Purification and Some Properties of South African Isolates of Ornithogalum Mosaic Virus

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ABSTRACT

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Horticulturally important *Ornithogalum* and *Lachenalia* spp. were found to be infected with filamentous viruses. Symptoms in infected plants were similar to those produced by Ornithogalum mosaic virus (OMV). An enzyme-aided purification protocol was developed, which eliminated a highly viscous mucilage from extracts of both species. Serological tests indicated that virus isolates from *Ornithogalum* (OMV-O) and from *Lachenalia* (OMV-L) were indistinguishable. A morphologically similar virus, OMV-W, isolated from local symptom-bearing wild *O. thyrsoides*, was serologically closely related to OMV-O and OMV-L. Local isolates of OMV were serologically closely related to the Dutch isolate of OMV and were also related to several other potyviruses. Initial observations that OMV belongs to the potyvirus group were confirmed by biological and physicochemical characterization: the virus was mechanically transmissible to a restricted host range; it was nonpersistently aphid-transmitted; purified flexuous particles had a modal length in the range of 720–760 nm; a single major protein band of M_r 30,000 was observed after sodium dodecyl sulfate polyacrylamide gel electrophoresis; and an M_r of 2.90×10^6 was calculated for OMV RNA after electrophoresis in denaturing formaldehyde agarose gels. Oligo (dT) cellulose chromatography confirmed that OMV RNA was polyadenylated.

Additional keywords: Hyacinthaceae, immunoelectroblotting, monospecific antibodies, plant tissue culture, virus relationships.

Ornithogalum and *Lachenalia* are two genera of the Hyacinthaceae (8) native to South Africa that have great potential as horticultural crops. Species from both genera, which are used in plant improvement programs at the Vegetable and Ornamental Plant Research Institute (VOPRI), Pretoria, were shown to be virus-infected (20). Viral infections caused severe mosaic symptoms on leaves and flower stems as well as flower deformation. Symptoms were similar to those described for Ornithogalum mosaic virus (OMV) by Smith and Brierley (37) in the United States and Derks (9) in the Netherlands. Preliminary electron microscopic studies on partially purified isolates of OMV-O and OMV-L, from *Ornithogalum* and *Lachenalia*, respectively, revealed filamentous virus particles. A morphologically similar virus, OMV-W, was isolated from symptom-bearing *O. thyrsoides* Jacq. collected from the wild (4). The filamentous nature and size of the particles suggested that the viruses might belong to the potyvirus group. In this paper we describe the use of enzyme-linked immunosorbent assay (ELISA) and immunoelectroblotting (IEB) for the investigation of the serological relationships among South African isolates of OMV, the relationship between the South African and Dutch isolates, and the relationships between OMV and other potyviruses. We further report additional biological and physicochemical characteristics of the virus isolates, which indicate that *Ornithogalum* and *Lachenalia* viruses belong to the potyvirus group.

MATERIALS AND METHODS

Virus sources. *O. thyrsoides, O. dubium* Houtt., *O. conicum* Jacq., *L. aloides* (L.f.) Asch. & Graeb., *L. viridiflora* W. Barker, *L. glaucina* Jacq., *L. mutabilis* Lodd., *L. orchidioides* Ait., *L. rubida* Jacq., *L. bulbifera* (Cirillo) Hort., and *L. reflexa* Thunb. are used in plant improvement programs at the VOPRI. Plant materials used in this work were *Ornithogalum* and *Lachenalia* hybrids derived in these programs. Virus-infected bulbs of both genera were obtained and diseased plants grown in plant growth rooms with a 14-hr photoperiod, day and night temperatures of 22 and 18 C (respectively), and 70% humidity. These plants were the source of virus for tests and purifications. The origins of other potyvirus isolates used are as follows: bean yellow mosaic virus (BYMV-G), potato virus Y (PVY-2), maize dwarf mosaic virus (MDMV-A and MDMV-B), and watermelon mosaic virus (WMV-Morocco) are reference isolates of the University of Cape Town; PVY-1 was obtained from G. Thompson, VOPRI.

Virus-free plants. Callus tissue of virus-free *Ornithogalum* and *Lachenalia* was obtained from the VOPRI (20). Callus tissue was cultured on a basal medium containing the inorganic salts of Murashige and Skoog (27) with amendments for the propagation and regeneration of *Ornithogalum* (28). For *Lachenalia* the auxin indole-3-butyric acid (2 mg/L) was added to the basal medium (29). Explants were kept in test tubes at a constant temperature of 22 C and a 16-hr photoperiod under VHO Gro-Lux fluorescent lights. Plantlets were subcultured under sterile conditions onto fresh medium at 8- to 10-wk intervals. When roots were adequately developed, plantlets were transplanted into polystyrene trays containing a porous soil mixture and kept in plant growth rooms for further development.

