Immunological Detection of a GarV-Type Virus in Argentine Garlic Cultivars

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ABSTRACT

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A cDNA library representing the genomes of several garlic viruses was generated using a viral RNA mixture as template. Analysis of randomly selected clones allowed the identification of different viral genomic sequences. On the basis of amino acid and nucleotide sequence comparisons, one of them was assigned to garlic virus A (GarV-A), a novel flexuous, rod-shaped virus recently reported in Japan. The coat protein (CP) of this virus was expressed in *Escherichia coli* cells and used as immunogen to produce polyclonal antibodies. The expression protein was recognized by an antiserum detecting garlic mite-borne filamentous virus, but did not react with antibodies specific for other garlic viruses. Antibodies raised against the viral CP reacted with extracts infected with garlic mite-borne viruses and fail to recognize preparations of onion yellow dwarf potyvirus, leek yellow stripe potyvirus, and carnation latent carlavirus. The same antibodies decorated viral particles exhibiting a modal length of 586 nm in immuno electron microscopy with decoration assays. Double-antibody enzyme-linked immunosorbent assays and tissue printing assays performed with these antibodies allowed detection of GarV-A in most garlic cultivars used in Argentina.

Commercial garlic varieties are strictly apomixic. For this reason, viruses tend to accumulate in bulb-seed stocks and are readily transmitted to propagative materials (28,29). Disease is caused by a viral mixture—the “garlic viral complex”—comprising potyviruses (25), carlaviruses (24), and several flexuous mite-borne viruses that remain unassigned (23,27). The composition of this infective complex varies according to different geographical regions, and usually includes viruses from more than one group (2,7,8,9,20,22,23,25). Symptoms resulting from infection include leaf mosaics and pronounced decreases in the weight and quality of cloves (29).

Several components of the garlic infective complex have been identified on the basis of physical and serological evidence. These viruses include the garlic strains of onion yellow dwarf potyvirus (OYDV-G; 14,25) and leek yellow stripe potyvirus (LYSV-G; 25,32), shallot latent carlavirus (SLV; 28,30), garlic common latent car-

lavirus (GCLV; 24) and several mite-borne viruses (27,31). Two viruses belonging to this latter viral type were initially classed into the *Rymovirus* genus of *Potyviridae* due to their morphological and cytopathological properties (27). Later, it was shown that these viruses were immunologically related to shallot virus X (ShVX; 13), which also remains unassigned (26). In addition, Sumi et al. (22) obtained partial genomic sequences corresponding to four flexuous garlic viruses (GarV-A, -B, -C, and -D) exhibiting a genomic organization similar to that of ShVX. More recently, Tsuneyoshi and Sumi (23) showed that GarV-C and -D have a close serological relationship with the garlic mite-borne viruses described by Van Dijk et al. (27). All this suggests that garlic mite-borne viruses do not belong to the *Rymovirus* genus and should be grouped together with the GarV-type viruses (18).

Garlic viruses are unable to infect (or infect very poorly) most alternative host plants (25). As a result, they have been difficult to isolate from each other. Consequently, their classification by criteria commonly accepted in plant virology, such as host-range, morphology, serology, and cytopathology, frequently led to taxonomic confusion. The difficulties of obtaining purified viral proteins limited the production of reliable antisera in the amounts needed for indexing programs or epidemi-

ological studies. Application of molecular biology techniques to the study of this viral complex has allowed an increased understanding of the identity of its individual members (14,20,22,23) and the development of specific diagnostic tools.

Current programs for certification of garlic seed-bulbs emphasize the incidence of OYDV-G and LYSV-G in symptom development and yield losses (15). In contrast, the effect of mite-borne and GarV-type viruses on garlic production remains largely unexplored. Taking into account that these viruses have been detected in countries so distant from each other as Argentina, Japan and the United Kingdom (23,27), their significance might be more relevant than previously considered. In this work, the coat protein (CP) gene of a local GarV-A strain was cloned and expressed in *Escherichia coli* cells, and the respective polypeptide was used to produce polyclonal antibodies in rabbits. These antibodies were successfully employed to optimize double-antibody enzyme-linked immunosorbent assays (DAS-ELISA) and tissue printing immunoassays, and to detect this virus in most of the important garlic cultivars grown in Argentina.

MATERIALS AND METHODS

Plant material and virus strains.

