3% acrolein, postfixed in 1% osmium tetroxide, and further processed (21).

**Polyacrylamide gel electrophoresis of viral protein.** Purified virus was solubilized overnight in 0.01 M phosphate buffer (pH 7.2) containing 2% SDS, 1% 2-mercaptoethanol and 8 M urea (1). Alternatively, the virus was precipitated with two volumes of acetone and the protein was obtained as described by Francki et al (6). Unless otherwise stated, electrophoresis was done in 15% gels at room temperature in a Bio-Rad Model 220 slab-gel apparatus in a

continuous system as described by Swank and Munkres (30) or in the discontinuous system of Laemmli (11). For molecular weight estimates (33), the following marker proteins were used (molecular weights in parenthesis): horse heart cytochrome *c* ( $1.17 \times 10^4$ ), bovine pancreatic  $\alpha$ -chymotrypsinogen ( $2.57 \times 10^4$ ), bovine erythrocyte carbonic anhydrase ( $2.90 \times 10^4$ ), pepsin from hog stomach mucosa ( $3.50 \times 10^4$ ), ovalbumin H chain ( $4.30 \times 10^4$ ), bovine liver glutamate dehydrogenase ( $5.30 \times 10^4$ ), all from Sigma Chemical Company (St. Louis, MO 63178), and TMV coat protein ( $1.75 \times 10^4$ ).

**Nucleic acid extraction and analysis.** Nucleic acid was isolated from purified ZYMV suspended in sterile 0.1 M Na acetate-0.001 M EDTA, pH 6.5, by the double-phase phenol-SDS procedure (1). Alternatively nucleic acid was released by overnight digestion with pronase (protease type VI from *Streptomyces griseus*, Sigma; preincubated at 37 C for 30 min) and 0.5% SDS in 0.1 M Na acetate and then extracted as above and precipitated with ethanol. The various preparations were lyophilized and kept frozen. Infectivity of the nucleic acid, before and after digestion with pancreatic ribonuclease A (RNase, Sigma), was assayed in zucchini.

Thermal denaturation curves of ZYMV nucleic acid in 1  $\times$  SSC buffer (SSC = 0.15 M NaCl and 0.015 M Na citrate, pH 7.0) were determined as described by Redolfi and Pennazio (26).

Polyacrylamide gel electrophoresis in nondenaturing 2.4% gels, buffered with 0.04 M tris(hydroxymethyl)aminomethane (Tris), 0.02 M Na acetate, 0.001 M EDTA, pH 7.2, was as described by Redolfi and Boccardo (27). The following viral RNAs (molecular weights in parenthesis) were used as molecular weight standards: TMV ( $2.1 \times 10^6$ ) (2), tomato bushy stunt virus ( $1.65 \times 10^6$ , TBSV) (4), and alfalfa mosaic virus (1.3, 1.0, 0.7,  $0.34 \times 10^6$ , AMV) (22).

## RESULTS

**Host range.** ZYMV infected 15 plant species belonging to seven different families, but caused severe systemic diseases only on some cucurbits (Table 1). In *C. pepo* (Fig. 2), chlorotic local lesions appeared 6-8 days after inoculation, at the same time as vein



Fig. 1. Zucchini fruit naturally infected with zucchini yellow mosaic virus.