Transmission tests. Virus isolates were mechanically inoculated onto virus-free explants of *Ornithogalum* and *Lachenalia* and seedlings of *Chenopodium quinoa* Willd., *C. giganteum* Don., *C. album* L., *C. schraderanum* Roem. & Schult., *C. capitatum* (L.) Aschers., *Nicotiana tabacum* L. cv. Xanthi, *N. clevelandii* A. Gray, *N. benthamiana* Domin, *Tetragonia tetragonioides* (Pallas) Kuntze, *Phaseolus vulgaris* L. cv. Bonus, *Pisum sativum* L. cv. Greenfeast, *Petunia hybrida* (Hort.) Vilm.-Andr., and *Tropaeolum majus* L. Leaves were inoculated with leaf sap or freshly purified virus in 0.1 M Tris-Cl buffer, pH 9, with Celite as an abrasive. Colonies of *Myzus persicae* (Sulz.) were reared on virus-free *Ornithogalum* plants for aphid transmission studies. Aphids were starved for 4–6 hr, allowed an acquisition feeding period of 5–10 min on freshly cut infected *Ornithogalum* or

Lachenalia leaves, transferred to virus-free test plants, and allowed to feed for 2 days before being exterminated.

Virus purification. Several preliminary purification attempts, using a variety of conventional methods, failed because of the very mucilaginous nature of *Ornithogalum* and *Lachenalia* plant sap. The following method was the most successful in eliminating the mucilage. Leaves from infected plants (freshly harvested or kept at 4 C for up to 4 mo) were homogenized in a Waring blender with 0.1 M sodium acetate buffer, pH 5.5 (1:3, w/v), containing hemicellulase (Sigma Chemical Company, St. Louis, MO) (1 mg of enzyme per gram of leaf tissue). The mucilaginous extract was liquefied by incubation at room temperature (22 C) for 4–6 hr and solid material separated by low-speed centrifugation (10,000*g* for 10 min). The supernatant was discarded and solid material rehomogenized with 0.1 M Tris-Cl buffer, pH 9, containing 1% (w/v) Na_2SO_3 and 0.1% (v/v) thioglycolic acid. The supernatant obtained from low-speed centrifugation of this extract was clarified and the virus precipitated by incubation for 1 hr with 2% (v/v) Triton X-100, 5% (w/v) polyethylene glycol 6,000, and 1.5% (w/v) NaCl. The crude preparations were concentrated by ultracentrifugation (64,000*g* for 60 min) and pellets resuspended in 0.1 M Tris-Cl, pH 9. Virus could be further purified by rate zonal centrifugation in 10–40% (w/v) sucrose gradients in 0.1 M Tris-Cl, pH 9, at 27,000 rpm for 2 hr in a Beckman SW28 rotor. Virion-containing fractions were collected with an ISCO model 640 density gradient fractionator and a model UA-5 UV-analyzer. Virus preparations were dialyzed overnight against 0.1 M Tris-Cl, pH 9, and concentrated by ultracentrifugation.

Electron microscopy. Virus preparations were adsorbed onto carbon-coated copper grids and negatively stained with 2% (w/v) phosphotungstic acid, pH 6.2, or 2% (w/v) ammonium molybdate, pH 6.5. Samples were viewed and photographed in a Zeiss EM 109 or a Jeol 200 CX electron microscope. Particle length measurements were made from electron micrographs of partially purified virus preparations on a Summagraphics tablet coupled to a Tektronix 4051 microcomputer. Tobacco mosaic virus (TMV) was used as an internal standard for calibration.

Antiserum and monospecific antibody preparation. Antisera to OMV-O, OMV-W, and OMV-L were prepared in rabbits as described by Rybicki and von Wechmar (35). Weekly injections of purified virus, emulsified in Freund's incomplete adjuvant, were administered for 4 wk. Subsequent booster injections were given monthly. Serum was collected 3 wk after the initial injections and weekly thereafter. Antisera to BYMV-G, PVY-2, WMV-2, MDMV-A, MDMV-B, cucumber mosaic virus (CMV), brome mosaic virus (BMV), TMV, and maize streak virus (MSV) were taken from the departmental collection. Antisera to PVY-1 and WMV-Morocco (38) were obtained from G. Thompson, VOPRI; antisera to WMV-1 and zucchini yellow mosaic virus (ZYMV) from H. Lecoq, Montfavet, France; antiserum to sugarcane mosaic virus (SCMV) from A. G. Gillaspie, Beltsville, MD; antiserum to wheat streak mosaic virus (WSMV) from M. Brakke, Lincoln, NE; and antiserum to passion fruit woodiness virus (PWV) from E. W. Kitajima, University of Brazil. Antisera to BYMV-Scott and soybean mosaic virus (SMV), which originate from R. O. Hampton, Oregon State University, Corvallis, and R. M. Lister, Purdue University, Lafayette, IN, respectively, were kindly donated by G. Pietersen, Plant Protection Research Institute, South Africa. Antisera to the Dutch isolate of OMV (OMV-D) and hyacinth mosaic virus (HMV) were obtained from A. F. L. M. Derks, Lisse, Netherlands. Approximate antiserum titers were determined by microprecipitin tests (30).

