Filamentous Viruses of Garlic in Argentina

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ABSTRACT

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Filamentous viruses of garlic (*Allium sativum*) in Argentina were identified by the serological techniques of immunosorbent electron microscopy plus quantitative decoration, and enzyme-linked immunosorbent assay. The most widely grown cultivars, Rosado Paraguayo, Colorado, and Blanco, were sampled from different areas in which the crops showed mosaic symptoms. Most samples proved to contain mixtures of filamentous viruses, particularly onion yellow dwarf and garlic yellow streak (potyviruses), and a carlavirus. The carlavirus reacted strongly (to near or beyond the stated homologous titers) with antisera to carnation latent virus produced in three different laboratories but did not react with antisera to garlic latent, shallot latent, and potato S viruses (all carlaviruses). The virus could be mechanically transmitted with difficulty to carnation. This appears to be the first report of a virus closely related to carnation latent virus infecting garlic.

In garlic (*Allium sativum* L.), viruses with flexuous filamentous particles have been known for many years and are reported in all parts of the world in which studies have been made. Such viruses appear to be universally present in garlic cultivars, unless the cultivars have been treated by meristem tip culture (4,6,7,10, 13,14,18,21,23,25,26). The following viruses have been identified in garlic.

Potyviruses. Onion yellow dwarf virus (OYDV) (2) infects garlic (14,15,25–27); it is reported to be distantly related serologically to leek yellow stripe virus (LYSV) (2,3,5), also found in garlic, but Walkey et al (27) stated that the viruses did not cross-react when tested by immunoelectron microscopy (decoration after trapping). Garlic yellow streak virus (GYSV), also reported in garlic (21), was found to be only distantly related serologically to OYDV and LYSV and to differ from these in experimental host range.

More problematic is "garlic mosaic virus" (1,15,16,18,28). It is not clear whether this is a distinct virus, or if the agent described was one or more of the viruses OYDV, LYSV, and GYSV. Two other potyviruses, hippeasterum mosaic and alstroemeria mosaic, are said to have been detected in garlic (D. G. A. Walkey, *unpublished*, 25).

Carlaviruses. Three carlaviruses have been noted in garlic. These are shallot latent virus (SLV) (24,26; D.-E. Lesemann, *unpublished*), garlic latent virus (GLV) (18), and narcissus latent virus (NLV) (Walkey, *unpublished*, 25). GLV has not been precisely characterized, and may or may not be distantly related serologically to SLV (Lesemann, *unpublished*). However, it is not clear that the GLV of Lee et al (18) is the same as that of Lesemann (*unpublished*). SLV and GLV are not apparently serologically related to carnation latent virus (CLV) or chrysanthemum virus B (6,18). NLV was found to be unrelated serologically to 12 carlaviruses (presumably, but not explicitly, including the type carlavirus, CLV) (8,9). It may be distantly related to SLV (6).

Since 1980 we have noted filamentous viruses in garlic from the province of Cordoba, Argentina. The aim of the present work was to better characterize these viruses and establish their relationships to garlic viruses reported elsewhere.

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MATERIALS AND METHODS

Plants. We worked with the most commonly grown cultivars in Argentina: Rosado Paraguayo, Colorado, and Blanco, all showing mosaic symptoms. Samples were taken from 10–20% of the producers in all areas in which these cultivars are grown. Plants were collected at random along a diagonal of each field sampled.

Serological detection and identification. Leaf samples were homogenized in either 0.1 M phosphate buffer, pH 7, or 0.05 M borate buffer, pH 8.1 (21). Antisera were serially diluted by twofold steps in one of the above buffers or in distilled water. The decoration method (20) was used, and an antiserum titer obtained for each virus-antiserum combination. In cases where there were very few virus particles, the viruses were first trapped by immunosorbent electron microscopy (ISEM) (20) using a serum dilution of 1:500 (v/v). The antisera (homologous titers given in Table 1) were kindly provided by B. Delecolle, INRA, Montfavet, France (OYDV); A. W. Mohamed, Ministry of Agriculture and Fisheries, Auckland, New Zealand (GYSV); T. Osaki, University of Osaka Prefecture, Japan (GLV); A. A. Brunt, Institute of Horticultural Research, Littlehampton, U.K. (CLV); D. Z. Maat, Research Institute for Plant Protection, Wageningen, The Netherlands (SLV, CLV, OYDV); L. F. Salazar, International Potato Center, Lima, Peru (Potato viruses S and Y); and E. Luisoni, Institute of Applied Plant Virology, Torino, Italy (CLV). Luisoni also provided antisera to carnation mottle virus (CarMV) and carnation vein mottle virus, which were used in enzyme-linked immunosorbent assays (ELISA) (11) to check that test plants of carnation were free of these viruses.