Healthy garlic plants (*Allium sativum* L. cv. Blanco Mendoza) were obtained from in vitro-cultured meristem tips that were previously subjected to thermotherapy (7). Infected garlic plants (cv. Blanco Mendoza) reacting with antibodies directed to LYSV, OYDV, GCLV, and mite-borne viruses were collected from a production field in Córdoba (central Argentina). Onion (*A. cepa* L. cv. Ebenezer) and leek (*A. ampeloprasum* L. var. *porrum*) plants were grown from seeds obtained from INTA-La Consulta (Mendoza, Argentina). Commercial *Dianthus barbatus* seeds were purchased from Dinatale (Argentina). Garlic plants infected with mite-borne viruses were grown from thermotherapy-treated meristem tips. Only those plants reacting with anti-garlic mite-borne filamentous virus (GMbFV) antisera (2) and showing no reaction with antibodies against OYDV, LYSV, common latent carlavirus (CLV), GCLV, and SLV were considered for this purpose. LYSV-infected leek plants, OYDV-infected onion plants,

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and CLV-infected *Dianthus barbatus* plants were obtained by inoculation of healthy plants with the respective viruses. Viral infection was confirmed by immuno-electron microscopy with decoration (IEM-D) assays conducted with antibodies specific for LYSV, OYDV, and CLV.

Virus purification. Ten plants of *Allium sativum* (cv. Rosado Paraguayo) exhibiting typical symptoms of virus infection were sampled from a production field. Several leaves from each plant were mixed and used as a source for virion purification. Isolation of viral particles was carried out according to the procedure described by Murphy et al. (19), with minor modifications. Viral RNA was extracted using the method described by Hull (12) and electrophoresed in denaturing agarose gels. After staining with ethidium bromide, an RNA smear ranging from 10,000 to 7,000 nucleotides was visualized. Samples of individual viruses to be analyzed in Western immunoblots were obtained as follows: 20 g of infected garlic leaves were homogenized in 40 ml of 0.5 M Na₂B₄O₇, 0.2% thioglycolic acid, pH 8, and thoroughly mixed with 10 ml of chloroform. After centrifugation for 15 min at 7,000 × g, the supernatant was loaded onto a 20% sucrose cushion and centrifuged for 1 h at 100,000 × g. Pellets containing approximately 0.2 to 0.5 mg of protein were resuspended in 100 µl of 0.05 M Na₂B₄O₇, pH 8.

Molecular cloning. Synthesis of cDNA was carried out using a mix of viral genomic RNAs as template (see previous section), and oligo(dT)₁₅ as primer for reverse transcription. A cDNA library was obtained using the commercial kit Librarian (Invitrogen Corp., San Diego, CA). Viral cDNA was ligated to *Bst*XI adapters, inserted into the cloning vector pcDNA II, and used to transform *E. coli* JM 109 cells. Subsequently, 50 recombinant plasmids were digested with *Bst*XI and analyzed in agarose gels to assess the size of the respective cDNA inserts, and those containing the largest fragments were partially sequenced. The CP gene sequence encoded in clone pCC6 was amplified by polymerase chain reaction (PCR) using two oligonucleotide primers designed on the basis of the sequence deduced from this clone (5'-ATAGGATCCCATATGAACGCA-CCTGTTGAC-3' and 5'-GTAACCTGATTCTAGAGGAATCATC-3'), which introduced a *Bam*HI site at its 5' end (underlined sequence). The PCR was carried out in a 100 µl mix containing 1 ng of plasmid pCC6, 1.5 mM of MgCl₂ and 200 ng of each primer. Nucleotide buffer and *Taq* polymerase were obtained from Promega (Madison, WI). The amplification program consisted of 5 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C; and 30 cycles of 1 min at 94°C, 1 min at 60°C, 2 min at 72°C. After completion of the reaction, the PCR products were electrophoresed in a 1.2% agarose gel and visu-

alized by staining with ethidium bromide. The DNA fragment corresponding to the CP gene was purified from the gel using Wizard PCR Preps (Promega), blunted with T4 DNA polymerase (Gibco BRL, Gaithersburg, MD) and digested with *Bam*HI. The fragment was then subcloned by *Bam*HI/blunt end ligation into the *E. coli* vector pRSETa (Invitrogen), which allowed expression of the viral protein as a C-terminal fusion to a poly-histidine tract. The resulting plasmid was called pRSET/C6CP.

DNA sequencing and recombinant DNA techniques. Nucleotide sequencing was performed by the dideoxy chain-termination method (21) using a commercial kit (Sequenase US Biochemical, Cleveland, OH). Sequencing was conducted according to instructions provided by the manufacturer. At least two independent sequences from both the sense and antisense DNA strands were obtained. Comparisons between nucleotide and amino acid sequences were made using the Prosis Maxim Homology program (Hitachi Software, Japan). The sequence reported in this paper is listed under GeneBank accession No. X98991. DNA digestions, electrophoresis, hybridizations, and other recombinant DNA procedures were conducted as described in Maniatis et al. (16) and Ausubel et al. (1).