In order to obtain maximum specificity and to minimize background reactions in serological assays, host-absorbed antisera were used to prepare monospecific antibodies by an adaptation of the method of Rybicki (32). Virus was adsorbed to a nitrocellulose filter (BA 85, 0.45 μm pore, Schleicher & Schuell, Keene, NH) by soaking filter strips in a diluted virus preparation for 2 hr. The strips were washed on a shaker with four changes of 150 mM NaCl and 0.1% Tween 20 (wash buffer) for 5 min and then soaked in 10 mM Tris, 150 mM NaCl, 0.1% (v/v) Tween 20, and 2% (w/v) bovine serum albumin (blocking buffer) overnight to block protein adsorption sites. The strips were then shaken in host-absorbed antivirus antiserum, diluted 1:5, for 2 hr. The strips were washed again, and virus-specific antibodies eluted by agitating the strips in 10 ml of 0.1 M glycine-Cl, pH 2.9, for 10 min. The eluate was poured off, immediately neutralized by the addition of 0.1 M NaOH, dialyzed against distilled water, and concentrated by lyophilization.

ELISA. Alkaline phosphatase (1,133 units per milligram) was conjugated to the monospecific antibody preparation as described by Avrameas (2). Direct double-antibody sandwich ELISA (DAS-ELISA) was performed essentially by the method of Clark and Adams (6). Coating and conjugate antibody concentrations were 0.005 mg/ml and 0.01 mg/ml, respectively. For the indirect ELISA, microtiter plates were coated with a fivefold dilution series of purified OMV. Antisera (Table 1) and goat antirabbit alkaline phosphatase conjugate were used at dilutions of 1:250 and 1:1,000, respectively. Plant tissue for serological assays was homogenized, with a Pollaehne Press (Sew-Eurodrive, West Germany), in phosphate-buffered saline (0.01 M phosphate and 0.15 M NaCl), pH 7.4, containing 0.1% (v/v) Tween 20, 2% (w/v) polyvinylpyrrolidone 44,000, and 0.2% (w/v) bovine serum albumin. Hemicellulase (1 mg per gram of sample tissue) was added, and samples were incubated overnight at room temperature (3). Microwell plates were obtained from Nunc, Denmark. Color intensities of the enzyme-substrate reactions were measured at 405 nm with a Titertek Multiskan ELISA Reader (Flow Laboratories, Sweden).

IEB. IEB of viral coat proteins was performed as described by Rybicki and von Wechmar (34). After electrophoresis the gel was soaked in transfer buffer—192 mM glycine, 25 mM Tris-Cl, pH 8.3, and 20% (v/v) methanol—and laid on a presoaked nitrocellulose filter. Two sheets of wet Whatman 3MM filter paper were placed on either side of the gel and filter, and the whole "sandwich" was placed in the transfer cassette of a Hoefer TE-50 Transphor apparatus (Hoefer Scientific Instruments, San Francisco). Transfers were done in transfer buffer for 2 hr at 0.6–0.8 A. The substrate for the goat antirabbit alkaline phosphatase conjugate was nitro blue tetrazolium (0.3 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (0.2 mg/ml) in 100 mM Tris-Cl, pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$ (24).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis. The molecular weight of OMV coat protein was determined by discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), by the system of Laemmli (23). Virus samples were dissociated by heating at 95 C for 5 min in 0.1 M Tris-Cl, pH 6.8, containing 2.5% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, and 5% (v/v) glycerol. Pharmacia LMW molecular weight markers were used (Pharmacia Fine Chemicals, Sweden). Electrophoresis was performed in a Hoefer SE 600 electrophoresis apparatus. Gels were stained in 0.2% (w/v) Coomassie Brilliant Blue R250 or 0.2% (w/v) PAGE Blue 83 (both from BDH, United Kingdom). Alternatively, photochemical silver staining of gels was performed (26).

RNA extractions. RNA was extracted from purified virus preparations by a modification of the method by Gallitelli et al (12). The virus preparation was heated at 60 C for 5 min in 10 mM Tris-Cl, 1% (w/v) SDS, and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.25, emulsified with phenol/chloroform with a vortex mixer, and the aqueous phase separated by centrifugation. The aqueous phase was reextracted twice more with phenol/chloroform, and traces of phenol removed by three cycles of ether extraction. RNA was precipitated from suspension by the addition of sodium acetate, pH 5.5, to 120 mM, and 2.5 volumes of 96% ethanol. RNA was pelleted by centrifugation, resuspended in sterile distilled water to 1 mg/ml, and stored as small aliquots at −70 C. All glassware, Eppendorf tubes, and tips were treated with 0.1% (v/v) diethyl pyrocarbonate for 16 hr at 37 C before being autoclaved.

