DISTRIBUTION OF *GARLIC VIRUS A* IN DIFFERENT GARLIC PRODUCTION REGIONS OF ARGENTINA

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SUMMARY

Comparative tests using inmunosorbent electron microscopy plus decoration and ELISA with antibodies against *Garlic virus A* (GarV-A) and *Garlic virus C* (GarV-C) coat proteins showed that they specifically recognize the respective viruses. On this basis, the GarV-A antiserum was used in DAS-ELISA tests to study the distribution of GarV-A in different regions of Argentina. A total of 2655 samples, representing 115 garlic fields were analyzed. GarV-A was detected in 64%, 51%, 43% and 35% of samples collected in San Juan, Mendoza, Córdoba and Buenos Aires Provinces, respectively. Positive samples were not found in plants from Santa Cruz Province. Distribution among different garlic cultivars showed that this virus was present in 33%, 46%, 53%, 70% and 43% of plants belonging to cvs 'Colorado', 'Chino', 'Violeta Francés', 'Blanco' and 'Rosado Paraguayo', respectively. Negative results were obtained in samples of cvs 'Castaño-INTA' and 'Violeta Santacruceño'.

Key words: *Allium* sp., GarV-A, GarV-C, distribution frequency.

INTRODUCTION

Garlic in Argentina is grown on a total area of about 10,000 ha distributed in a broad range of latitudes. According to their adaptation to specific environments, different garlic cultivars are employed. Thus, cultivars such as 'Colorado' and 'Violeta Santacruceño', with a long life-cycle, are grown in regions of high altitude or very cold winters, while others, like 'Blanco' and 'Violeta Francés', develop in slightly shorter periods and are planted in milder conditions. Due to their long storage like and the limited number and large size of their cloves, these garlic cultivars are usually selected for export. In contrast, the cultivars 'Rosado Paraguayo' and 'Chino' grow efficiently in warmer climates and have the shortest life-cycles. All these cultivars belong to the species *Allium sativum* L. var. *sativum*. On the other hand, cv. 'Castaño-INTA', which is also grown in very cold regions and has a long life-cycle, is original from eastern Europe and is classified as *A. sativum* L. var. *ophioscorodom*. This cultivar is grown for both direct consumption and industrial processing.

Garlic-infecting viruses are distributed worldwide in all producing areas. Several members of the genera *Potyvirus* and *Carlavirus*, such as *Onion yellow dwarf virus*, *Leek yellow stripe virus*, *Shallot latent virus*, *Garlic common latent virus* and different viruses serologically related to *Carnation latent virus*, have been described infecting garlic (Walkey 1990; Conci *et al.,* 1992; Van Dijk, 1993a, b; Barg *et al*., 1994; Kobayashi *et al*., 1996; Tsuneyoshi *et al*., 1998a, b). In addition to *Poty-* and *Carlaviruses*, a *Reovirus* named *Garlic dwarf virus* (GDV) was reported by Lot *et al.* (1994).

Additionally a hitherto unassigned new type of viruses infecting *Alliaceae* has been described. Two of them, Onion mite-borne latent virus and Shallot mite-borne latent virus were reported by Van Dijk *et al.* in 1991. Later, employing RT-PCR and restriction enzyme analysis, Tsuneyoshi and Sumi (1996) characterized four different viruses. These were designated garlic viruses *A, B, C* and *D* (GarV-A, -B, -C, and -D). They showed close serological relationships with other mite-borne *Allium* viruses and shared sequence homology with *Shallot virus X* (ShVX, Kanyuka *et al*., 1992), which is also mite-transmitted. In addition, it was found that the 3' genomic region of an isolate of *Garlic mite-borne filamentous virus* (MbFV) has sequence similarity with GarV-D (Ryabov *et al*., 1996).

More recently, *Garlic virus X*, another virus of this type, was shown to be present in 40 different regions including America, China, Japan and Korea (Song *et al*., 1997). Its genome has been completely sequenced. It comprises six open reading frames and differs from that of potexviruses and carlaviruses by the presence of an ORF4 encoding an unusual protein (Song *et al*., 1998). During the 27th Meeting of the Executive Com-

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mittee of the International Committee on Taxonomy of Viruses (San Diego, California, 1998) it was proposed to classify these viruses such as a new genus named *Allexivirus*, with ShVX as type species (Pringle, 1998).

The coat protein of an Argentine isolate of GarV-A has been expressed in bacteria and used as antigen to raise polyclonal antibodies (Helguera *et al*., 1997). In this paper, we compare this antiserum with one obtained against the coat protein of a Japanese isolate of GarV-C (Tsuneyoshi and Sumi, 1996). The GarV-A antiserum proved specific to this virus and was then used to evaluate the presence of GarV-A in major garlic-producing regions of Argentina and its incidence in the major garlic cultivars.