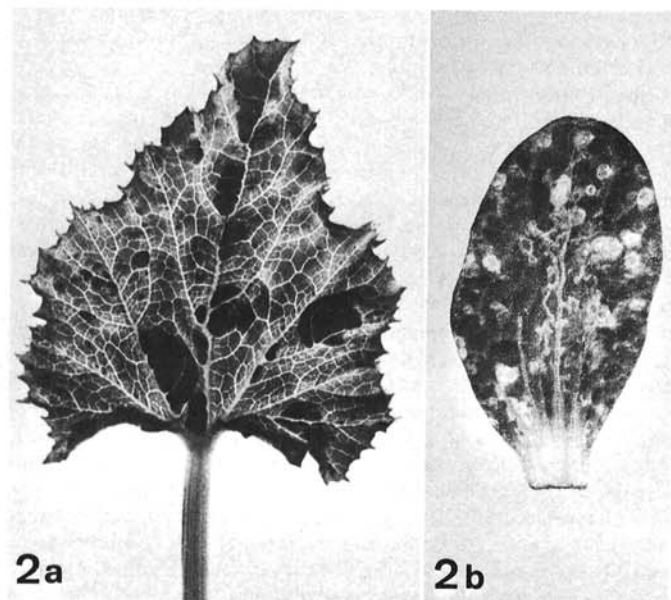


Fig. 2. Experimental zucchini yellow mosaic virus infection on zucchini cultivar Genovese: a, systemic infection; b, cotyledonary local lesions.

TABLE 1. Host range of zucchini yellow mosaic virus

Family, species, and cultivars	Symptoms <sup>a</sup>	
	Local infection	Systemic infection
Aizoaceae		
<i>Tetragonia expansa</i> Murr.	c/l	0
Amaranthaceae		
<i>Gomphrena globosa</i> L.	c/l	0
Chenopodiaceae		
<i>Chenopodium amaranticolor</i> Coste et Reyn.	c/l	0
<i>C. quinoa</i> Willd.	c/l	0
<i>Spinacia oleracea</i> L.	L	0
Cucurbitaceae		
<i>Citrullus lanatus</i> (Thunb.) Mansfeld		
'Congo'	n/l	M
<i>Cucumis melo</i> L. 'Cantaloup'	c/l	M
'Hale's Best'	n/l	M
<i>C. sativus</i> L. 'Marketer'	n/l	M
<i>Cucurbita pepo</i> L. 'Genovese,'		
'Prince Noir,' 'Tarmino,' 'Tromba,'		
'd'Albenga,' and 'Vemor'	(c/l)	M
<i>Luffa acutangola</i> Roxb.	L	L
<i>L. aegyptiaca</i> Mill.	L	0
Leguminosae		
<i>Phaseolus vulgaris</i> L. 'Saxa,'	c/l	0
'Double White Princess,'		
and 'Great Northern 123'	0	0
<i>Trigonella foenum-graecum</i> L.	L	(M)
Umbelliferae		
<i>Ammi majus</i> L.	L	L
Solanaceae		
<i>Nicotiana clevelandii</i> Gray	L	0

<sup>a</sup> Explanation of symbols: c/n/l = chlorotic/necrotic local lesions; L = latent; M = mosaic; 0 = no symptoms and no virus recovered. Parentheses indicate that the symptoms were not consistently present.