RNA molecular weight determination. RNA samples were electrophoresed in 1% (w/v) agarose gels under denaturing conditions. Formaldehyde gels were prepared and run as described by Maniatis et al (25). RNA sequences derived from bacteriophage

T7, yeast 2μ plasmid, and bacteriophage λ DNA ("RNA ladder," Bethesda Research Laboratories, Gaithersburg, MD) were used as molecular weight markers. Marker sizes were 9.5, 7.5, 4.4, 2.4, 1.4., and 0.3 kb. RNA molecular weight was determined graphically by reference to markers.

Oligo (dT) cellulose chromatography. A column was prepared by pouring oligo (dT) cellulose (Collaborative Research Inc., Waltham, MA), suspended in low-salt elution buffer—0.01 M Tris, pH 7.5, 0.05% (w/v) SDS, and 1 mM EDTA—in a sterile 1-ml syringe. The column was equilibrated by washing with 10 bed volumes of high-salt binding buffer—0.5 M NaCl, 0.01 M Tris, pH 7.5, 0.5% (w/v) SDS, and 1 mM EDTA. RNA was resuspended in high-salt binding buffer and applied to the column. Nonadsorbed material was eluted by washing with at least 10 bed volumes of binding buffer before poly(A)$^+$ RNA was eluted with a low-salt elution buffer. Aliquots of 300 μl were collected and absorbance at 260 nm determined.

RESULTS

Virus transmission. OMV isolates were readily mechanically transmissible to virus-free *Ornithogalum* and *Lachenalia* explants by means of purified virus preparations or sap from infected plants. Typical systemic mosaic symptoms appeared 2–3 wk after inoculation. Virus-infected leaves kept at 4 C for 4 mo were shown to remain infective when inoculated to virus-free plants. No mechanical transmission to *T. tetragonioides, P. vulgaris, P. sativum, P. hybrida, T. majus*, or any of the tested *Chenopodium* and *Nicotiana* spp. occurred. *M. persicae* transmitted OMV in the nonpersistent manner to both *Ornithogalum* and *Lachenalia* spp.

Virus purification. The very mucilaginous nature of *Ornithogalum* and *Lachenalia* plant sap caused problems in preliminary attempts at virus purification. Homogenization of leaves resulted in an extremely viscous mucilage even when extractions were made in five volumes of buffer. Several approaches to overcome this problem were investigated. Sap extraction from frozen leaf tissue or homogenization in liquid nitrogen had no significant effect. Organic solvents, such as chloroform, *n*-butanol, carbon tetrachloride, and mixtures thereof, were used in extractions with phosphate, acetate, and Tris-Cl buffer systems. Digestion of the polysaccharides in the plant sap was attempted with β-glucosidase, cellulase, and hemicellulase. The best results were obtained when sap was extracted in three volumes of 0.1 M sodium acetate buffer, pH 5.5, containing hemicellulase, followed by incubation for 4–6 hr at room temperature, which resulted in digestion of the mucilage. Plant material was separated by low-speed centrifugation, and virus extracted from it with 0.1 M Tris-Cl buffer, pH 9.0, containing Na_2SO_3 and thioglycolic acid. In the development of the purification protocol, electron microscopy was used to monitor the presence of virus through all the stages of the purification. If the enzyme extraction method was used, approximate virus yields of 4–6 mg per kilogram of infected *Ornithogalum* leaves and 1–2 mg per kilogram of infected *Lachenalia* leaves were regularly obtained. Similar yields were obtained from cultivated and wild *Ornithogalum* plants, but *Lachenalia* yielded less virus. Although virus could be purified from infected leaves kept at 4 C for 4 mo, the best yields were obtained from freshly harvested leaves. Very little virus was purified from leaves kept frozen for a few months.

Electron microscopy. Flexuous, rod-shaped particles were consistently observed in leaf extracts from plants showing mosaic symptoms (Fig. 1). Length measurements of 356 particles from partially purified virus preparations were made. The modal length was in the range of 720–760 nm (Fig. 2). A few end-to-end aggregates were observed.

Serology. Homologous end-point titers of antisera to OMV-O, OMV-W, and OMV-L in microprecipitin tests were 1:1,024, 1:1,024, and 1:512, respectively. OMV-O and OMV-L were serologically indistinguishable in DAS-ELISA. OMV-W differed from both but was still closely related (Fig. 3). In reciprocal titrations with all three viruses, OMV-W reacted less than OMV-O or OMV-L with antisera to the latter, and more than OMV-O or OMV-L with its homologous antiserum. Antiserum to an OMV isolate from the Netherlands reacted strongly with the local isolates in IEB (Fig. 4), indicating a close relationship.

The relatedness of OMV isolates to other potyviruses was investigated by ELISA and IEB (Table 1). In direct DAS-ELISA OMV cross-reacted only with BYMV, whereas in indirect assays (indirect ELISA and IEB) serological relationships with HMV, BYMV, PVY, WMV, ZYMV, SCMV, SMV, and PWV were observed. Serological relationships with members of other virus groups were investigated. OMV isolates did not react with antisera to CMV, BMV, TMV, or MSV.