Viral protein expression and purification from bacterial cells. Plasmid pRSET/C6CP was introduced into *E. coli* BL21 pLys cells by a standard transformation procedure (16). Expression of the C6CP polypeptide was induced by addition of 0.3 mM IPTG to a bacterial culture grown to OD₆₀₀ 0.5 and by further incubation for 2 h at 37°C. The C6CP protein formed intracellular aggregates that could be easily precipitated from cell extracts. Bacterial cells were resuspended in STE buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8, 1 mM EDTA), disrupted by sonication and centrifuged for 10 min at 9,000 × g. The resultant pellet was resuspended in 2 ml of STE buffer supplemented with 8 mM MgCl₂, 10 µg/ml DNase I, and maintained for 15 min at 4°C. The incubation mix was centrifuged for 10 min at 9,000 × g and the pellet was resuspended in 1 ml of STE buffer. Expression products were analyzed in Western immunoblots using antibodies specific for the different garlic-infecting viruses.

Rabbit immunization. Bacterially expressed C6CP protein was electrophoresed in a preparative SDS-polyacrylamide gel and the C6CP band was excised from the gel, crushed and lyophilized. Antisera were produced in two California female rabbits by intradermal injection of 0.6 mg of fusion protein emulsified in 1 ml of Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO). Two weeks later, each animal was boosted by intramuscular injection of an equal amount of protein in

Freund's incomplete adjuvant. Bleeding was conducted at 15 and 21 days after the last injection. Anti-C6CP antiserum was used at a 1:2,000 dilution.

Antisera and immunoassays. The anti-OYDV and anti-LYSV antisera were provided by K. Graichen (Institute für Pathogendiagnostik, Germany) and Dr. H. Lot (INRA-Montfavet, France), respectively. The anti-SLV antiserum was obtained from D. Maat (Research Institute for Plant Protection, The Netherlands), and antisera recognizing GCLV and GMBFV (2) from D. Lesemann (Federal Biological Research Centre for Agriculture and Forestry, Germany). The antiserum against CLV was obtained from E. Luisoni (Istituto di Fito-virologia Applicata, Italy). Antibodies recognizing multiple components of the garlic viral complex (anti-GVCx) were obtained by standard procedures at IFFIVE-INTA, using a mixture of purified garlic viruses as a source of antigen. Purification of the IgG fraction was carried out by (NH₄)₂SO₄ precipitation followed by fractionation in a DEAE-Sepharose chromatography column (Pharmacia LKB Biotechnology, Inc., Uppsala, Sweden). Alkaline phosphatase-IgG antibodies were obtained by coupling with glutaraldehyde following the procedure reported by Harlow and Lane (11). DAS-ELISAs were performed according to Clark and Adams (6).

Tissue printing. Cloves from chronically infected bulbs were transversely or longitudinally cut with a scalpel blade and printed onto BA85 nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). The membranes were blocked for 1 h at room temperature in 50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Tween 20 and 5% not-fat dried milk, washed three times in 50 mM Tris-HCl, pH 8, 150 mM NaCl, 0.05% Tween 20 (TBS/Tween), and incubated for 1 h in the same buffer supplemented with 5% not-fat dried milk, 5% (wt/vol) extract from healthy garlic plants, and anti-C6CP as primary antibody. The blots were then washed and incubated with alkaline phosphatase-linked goat anti-rabbit antibody (BioRad, Hercules, CA) for another period of 1 h. Following several washes in TBS/Tween, phosphatase activity was visualized by a chromogenic reaction using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Gibco BRL) as substrates (1).

SDS-PAGE and Western blots. Infected leaf tissues and purified virion preparations were resuspended in 60 mM Tris-HCl, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, and 0.1% SDS. Samples were boiled for 5 min and centrifuged for 2 min at 9,000 × g. Total protein content was determined following the method of Bradford (3). Aliquots containing 30 to 40 µg of protein were electrophoresed in 12.5% SDS-polyacrylamide gels that were then stained with Coomassie Blue or blotted

onto nitrocellulose membranes. Viral proteins were detected by a 1-h incubation with antisera recognizing different garlic viruses as primary antibodies, followed by several washes with TBS/Tween and a second 1-h incubation with alkaline phosphatase-linked goat anti-rabbit antibody as secondary antibody. Following this treatment, phosphatase activity was detected as described above.