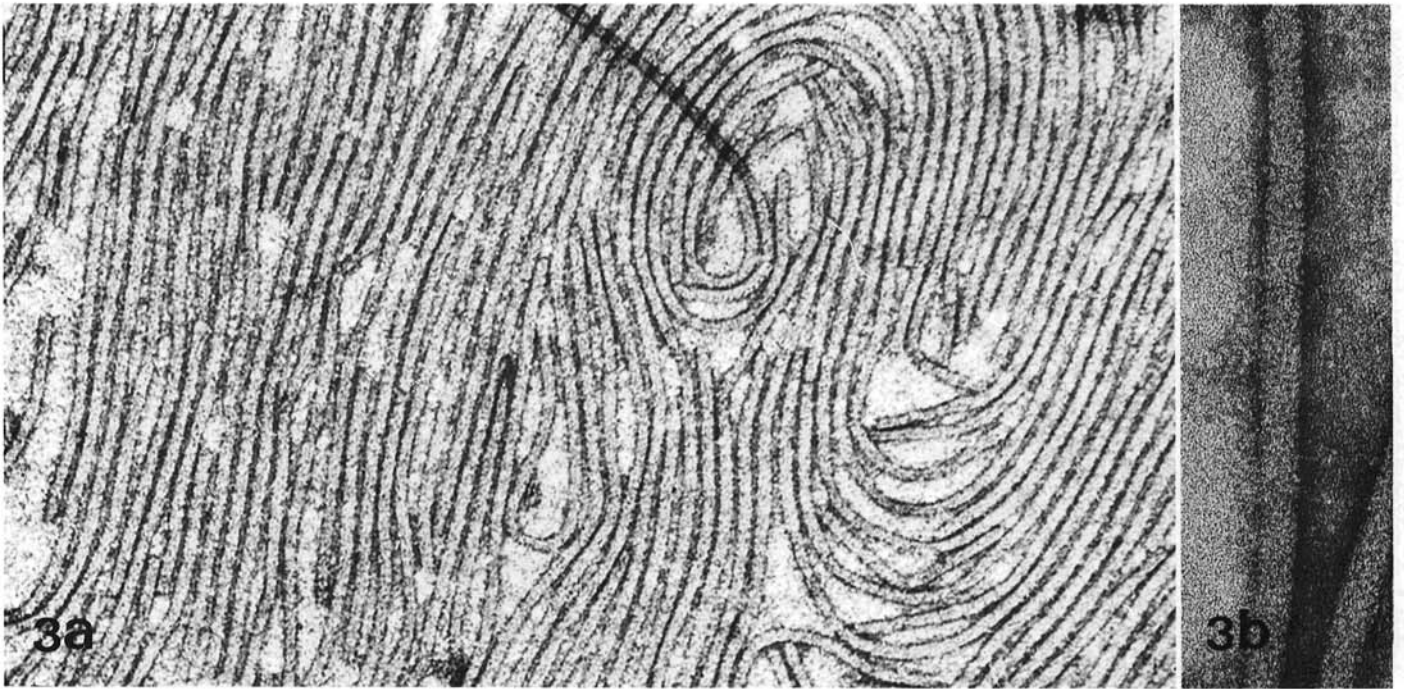


Fig. 3. Purified zucchini yellow mosaic virus particles after isopycnic centrifugation and negative staining with 1% uranyl acetate. Magnifications: a,  $\times 110,000$ ; b,  $\times 370,000$ .

netting, which was followed by yellowing, mosaic, blistering, and often necrosis and plant death. Local symptoms were not consistent, and were not observed in late summer. A similar syndrome was induced in *C. melo* L. Failure to recover the virus by back inoculation to *C. quinoa* indicated that it did not infect *Amaranthus caudatus* L.; *Apium graveolens* L.; *Brassica chinensis* L.; *Catharanthus roseus* (L.) G. Don; *Datura stramonium* L.; *Lavatera trimestris* L.; *Lycopersicon esculentum* Mill.; *N. glutinosa* L.; *N. megalosiphon* Huerck et Nuell.; *N. rustica* L.; *N. tabacum* L. 'White Burley'; *Pisum sativum* L. 'Juwel' and 'Koroza' *Ocimum basilicum* L.; or *Vigna unguiculata* (L.) Walp.

**Aphid transmission.** *M. persicae* (one individual per plant) transmitted the virus from *C. pepo* to three of 10 seedlings of the same species that were exposed.

**Purification.** Under the most favorable circumstances, 1 kg of tissue yielded about 10 mg of virus, as estimated spectrophotometrically taking 2.8 as the extinction coefficient at 260 nm (29). Use of chloroform for sap clarification decreased virus yield. The virus also could be precipitated from the clarified sap with polyethylene glycol 6000, but with no yield improvements as judged by serology. Use of high-molarity (0.5 M) buffers for resuspending concentrated ZYMV prior to sucrose density gradient centrifugation resulted in lower yields. In the citrate solution a large number of particles aggregated and sedimented during the low-speed centrifugation. To recover as much virus as possible, the sediments were washed several times in the citrate solution and the clarified supernatants were pooled and layered onto sucrose gradients. Upon isopycnic centrifugation in CsCl, purified ZYMV (Fig. 3) formed a single band with a buoyant density of 1.323 g/ml (Fig. 4).