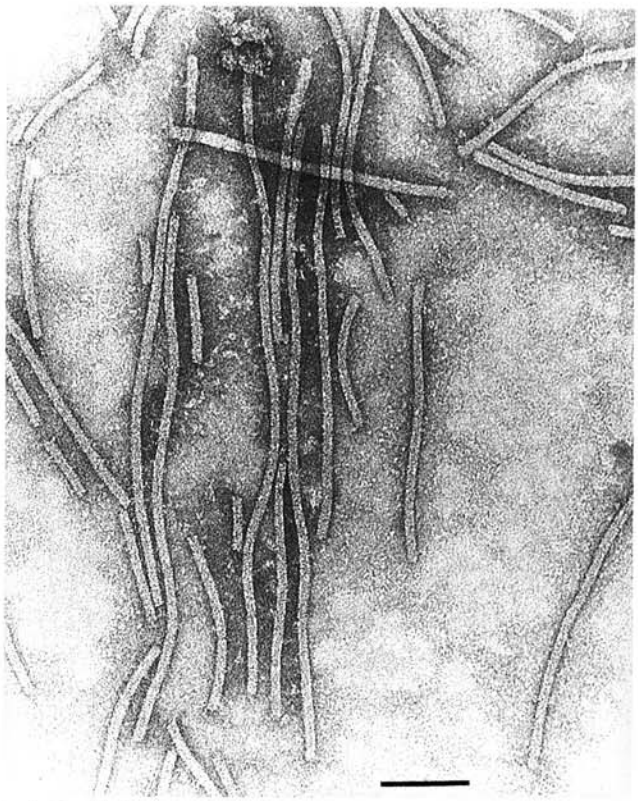

Fig. 1. Purified preparation of OMV stained with 2% (w/v) phosphotungstic acid, pH 6.2. Scale bar = 100 nm.

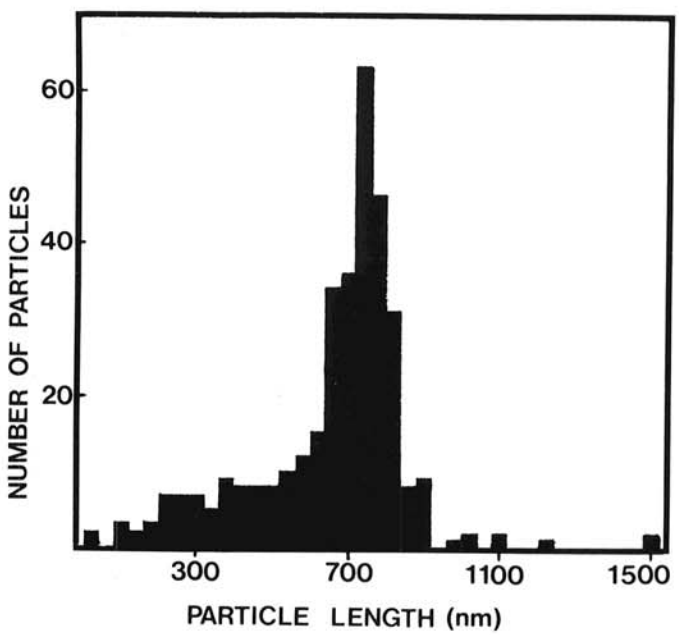

Fig. 2. Particle length distribution of OMV from a partially purified preparation. The modal length is in the range of 720–760 nm.