Results of mechanical inoculation of infected garlic sap to onion and carnation (see below) were checked by ISEM followed by decoration (ISEM-D), using the OYDV and CLV (Luisoni) antisera

diluted 1:500 (v/v) for ISEM and 1:25 (v/v) for decoration. Prior to inoculation, the absence of these viruses in the carnation plants was checked by the same method.

Mechanical inoculation. Transmission was attempted with leaf tissue homogenized in 0.05 M phosphate buffer, pH 7, or 0.05 M borate buffer, pH 8.1, each with the addition of sodium diethyldithiocarbamate (1:1,000, w/v) and vegetable charcoal (1:100, w/v). The following test plants were used, either in a glasshouse at 20–30 C or in growth rooms at 18 C with 16 hr of illumination: *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., *C. murale* L., *Tetragonia expansa* Thunb. ex J. A. Murray, *Nicotiana tabacum* L. cvs. Xanthi and Samsun, *N. glutinosa* L., *Gomphrena globosa* L., *Physalis floridana* Rydb., *Nicandra physalodes* (L.) Gaertn., *Allium cepa* L., *A. porrum* L, and *Dianthus caryophyllus* L.

Onion plants (the cultivars Blanca Chata and Valenciana) obtained from seed and checked by ISEM-D for absence of OYDV were inoculated with sap from garlic infected with OYDV (and other viruses) and then again tested by ISEM-D for the presence of OYDV after 1 and 2 mo.

Thirteen healthy carnation plants derived from meristem tip culture, checked for the absence of CLV and CarMV by ISEM-D, were inoculated with sap from garlic plants known to contain particles that could be decorated with CLV antisera. After 1 and 2 mo, the plants were again tested by ISEM-D for the presence of CLV. Sap from carnation plants now CLV-positive was inoculated to *C. quinoa* and to Rosado Paraguayo garlic previously freed from viruses by meristem culture and thermotherapy (12,22).

RESULTS

Serology. Table 1 shows the antisera used and the titers obtained with virus particles extracted from the garlic samples. Figure 1 shows a representative field of decorated and undecorated virus particles. Table 1 shows that the viruses present were OYDV, GYSV, and a carlavirus reacting strongly with all three CLV antisera but not reacting with antisera to GLV, SLV, or PVS. This virus, for the moment, will be referred to as "CLV." During the study we had the opportunity to check the SLV antiserum against carlavirus particles in shallot; it reacted strongly in decoration tests (*data not shown*).

On various occasions the decorating antisera were diluted in phosphate or borate buffer or in water as described in Materials and Methods. As a control, a sample of garlic sap containing "CLV" and at least one potyvirus was titrated with the Luisoni CLV antiserum diluted in each of these media. All diluents gave comparable decoration levels over a dilution range from 1:32 to 1:2,048 (v/v), and all gave an endpoint (just distinguishable decoration) on the "CLV" at a dilution of 1:2,048 (v/v) (Table 1). There was no decoration of the potyviruses at any dilution of this antiserum.

Table 2 gives the preliminary results of a still ongoing survey of garlic in Argentina. Nearly all garlic samples contained OYDV and GYSV, and about 25% contained "CLV." All of these viruses infected all three cultivars in all areas tested.

Mechanical transmission. Transmission from garlic to *C. quinoa* and *C. amaranticolor* was obtained under all conditions tested. The plants produced only chlorotic local lesions 5–10 days after inoculation; the lesions later became necrotic. This mechanically transmitted virus was identified as GYSV by serological tests. Where "CLV" was known to be present in the inoculum, lesion-bearing leaves from 10 inoculated *C. quinoa* plants were tested for by ISEM-D with CLV antiserum, but only two plants were positive, and these gave very few decorated particles.

The other species inoculated produced no symptoms. When inoculated onion plants were tested by ISEM-D for OYDV, only two of 15 plants proved positive, and very few virus particles were detected in them. Inoculation of additional onion plants from the infected onions gave no detected transmission.

The ISEM-D test for "CLV"-inoculated carnation plants gave positive results in one of 13 cases; sap from the infected carnation, inoculated to *C. quinoa* and to garlic, caused no infection.

DISCUSSION

The high antiserum titers obtained with particles of the appropriate morphologies suggest that the principal filamentous viruses infecting garlic in Argentina are closely related to or identical with OYDV, GYSV, and CLV. Potyviruslike particles almost always present in Argentine garlic reacted up to the homologous titer with the French OYDV antiserum of Delecolle. This is not surprising, since OYDV has been widely reported throughout the world.