The purified virus, suspended in 0.01 M phosphate buffer, pH 7.0 had an ultraviolet absorption profile typical of a nucleoprotein, with minima and maxima at 245 and 260 nm, respectively, and a tryptophan shoulder at 288 nm. The  $A_{max}/A_{min}$  and  $A_{260}/A_{280}$  ratios (uncorrected for light scattering) were 1.07 and 1.13, respectively, which suggested a low nucleic acid content, as expected for an elongated virus.

**Electron microscopy.** Elongated flexuous particles were easily detected in crude sap of infected *C. pepo*. When negatively stained in UA in crude preparations, the modal length of the particles was close to 750 nm (Fig. 5).

Figure 6 illustrates part of a typical ultrathin section of ZYMV-

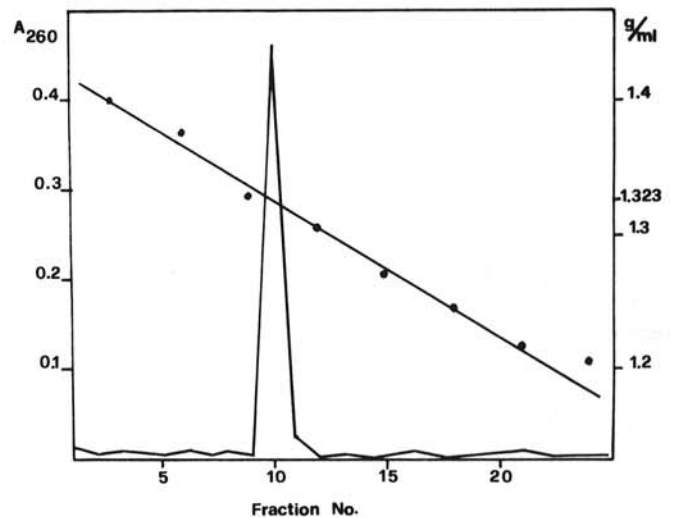


Fig. 4. Buoyant density of zucchini yellow mosaic virus by fractionation of a CsCl gradient ( $\rho = 1.31$  g/ml). Sedimentation from left to right.

infected cell of *C. pepo*. Inclusion bodies, consisting of pinwheels and scrolls (5) were readily detectable in the cytoplasm. No laminated aggregates or amorphous compact inclusions (18) were observed.

**Serology.** The two anti-ZYMV sera had homologous titers of 1:1,024 and 1:2,048 (as determined by the slide precipitin test) and titers of none or 1:2 against host components, respectively (as determined by slide precipitin and gel-diffusion tests). Table 2 summarizes the data on homologous and heterologous titers of the sera to ZYMV and two Italian isolates of WMV-2 (as determined by the slide precipitin test), and the serological differentiation indexes (SDIs) (32) between the two viruses: the results indicate only a distant relationship. The cross-reactions were confirmed in an electron microscopy experiment by using the immunological 'decoration' technique (19), as shown in Fig. 7. The two anti-ZYMV sera, absorbed against healthy plant preparations, reacted also against partially purified BYMV preparations up to 1:4 and 1:8, respectively, in the slide precipitin test (SDIs = 8). An anti-

BYMV serum (homologous titer 1:512) did not react against ZYMV, either in slide precipitin tests or in the immunological electron microscopy experiments, possibly because of its lower titer. No reaction occurred between ZYMV and the other sera tested as detailed in Materials and Methods, including sera to an Italian isolate of WMV-1 and to WMV-1 and -2 from Florida, either in the slide precipitin or in the SDS-agar gel diffusion test (23).

**The coat protein.** Irrespective of the method adopted for virus dissociation, ZYMV coat protein migrated as a single homogeneous species when subjected to electrophoresis in 15% polyacrylamide gels. Similar results were obtained when protein preparations were analyzed in either the discontinuous system with Tris-glycine-SDS buffer (11) or the continuous system of Swank and Munkres (30) with Tris-phosphate-urea buffer. By co-electrophoresis with marker proteins ZYMV coat protein was estimated to have a molecular weight of  $3.48 \times 10^4$  in the continuous system (mean of seven determinations on three different protein preparations; s.e. =  $0.1 \times 10^4$ ), and  $3.65 \times 10^4$  in the discontinuous system (mean of six determinations on the same

preparation; s.e. =  $0.02 \times 10^4$ ) (Fig. 8).