Immuno electron microscopy with decoration assays. Decoration assays of viral particles were performed basically as described by Milne and Luisoni (17) using garlic plants infected with OYDV, LYSV, and other non-identified viruses. Briefly, carbon filmed grids (Ernest F. Fullam, Latham, NY) were incubated for 5 min at 20°C in 10 µl of anti-GVCx antiserum diluted 1:500 to 1:1000 in 0.05 M Na₂B₄O₇, pH 8. Antiserum-coated grids were washed with 2 ml of 0.05 M Na₂B₄O₇, pH 8, immersed in 100 µl of infected plant extracts and kept for 15 min at 20°C. Following this, the grids were washed as before and treated with specific antisera at 1:20 dilution in 0.05 M Na₂B₄O₇, pH 8, for 15 min. After this, the grids were washed with distilled water and stained with 2% uranyl acetate. Viral preparations were observed at 1200× in a electron microscope (Jeol, Japan). Size of GarV-A particles was estimated from samples of virus mixtures using the C6CP antiserum in IEM-D assays. Virion lengths were calculated according to Brandes and Wetter (4) by comparison with spherical latex particles of 0.482 µm in diameter (Ted Pella, Redding, CA).

RESULTS

Cloning of a GarV-type genomic sequences. A mix of virions purified from infected garlic plants was used as the source of RNA to generate a cDNA library. Three clones from this library, pCC5, pCC6, and pCC15, contained cDNA fragments showing 82% nucleotide sequence homology with GarV-A, a novel rod-shaped virus that was recently characterized in Japan (22). Clone pCC6 contained a sequence comprising the complete CP gene, the 15 kDa ORF, the 3' non-coding region, and a short poly(A) tract (Fig. 1A to D). A comparison between the pCC6 translated sequences with those corresponding to GarV-A showed homologies of 91% for the viral CP and 75% for the 15 kDa polypeptide (Fig. 1B and C). Similar comparisons with the respective sequences of GarV-B, -C and -D showed homologies of 63, 61, and 76% for the coat protein and 50, 53, and 66% for the 15 kDa product, respectively. The highest level of nucleotide sequence homology (98%) was found between the pCC6 and GarV-A 3' non-coding sequences (Fig. 1D).

Bacterial expression of the pCC6 CP gene. The sequence corresponding to the complete pCC6 CP gene was amplified by

PCR and subcloned into the *E. coli* expression vector pRSETa (plasmid pRSET/C6CP). In agreement with the size predicted from the amino acid sequence, expression of the pCC6 CP gene in bacterial cells led to the production of a 31 kDa fusion protein. This expression protein did not react with antisera specifically recognizing LYSV, OYDV, CLV, SLV, or GCLV, but it was detected by antibodies raised against GMbFV (data not shown). To further explore the identity of the corresponding virus, the 31 kDa protein was used as immunogen to raise polyclonal antibodies. The resulting antiserum (C6CP antiserum) was used to analyze OYDV, LYSV, or CLV virion preparations in Western immunoblots (Fig. 2A). This antiserum did not react with semi-purified prepara-

tions of CLV (lane CL), OYDV (lane OY), or LYSV (lane LY), but it recognized the C6CP expression protein (lane C6) and a protein of about 30 kDa in a virion preparation obtained from plants infected with mite-borne viruses (lane Mx). However, antisera to CLV, OYDV, and LYSV reacted with their respective homologous antigens. On the other hand, the preparation of mite-borne viruses did not react with antisera to OYDV, LYSV, GCLV, CLV, or SLV (Fig. 2B, lanes O, L, G, C, and S), but showed a protein band of approximately 30 kDa when assayed with anti-GMbFV antibodies (Fig. 2B, lane Mb). Depending on the antisera used in each case, several faint bands were apparent in these latter assays. These bands were attributed to non-specific binding of these

Fig. 1. Sequence comparisons between clone pCC6 and the garlic virus A (GarV-A) genome. (A) schematic representation of coding and non-coding nucleotide sequences in clone pCC6. Open boxes represent ORFs encoding the viral coat protein (CP) and to the 15 kDa protein; (B) and (C) amino acid sequence comparison between the CPs (B) and the 15 kDa proteins (C) encoded in clone pCC6 and in the GarV-A sequence; (D) nucleotide sequence comparison between the 3' non-coding sequence of clone pCC6 and the GarV-A genome. GarV-A sequence was taken from Sumi et al. (22). Differences in the amino acid and nucleotide sequences are indicated in bold.

antibodies to epitopes located in plant proteins.