MATERIALS AND METHODS

Antisera. Polyclonal antiserum to GarV-A was previously described (Helguera *et al*., 1997). Antiserum to MbFV was kindly provided by Drs. D.E. Lesemann, E. Barg and H.J. Vetten (Federal Biological Research Centre for Agriculture and Forestry, Germany) and GarV-C antiserum by Dr. S. Sumi (Wakunaga Pharmaceutical, Hiroshima, Japan).

Virus-infected plants. Garlic plants of cv. 'Blanco' specifically reacting with GarV-A antiserum or GarV-C antiserum in inmunosorbent electron microscopy plus decoration (ISEM-D) assays were considered as respectively infected with GarV-A or GarV-C. In some cases, plants of cv. 'Castaño-INTA' serologically negative for GarV-A, and serologically positive for GarV-C and MbFV**,** were used. Garlic plants of cv. 'Blanco', obtained by thermotherapy and meristem tip culture (Conci and Nome, 1991), were considered as healthy.

Collection of garlic samples. Samples were collected from all major producing areas in Argentina, according to the following distribution: 989 samples from San Juan (Rawson, Santa Lucía and Pocito), 627 samples from Mendoza (Valle de Uco and Uspallata); 220 samples from Córdoba (Jesús María); 464 samples from Buenos Aires (Bahía Blanca, Hilario Ascasubi and Médanos) and 355 samples from Santa Cruz (El Calafate, Cancha Carrera, San Rafael, Mansilla and Gobernador Gregores). A total of 115 fields, representing between 15-50% of the planted area in the respective provinces, were evaluated. Fifteen to 30 bulbs were collected at random from each field; one clove from each bulb was then analyzed by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) with GarV-A antiserum. Samples from these regions included the following garlic cultivars 'Colorado', 'Chino', 'Violeta Francés', 'Blanco', 'Rosado Paraguayo', 'Violeta Santacruceño' and 'Castaño-INTA'. These cultivars represent most of those grown in Argentina.

Immunoassays. ISEM-D was done as described by Milne and Luisoni (1977). Briefly, carbon filmed grids were incubated for 5 min with GarV-A or GarV-C anti-

Table 1. Survey of GarV-A-infected garlic in Argentina using DAS-ELISA.

Province	Fields analyzed	Area planted (ha)	Area analyzed (ha)	No. samples analyzed	Positive samples	% positives
San Juan	46	1280[2]	299.5	989	633	64.00%
Mendoza	38	8763[2]	1300.0	627	320	51.00%
Córdoba[1]	2	70[3]	40.0	220	95	43.00%
Buenos Aires	22	590[2]	141.0	464	164	35.00%
Santa Cruz	7	130[4]	65.0	355	–	–
TOTAL	115	10,833	1845.5	2655	1212	43.75%

[1] Only the Jesús María region was evaluated in Córdoba. Total area under garlic in this Province is about 600 ha.
[2] Burba, 1997.
[3] Rusell Italia (personal communication).
[4] Horacio Gábriel (personal communication).

sera (dilution 1:500), washed with 0.05 M $Na_2B_4O_7$ and kept for 15 min on a drop of infected plant extract. The grids were then washed as before and incubated with serial two fold dilutions of GarV-A or GarV-C antiserum. Then, the grids were washed with distilled water and stained with 2% uranyl acetate. Viral preparations were observed with a Jeol Jem 1200 EX II electron microscope. Twenty five plants of 'Violeta Santacruceño' and 50 plants of 'Castaño-INTA' were evaluated with GarV-A antiserum using a dilution of 1:500 for coating and 1:50 for decoration.

DAS-ELISA was performed as described by Clark and Adams (1977). Extracts from GarV-A-infected plants, and from cv. 'Castaño-INTA' plants reacting to GarV-C and MbFV antisera in ISEM-D assays, were used as antigens. Plate-trapped antibody enzyme-linked immunosorbent assay (PTA-ELISA) was performed according to Lommel *et al.* (1982).

RESULTS AND DISCUSSION

Specificity of GarV-A and GarV-C antisera. Extracts of garlic plants infected with GarV-A or GarV-C were analyzed by ISEM-D. Extracts containing GarV-A reacted to GarV-A and GarV-C antisera up to dilutions of 1:2048 and 1:32, respectively. Extracts from plants infected with GarV-C reacted with GarV-C and GarV-A antisera up to dilutions of 1:4096 and 1:8, respectively. Hence, although the two viruses were found to be serologically distantly related, GarV-A and GarV-C could be differentiated using dilutions higher than 1:32 and 1:8, respectively.