Protein preparations of ZYMV were pooled and analyzed in 15.0, 12.5, 10.0, and 7.5% polyacrylamide gels in the discontinuous system. The apparent molecular weight of the coat protein was virtually independent of the gel concentration used; it varied from 3.69 to  $3.63 \times 10^4$ , with decreasing gel concentrations.

**The nucleic acid.** Phenol-extracted ZYMV nucleic acid, suspended in 0.01 M phosphate buffer, pH 7.0, at a concentration of 15  $\mu\text{g}/\text{ml}$  caused the appearance of typical symptoms in *C. pepo*, but not after digestion at 37 C for 30 min with 3.3  $\mu\text{g}/\text{ml}$  RNase. Thermal denaturation profiles had the shape expected for a single-stranded nucleic acid, with denaturation completed at 76 C. The total hyperchromicity developed at 260 nm was about 22%. These data suggest that the ZYMV genome, as expected for a potyvirus, is a single-stranded RNA.

Irrespective of the extraction method adopted, RNA of ZYMV always migrated in 2.4% polyacrylamide gels as a single molecular species and, by coelectrophoresis with marker viral RNAs (Fig. 9), its molecular weight was estimated to be  $2.93 \times 10^6$  (mean of six determinations; s.e. =  $0.05 \times 10^6$ ).

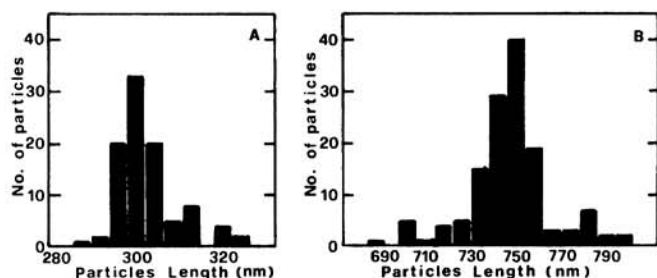


Fig. 5. Histogram of zucchini yellow mosaic virus particle length distribution: **A**, crude preparation from TMV-infected tobacco; **B**, crude preparation from ZYMV-infected zucchini.