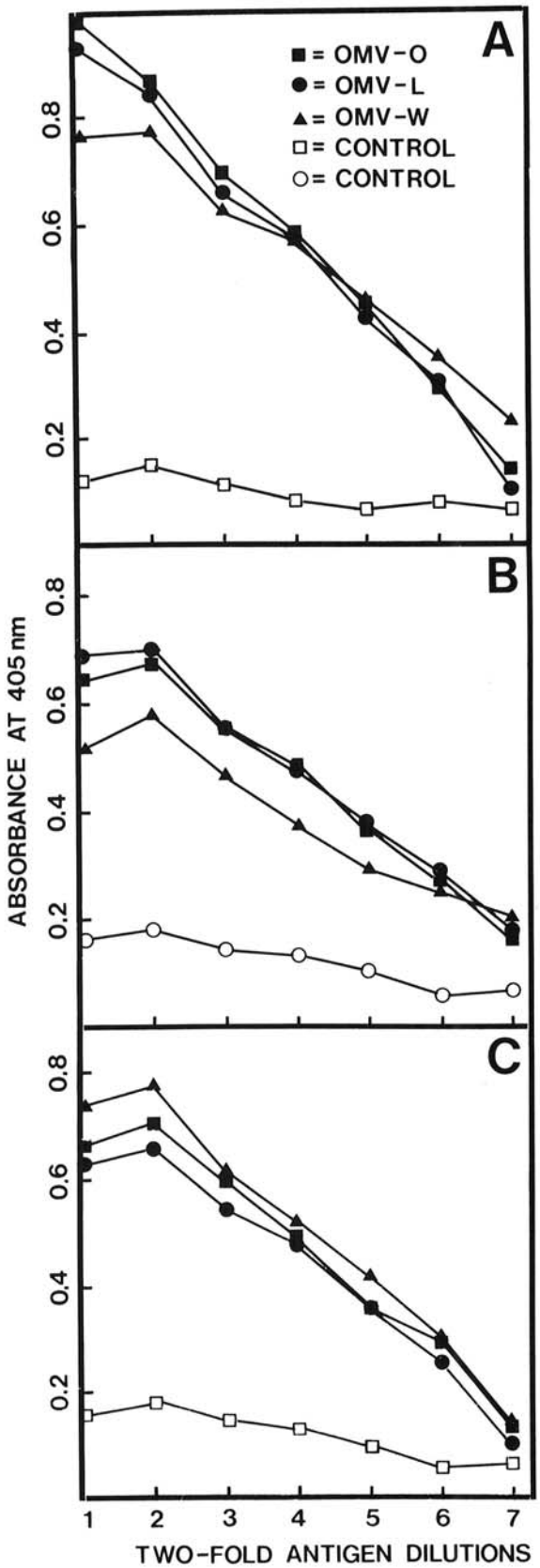

Fig. 3. Direct double-antibody sandwich enzyme-linked immunosorbent assay showing the serological relationships among three South African isolates of OMV, using antiserum to OMV-O (A), antiserum to OMV-L (B), and antiserum to OMV-W (C). Negative controls were virus-free plant sap from *Ornithogalum* (o) and *Lachenalia* (□).

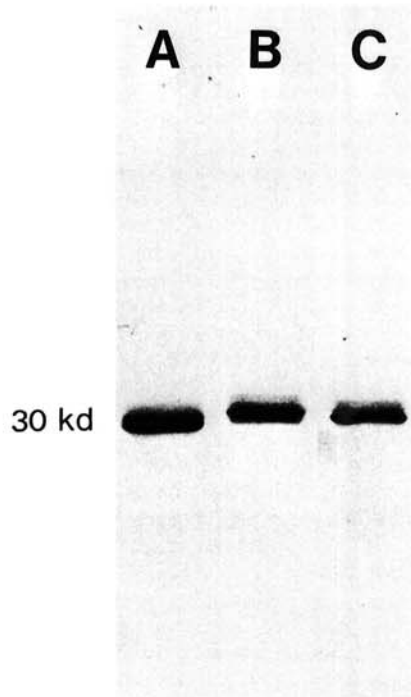

Fig. 4. Immunoelectroblot of three South African isolates of OMV probed with antiserum to OMV-D. **A,** OMV-O. **B,** OMV-L. **C,** OMV-W.

TABLE 1. Serological relationships between OMV and other potyviruses determined by direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), indirect ELISA (Ind-ELISA), and immunoelectroblotting (IEB)

Virus[a]	Antiserum[a]	DAS-ELISA[b]	Ind-ELISA	IEB[c]
OMV-O	OMV-O	0.89	0.76	++
BYMV-G	OMV-O	0.53	0.41	++
PVY-1	OMV-O	0.03	0.40	+
PVY-2	OMV-O	0.06	0.46	+
MDMV-A	OMV-O	0.00	0.00	−
MDMV-B	OMV-O	0.00	0.00	+
WMV-Morocco	OMV-O	0.00	0.00	+
OMV-O	OMV-D	ND[d]	0.56	++
	HMV	ND	0.21	+
	BYMV-G	ND	0.52	++
	BYMV-Scott	ND	0.34	++
	PVY-1	ND	0.42	++
	PVY-2	ND	0.46	++
	WMV-1	ND	0.42	+
	WMV-2	ND	0.00	−
	WMV-Morocco	ND	0.18	−
	ZYMV	ND	0.50	++
	SCMV	ND	0.20	+
	WSMV	ND	0.00	−
	SMV	ND	0.33	+
	PWV	ND	ND	++
	MDMV-A	ND	0.00	−
	MDMV-B	ND	0.00	−

[a]OMV = Ornithogalum mosaic virus; BYMV = bean yellow mosaic virus; PVY = potato virus Y; MDMV = maize dwarf mosaic virus; WMV = watermelon mosaic virus; HMV = hyacinth mosaic virus; ZYMV = zucchini yellow mosaic virus; SCMV = sugarcane mosaic virus; WSMV = wheat streak mosaic virus; SMV = soybean mosaic virus; PWV = passion fruit woodiness virus.

[b]Optical density at 405 nm; readings of healthy reactions were subtracted from corresponding infected reactions.