Particles decorated with GarV-C and MbFV antisera were detected in extracts of cv. 'Castaño-INTA' but no decorated virus particles were found when GarV-A antiserum was used at a dilution of 1:50 (Fig. 1).

GarV-A could also be clearly differentiated from GarV-C and MbFV by DAS-ELISA. Extracts of GarV-A infected plants reacted at IgG dilutions up to 1:25,600 (0.05 mg ml^{-1}) of the homologous antiserum, while extracts of GarV-C and MbFV infected plants and healthy plants did not react at any dilution tested (between 26 and 0.05 mg ml^{-1} of GarV-A antiserum IgG) (Fig. 2). Therefore, the GarV-A antiserum could efficiently discriminate between GarV-A and GarV-C plus MbFV under these conditions. Similar results were obtained when PTA-ELISA was employed (results not shown).

Frequency and distribution of GarV-A. A total to 2655 garlic samples from all major production areas of Argentina were analyzed by DAS-ELISA. GarV-A was present at most locations included in this study, with a distribution frequency ranging from 35% (South of Buenos Aires) to 64% (San Juan). Noteworthy, no GarV-A was detected in samples collected from Santa Cruz (Fig. 3).

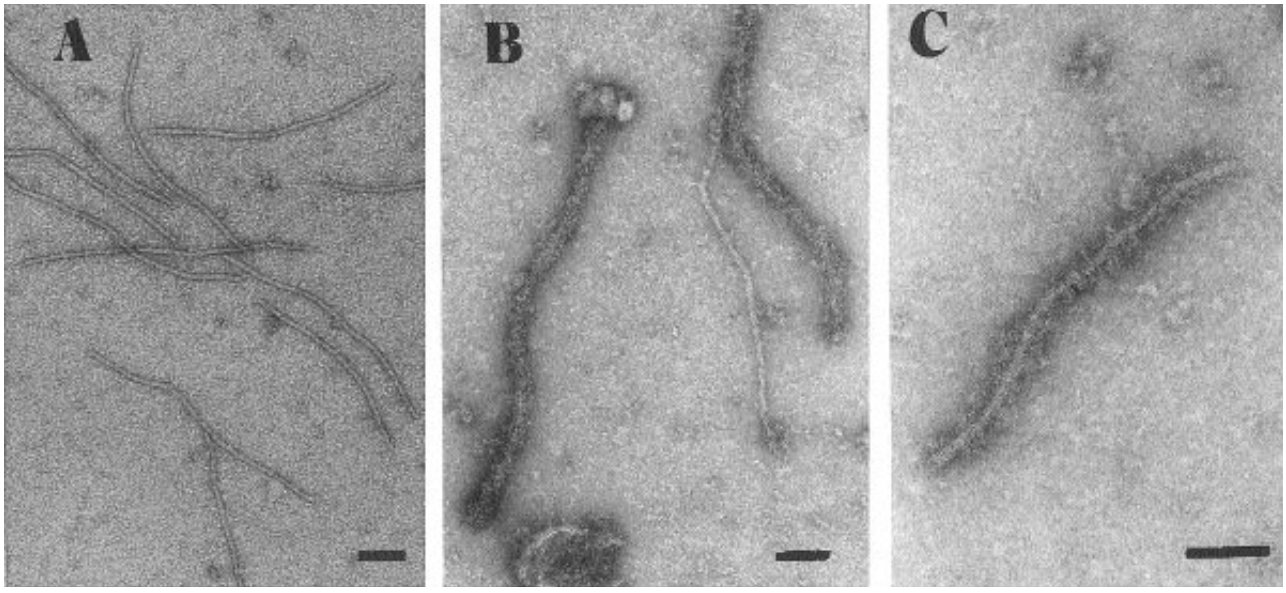

Fig. 1. Decorated and undecorated garlic virus particles in an extract of cv. 'Castaño-INTA' detected by ISEM-D. **A**: with GarV-A antiserum diluted 1:50 for decoration; **B**: MbFV antiserum diluted 1:25 for decoration and **C**: GarV-C antiserum diluted 1:50 for decoration. Bars = 100 nm.