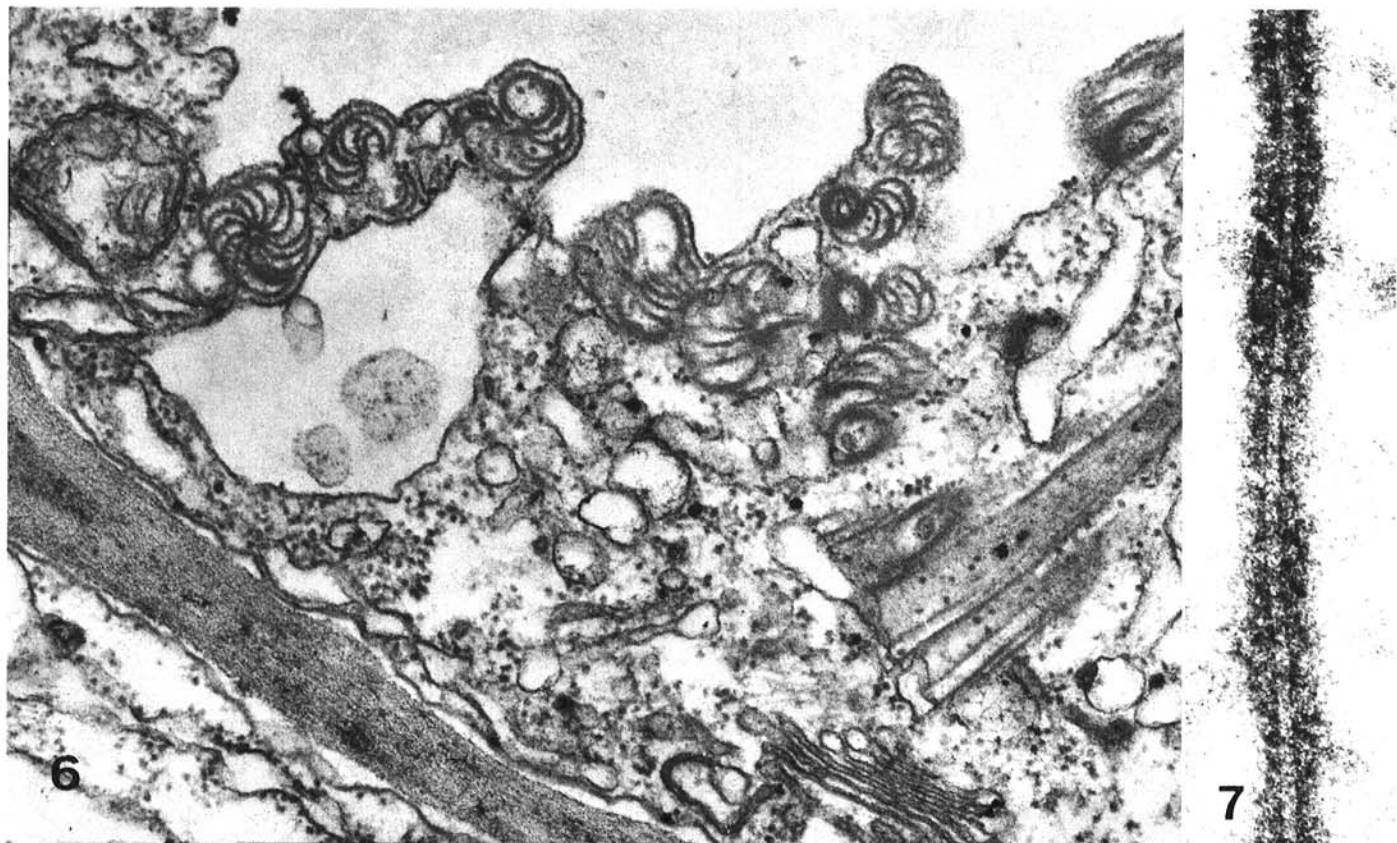
TABLE 2. Serological relationships between zucchini yellow mosaic virus (ZYMV) (two antisera, indicated as I and II) and two different Italian isolates of watermelon mosaic virus (WMV-2 A and B)

Serum	Titers <sup>a</sup> to:		
	ZYMV	WMV-2	SDI <sup>b</sup>
ZYMV - I	1:1,024	1:8 <sup>c</sup>	7
ZYMV - II	1:2,048	1:32 <sup>c</sup>	6
WMV-2 - A	1:32	1:512	4
WMV-2 - B	1:64	1:1,024	4

<sup>a</sup> Determined by the slide precipitin test.

<sup>b</sup> SDI = serological differentiation indexes.

<sup>c</sup> Same titer either with isolate A or B.



Figs. 6 and 7. 6, Ultrathin section of a zucchini yellow mosaic virus-infected zucchini mesophyll cell ( $\times 43,000$ ) showing pinwheels and scrolls. 7, WMV-2 particles decorated (19) with undiluted antizucchini yellow mosaic virus serum. (Magnification  $\times 145,000$ ).

## DISCUSSION

ZYMV can clearly be assigned to the potyvirus group (16) on the basis of the data presented above. The buoyant density (1.323 g/ml) and the molecular weight values, both for the RNA (about  $3 \times 10^6$ ) and the protein (about  $3.6 \times 10^4$ ), fit well with the available data for viruses belonging to this group (10) (Table 3). The slight discrepancy in coat protein molecular weights estimated in the two polyacrylamide gel electrophoretic systems that were used can be explained by the fact that Swank and Munkres (30) originally proposed their continuous system for the resolution of oligopeptides; the molecular weight of ZYMV coat protein may be too large to be accurately estimated in this system. Therefore, we prefer to rely on the value estimated in the discontinuous system, which was also found to be virtually independent of the gel concentration used. The modal length of the particles (about 750 nm) is also typical of the potyvirus group. Inclusion bodies induced by ZYMV would characterize it as a member of potyvirus subdivision I, according to the classification of Edwardson (5).