[c]++ = Strong positive reaction; + = slight reaction; − = no reaction.

[d]Not determined.

PAGE analysis of viral coat protein. A single major protein band with M_r 30,000 was observed for SDS-denatured OMV preparations in polyacrylamide gels (Fig. 5). Molecular weights were determined from five repetitions for each of the three OMV isolates. Minor bands of lower molecular weight were occasionally observed. These are believed to be partially proteolyzed products of the coat protein, such as those observed with other potyviruses (15,16).

RNA characterization. The molecular weight of OMV RNA was calculated from the relative position of RNA bands in formaldehyde agarose gels (seven repetitions). The average RNA size was 8.54 ± 0.075 kb (M_r 2.90×10^6) (Fig. 6). An average size of 6.22 ± 0.102 kb (M_r 2.11×10^6) was obtained for TMV RNA, which was included as an alternative marker. The presence of a poly(A)$^+$ tract on the viral RNA was investigated by passing RNA over an oligo (dT) cellulose column; 67% of the RNA bound to the oligo (dT) cellulose (Fig. 7), confirming that OMV RNA is polyadenylated.

DISCUSSION

Mechanical transmission of an OMV isolate to *C. quinoa, T. tetragonioides*, and *N. clevelandii* has been reported (11). Sap from infected *Ornithogalum* plants, which were imported from South Africa, was used in these transmission experiments. OMV isolates used in the current study could only be transmitted to virus-free *Ornithogalum* and *Lachenalia*. Considering these findings, it appears essential that more isolates should be tested for alternate hosts to ascertain whether sap-transmissible strains exist naturally. Virus purification from systemically infected alternative host plants might eliminate the cumbersome method currently in use and also result in better virus yields.

The many conflicting reports in the literature are clear evidence that serological relationships among potyviruses are very complex. Close serological relationships have been reported for potyviruses from completely unrelated hosts; e.g., tobacco etch virus (TEV) has been reported to be closely related to bean common mosaic virus (BCMV), pokeweed mosaic virus (PMV), turnip mosaic virus (TuMV), PVY, WMV, and MDMV (36), and lettuce mosaic virus

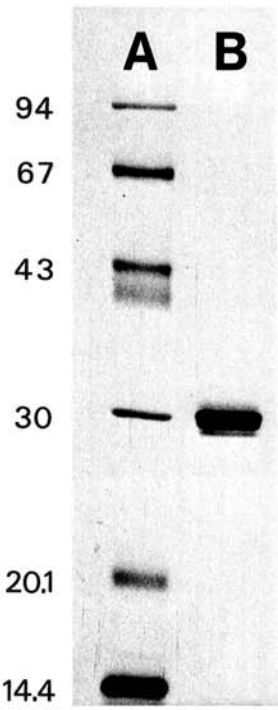

Fig. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of OMV coat protein. Lane A, molecular weight markers: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). Lane B, OMV-O (30 kDa).

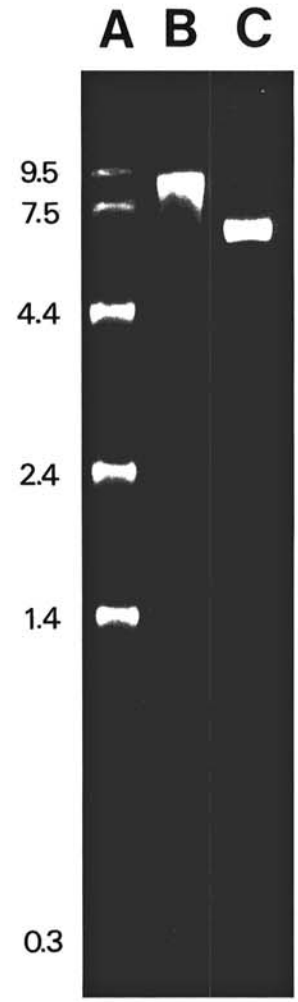

Fig. 6. Molecular weight determination of OMV RNA in formaldehyde agarose gels. Lane A, molecular weight markers (kb); lane B, OMV RNA (M_r 2.9×10^6); lane C, TMV RNA (M_r 2.11×10^6).