Other potyviruslike particles nearly always present reacted strongly with the GYSV antiserum of Mohamed and Young from New Zealand. The microprecipitin titer of this serum was stated as 1:256 (v/v), and our decoration titer

Table 1. Reciprocal serological titers obtained with garlic viruses and different antisera using immunoelectron microscopic decoration

Antiserum to	Source[a]	Reported homologous titer[b]	Decoration titer[c]
Potyviruses			
Garlic yellow streak	Mohamed	256	512
Onion yellow dwarf	Delecolle	1,024[c]	1,024
Onion yellow dwarf	Maat	512	512
Potato Y	Salazar	256	8
Carlaviruses			
Carnation latent	Brunt	8,192	2,048
Carnation latent	Luisoni	512	2,048
Carnation latent	Maat	256	1,024
Garlic latent	Osaki	256	No reaction
Potato S	Salazar	256	No reaction
Potato S	Salazar	500[c]	No reaction
Shallot latent	Maat	256	No reaction

[a] Antisera were provided, as indicated, by A. W. Mohamed, Ministry of Agriculture and Fisheries, Auckland, New Zealand; B. Delecolle, INRA, Montfavet, France; D. Z. Maat, Research Institute for Plant Protection, Wageningen, The Netherlands; L. F. Salazar, International Potato Center, Lima, Peru; A. A. Brunt, Institute of Horticultural Research, Littlehampton, U.K.; E. Luisoni, Institute of Applied Plant Virology, Torino, Italy; and T. Osaki, University of Osaka Prefecture, Japan.

[b] By microprecipitin test, except as noted.

[c] By decoration test.

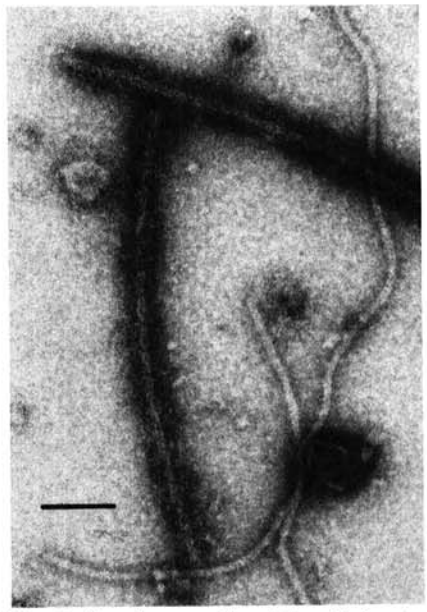

Fig. 1. Sap from garlic cultivar Colorado adsorbed to a grid and decorated with the Luisoni antiserum to carnation latent virus (see Table 1) diluted 1:32 (v/v) in phosphate buffer. A uranyl acetate stain was used. B = 100 nm. The undecorated particles are those of a potyvirus.

Table 2. Percentage of garlic plants of three cultivars found by enzyme-linked immunosorbent assay to give strong reactions with antisera to garlic yellow streak virus (GYSV), onion yellow dwarf virus (OYDV), and carnation latent virus (CLV)

Cultivar	GYSV		OYDV		CLV	
	Positive/sampled	%	Positive/sampled	%	Positive/sampled	%
Colorado	155/172	90	178/181	98	6/129	5
Blanco	465/494	94	424/500	85	182/485	37
Rosado Paraguayo	378/473	80	246/437	56	102/469	22

was 1:512 (v/v). Decoration titers often exceed precipitin titers for the same virus-antiserum pairs by one or two twofold steps (19). Although an uncertainty of one twofold step is inherent in these titrations, we think that our virus is serologically very close to GYSV. Also, we recently noted that an antiserum to LYSV gave positive reactions with some garlic plants.

CLV has not previously been reported in garlic, but the high titers obtained with three different CLV antisera to particles extracted directly from garlic plants suggest that the carlavirus was CLV or very closely related. The failure of the three other carlavirus antisera (to GLV, PVS, and SLV) to decorate the particles supports this idea; our difficulty in infecting carnation with the virus (one of 13 plants infected) suggests that it is a strain of CLV closely adapted to garlic, though we do not think it merits naming as a separate virus. Kassanis (17) reported a distant serological relationship between CLV and PVS; we obtained no reaction with two PVS antisera, and this may also suggest that the carlavirus in Argentine garlic differs somewhat from typical CLV.

There remains the possibility that the one CLV-positive carnation plant obtained may not have been the result of passage of the virus from garlic but of residual CLV infection in the supposedly virus-free meristem-derived plants. These plants were rechecked just before inoculation, so this possibility is considered minimal.

Although GLV and SLV have been reported elsewhere to infect garlic, we did not detect these viruses. The data of Walkey (25) came to our attention after this work was done, and therefore we made no tests for hippeasterum mosaic, alstroemeria mosaic, or NLV. However, it appears unlikely that our "CLV" was in fact NLV, since the (somewhat equivocal) literature (8,9) suggests that they are not or are only distantly serologically related.

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