Antisera to ZYMV reacted slightly against an isolate of BYMV from bean (13) (SDIs = 8); the reciprocal reaction did not occur, possibly because of the lower titer of the anti-BYMV serum. Although ZYMV reacted with two antisera against Italian isolates of WMV-2 (in Table 2, SDIs ranged from 4 to 7), it did not react with antisera prepared against the Florida isolate. We also observed some differences in host-range of ZYMV, WMV-2, and BYMV. Our virus was able to systemically invade *Luffa acutangola* Roxb., a species immune to WMV-2. *Lavatera trimestris* L. and *Pisum sativum* L. 'Koroza' were immune to ZYMV but both were

susceptible to WMV-2. BYMV did not infect cucurbits but caused systemic mosaic on *N. clelandii* Gray, on which ZYMV induced only latent local infection. No serological relationship was detected between ZYMV and lettuce mosaic, pea necrosis, clover yellow vein, wisteria vein mosaic viruses and the *Primula obconica* potyvirus, all of which belong to the BYMV 'cluster' (3). Moreover, ZYMV appeared to differ from soybean mosaic and blackeye cowpea mosaic viruses that are serologically related to WMV-2 (24). There was no serological relationship between ZYMV, WMV-1, and potyvirus Z-1, a strain of CIYVV isolated from zucchini in northern Italy (13). It also appeared to have a host range and inclusion bodies that differ from those of the zucchini yellow fleck agent, a potyvirus isolated from the same in southern Italy (G. P. Martelli, *personal communication*). We therefore suggest that ZYMV is a distinct potyvirus able to infect cucurbits.

TABLE 3. Molecular weights of some potyvirus nucleic acids and proteins, determined by polyacrylamide gel electrophoresis

Virus	RNA ( $\times 10^6$ )	Protein ( $\times 10^4$ ) <sup>a</sup>	References
Grapevine leafroll-associated virus	2.9 - 3.5	3.1	(31)
Maize dwarf mosaic virus	2.89 <sup>b</sup> ; 2.7 <sup>c</sup>	3.65 <sup>c</sup> ; 3.3 <sup>c</sup>	(9) <sup>b</sup> (8,20) <sup>c</sup>
PVY	3.1	3.4; 2.8	(7,17,20)
Tobacco etch virus	3.14	3.2 (2.6)	(7,9)
Turnip mosaic virus	3.31	3.6 (2.7)	(7,9)
ZYMV	2.93	3.6	(this study)

<sup>a</sup> In parentheses the 'minor component' molecular weight.

<sup>b</sup> Type strain.

<sup>c</sup> B strain (sugarcane mosaic).

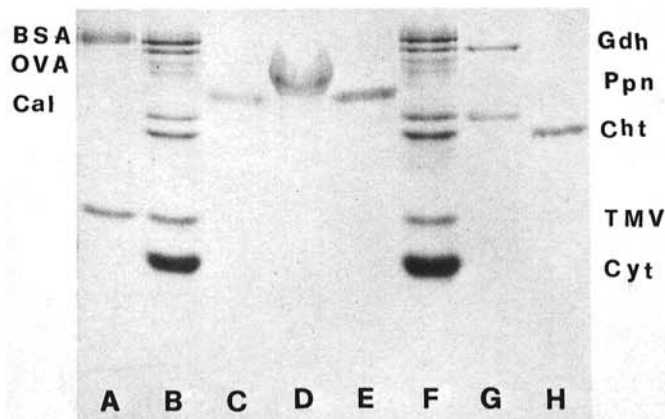


Fig. 8. Electrophoresis of zucchini yellow mosaic virus (ZYMV) coat protein (lanes C and E) in 15% polyacrylamide gel (discontinuous system [11]). Explanation of symbols: BSA = bovine serum albumin; Gdh = glutamate dehydrogenase; OVA = ovalbumin; Ppn = papain; Cah = carbonic anhydrase; Cht =  $\alpha$ -chymotrypsinogen; TMV = TMV coat protein; Cyt = cytochrome *c*. Migration from top to bottom. Electrophoresis was at 10 mA/slab for 1 hr, and then at 30 mA/slab for 4 hr. Lane A: BSA and TMV. Lanes B and F: BSA, Gdh, OVA, Cah, Cht, TMV, Cyt. Lanes C and E: ZYMV coat protein. Lane D: Ppn. Lane G, Gdh, Cah, Lane H: Cht.