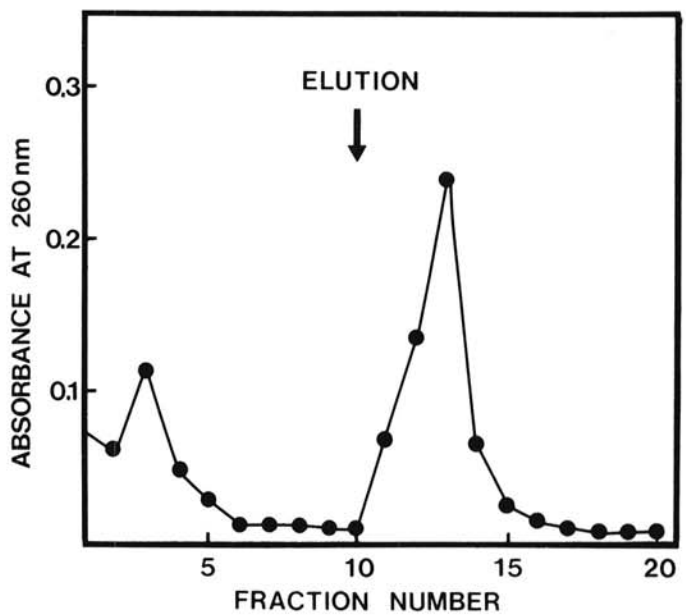

Fig. 7. Elution profile of OMV RNA in oligo (dT) cellulose chromatography. The arrow indicates the beginning of poly(A)$^+$ RNA elution.

(LMV) has been reported to be closely related to BYMV (1).

Serological differentiation by direct DAS-ELISA is strain-specific and an excellent method for demonstrating serological differences between isolates of the same virus (6,21,31,33). This technique was used to show that OMV-O and OMV-L isolates were indistinguishable, but that a slight serological difference existed between these two and the OMV-W isolate. Indirect immunoassays are known to be well suited for the detection of distant serological relationships between plant viruses. The use of indirect ELISA for the detection of a wide range of serologically related viruses is well documented (7,22,33,40,41). IEB has more recently been established as a reliable technique for the detection of distant serological relationships (5,34). This technique involves the separation of viral proteins by SDS-PAGE, electrophoretic transfer of these proteins to a nitrocellulose filter, and the detection of the proteins by enzyme immunoassay with an insoluble substrate product. OMV was serologically compared with several other potyviruses, by both these methods. Although results from indirect ELISA correlated well with IEB results, we feel that the latter is a more reliable test. In IEB, proteins can be identified by both their molecular weight (migration rate) and their antigenic properties. Denatured viral coat proteins have been shown to be more suitable than intact virions for the detection of serological relationships in the potyvirus group (36). It is presumed that cryptotopes—epitopes exposed only on unpolymerized protein—are more conserved than surface epitopes and may be responsible for the observed relationships (33,34,36,39). Results from these two indirect assays indicated that OMV is serologically strongly related to BYMV, PVY, and ZYMV; related to a lesser degree to HMV, WMV, SCMV, SMV, and PWV; and not related to MDMV and WSMV. A serological relationship between OMV and HMV, shown by microprecipitin tests, was previously described (10). The positive reaction obtained with OMV antibodies and a local BYMV isolate in DAS-ELISA and the lack of reaction with any other potyvirus indicate that of all the potyviruses tested, BYMV is the closest serological relative of OMV. The possibility that the virus infecting *Ornithogalum* and *Lachenalia* is in fact a strain of BYMV was investigated. Sap inoculation of OMV-O to *Ornithogalum, Lachenalia*, beans, and peas resulted in symptoms in *Ornithogalum* and *Lachenalia* only. Sap inoculation of BYMV-G to the same hosts resulted in symptoms on beans only. These data, as well as the differences in coat protein and RNA sizes, suggest that OMV is not a strain of BYMV.

The M_r of 30,000 calculated for OMV coat protein compares favorably with those published for other potyviruses, i.e., 34,000 for PVY, BYMV, and LMV (14,19) and 32,000 for TEV (14). Similarly the M_r of 2.90×10^6 calculated for OMV RNA compares well with the values of 3.07×10^6, 3.04×10^6, and 2.90×10^6 reported for TuMV, TEV, and MDMV, respectively (17). The presence of a poly(A)$^+$ tract on the RNA is typical of potyviruses and was first reported by Hari et al (13) for TEV.

The presence of viral inclusion proteins in infected *Ornithogalum* and *Lachenalia* leaf material was investigated. Ultramicrotomy of embedded material was difficult, probably because of improper fixing and embedding caused by the mucilaginous nature of *Ornithogalum* and *Lachenalia* leaf tissue. No typical inclusion bodies could be seen in the sections that were viewed.

It is speculated that the virus infecting *Ornithogalum* and *Lachenalia* originated in South Africa. The host range of OMV is limited to these two genera of the Hyacinthaceae, and most *Ornithogalum* and *Lachenalia* spp. are indigenous to the southern and western Cape regions. Virus-infected plants with typical symptoms were found regularly in remote semiarid areas along the West Coast region, where agricultural activities are limited mainly to stock farming. It is possible that the virus reached Europe through the early export trade of *Ornithogalum* and *Lachenalia* bulbs and flowers.

In conclusion, and after comparing the properties determined here for the OMV-O isolate with properties typical of potyviruses (18), i.e., filamentous 740-nm particles, nonpersistent aphid transmissibility, coat protein with M_r 30,000, poly(A)$^+$ RNA with M_r 2.9×10^6, and serological relationships, we suggest that OMV be considered a member of the potyvirus group.

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