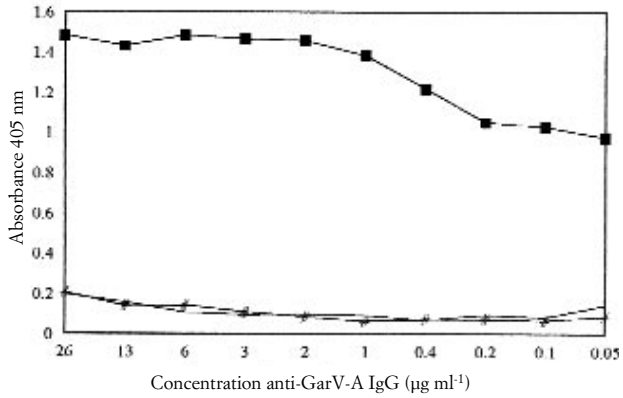

Fig. 2. DAS-ELISA absorbance values from garlic leaf extracts using serial dilutions of anti-GraV-A IgG. Alkaline phosphatase conjugate was used at 1:3000. Comparison of extracts of GarV-A infected cv. 'Blanco' (■), healthy cv. 'Blanco' (#) and GarV-C plus MbFV infected cv. 'Castaño-INTA' plant (-).

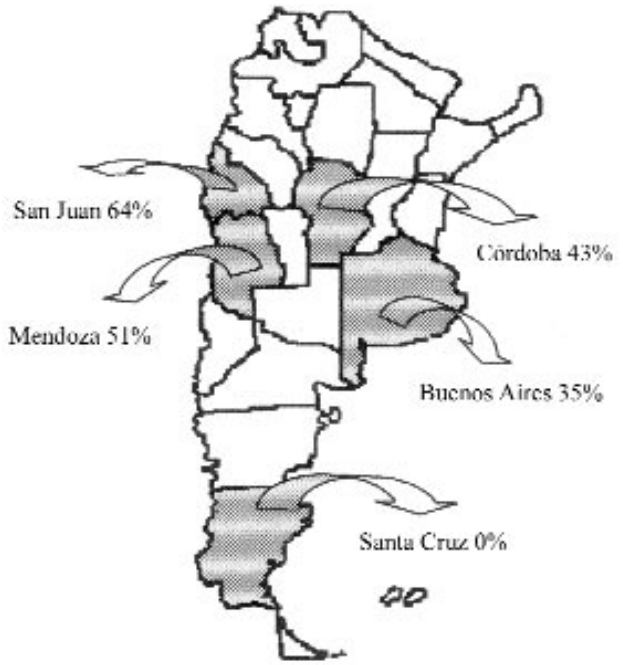

Fig. 3. Distribution of GarV-A in the main garlic growing regions of Argentina.

The results were also analyzed in respect of garlic cultivars. 'Colorado', 'Chino', 'Violeta Francés', 'Blanco' and 'Rosado Paraguayo' were infected with GarV-A with values varying between 33% and 70%. Strikingly, no GarV-A was observed in 'Violeta Santacruceño' and 'Castaño-INTA' (Fig. 4). These negative ELISA results

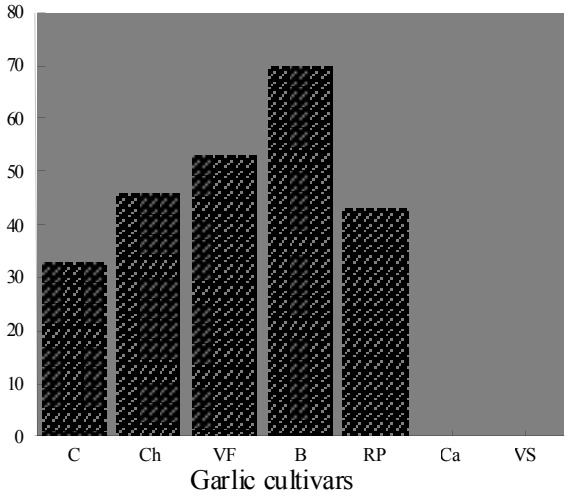

Fig. 4. Incidence of GarV-A in garlic cultivars grown in Argentina. C: 'Colorado'; Ch: 'Chino'; VF: 'Violeta Francés'; B: 'Blanco'; RP: 'Rosado Paraguayo'; Ca: 'Castaño-INTA'; VS: 'Violeta Santacruceño'.

were confirmed by ISEM-D tests with 25 samples of 'Violeta Santacruceño' and 50 samples of 'Castaño-INTA'. No particles decoration with the GarV-A antiserum was detected in any case. 'Violeta Santacruceño' is a garlic ecotype from southern Patagonia that is only grown in Santa Cruz Province. Geographic isolation from other garlic crop regions, plus the severe climatic conditions (low temperature, snow and strong winds), could explain why these plants escaped infection.

On the other hand, cv. 'Castaño-INTA' was also apparently GarV-A, even in areas in which this virus was abundantly detected in other garlic cultivars. Many plants of 'Castaño-INTA' reacted with GarV-C and MbFV antisera. These results might suggest the presence of natural resistance to GarV-A in cv. 'Castaño-INTA' and this should be further investigated.

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