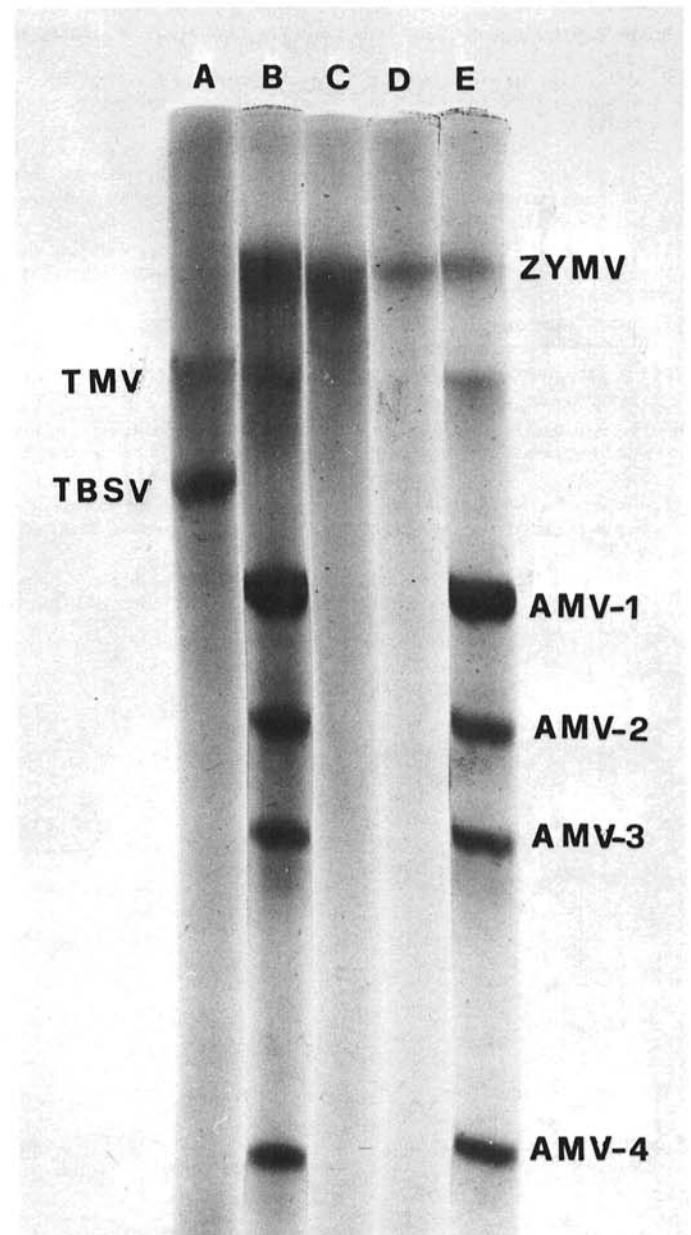


Fig. 9. Electrophoresis of zucchini yellow mosaic virus (ZYMV) and marker viral RNAs in nondenaturing 2.4% polyacrylamide gels. Electrophoresis was at a constant current of 3 mA per tube for 5 hr and migration was from top to bottom. Tube A: RNAs from TMV and TBSV. Tube B: ZYMV (pronase-extracted), TMV, and alfalfa mosaic viruses (AMV). Tube C: pronase-extracted ZYMV. Tube D: phenol-extracted ZYMV. Tube E: ZYMV (phenol-extracted), TMV, and AMV.

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