Immuno-electron microscopy. In order to establish the morphology of the viral particles reacting with the C6CP antiserum, garlic samples infected with a mixture of different viruses were analyzed in IEM-D assays. A sub-population of flexuous rod-shaped virions strongly reacted with anti-C6CP, while other viral particles were not stained (Fig. 3A). The decorated particles displayed a modal length of 586 nm (Fig. 3B). Confirming its specificity, the C6CP antiserum did not react with samples exclusively con-

taining OYDV, LYSV, or CLV particles (data not shown).

DAS-ELISA and tissue printing assays. On the basis of the evidence reported above, it was concluded that the cDNA sequence encoded in pCC6 corresponded to a local strain of GarV-A, and that the C6CP antiserum showed high specificity to this virus. To study its performance in DAS-ELISA tests, the C6CP antiserum was tested against serial dilutions of plant extracts infected with either garlic mite-borne viruses, OYDV, or CLV. While extracts infected with mite-borne viruses reacted with anti-C6CP at dilutions ranging

from 1:1,000 to 1:2,000, only background values were observed in the case of plants infected with OYDV or CLV. Reciprocally, anti-OYDV and anti-CLV antisera did not react with extracts containing garlic mite-borne viruses (data not shown). Therefore, DAS-ELISA results closely correlated with those obtained by Western blot analysis, suggesting that anti-C6CP could be included in routine diagnostic tests for de-

Fig. 2. Western blot analysis of partially purified virion preparations with antisera recognizing different garlic-infecting viruses. (A) immunoblots of partially purified onion yellow dwarf potyvirus (OYDV), leek yellow stripe potyvirus (LYSV), and carnation latent carlavirus (CLV) virions developed with C6CP antiserum. (B) immunoblots of partially purified mite-borne virions developed with antisera recognizing different garlic-infecting viruses. H = extract from healthy garlic plants; C6 = recombinant C6CP expression protein; Mx = semi-purified virions from garlic plants infected with mite-borne viruses; CL, OY, LY = semi-purified virions from CLV-, OYDV-, and LYSV-infected plants, respectively. Each lane was loaded with 30 µg of protein. The antisera used in each case are indicated at the bottom of the panel. O = anti-OYDV; L = anti-LYSV; G = anti-GCLV; C = anti-CLV; S = anti-SLV; Mb = anti-GMbFV. All antisera were employed at dilutions of 1:2,000. The arrow indicates the position of the 30 kDa molecular marker. Membrane cuts shown in (A) and (B) correspond to different gels.

Fig. 3. Morphological characteristics of virions recognized by anti-C6CP antibodies. (A) immuno-electron microscopy with decoration (IEM-D) assay of garlic viruses reacting with anti-C6CP antiserum. Non-decorated particles are indicated by solid arrowheads. An individual viral particle decorated by anti-C6CP is shown in the insert. Scale bars indicate 200 nm. (B) length distribution histogram of viral particles recognized by the C6CP antiserum. A total of 105 viral particles grouped in ranges of 39 nm were measured.

tection of GarV-A. Preliminary DAS-ELISA tests performed with samples from different garlic production areas indicated that this virus is present in all commercially important varieties grown in Argentina (cv. Rosado Paraguayo, Blanco Mendoza, and Colorado Mendoza).

In order to study the distribution of GarV-A viruses in chronically infected garlic plants, the C6CP antiserum was used to perform a series of squash blot and tissue print immunoassays. Viral presence could be easily detected in squash blots of leaves and roots from infected plants (data not shown). Interestingly, strong binding of anti-C6CP antibodies was observed in tissue printings from individual cloves or whole bulbs (Fig. 4A and B), suggesting that this technique could be advantageously developed to monitor viral presence in propagative materials.

DISCUSSION

Due to problems derived from their narrow host range, individual viruses of the garlic complex have been difficult to isolate employing the traditional tools of plant virology. On the other hand, simultaneous cloning of viral genomes, and subsequent analysis of the respective cDNA libraries, provided a suitable means to study the composition of this complex in different geographical locations. The feasibility of such an approach was shown by Sumi et al. (22) and Nagakubo et al. (20) in Japan, and by ourselves in Argentina (14). In our case, analysis of randomly selected cDNA clones allowed characterization of partial genomic sequences derived from three different viruses. While two of them were assigned to OYDV-G (13) and LYSV (unpublished results), the third sequence is identified here as belonging to a local strain of GarV-A. No sequences showing features typical of the genome organization of carlaviruses were isolated in this first screening of our cDNA library.

The sequence encoded in plasmid pCC6 was assigned to GarV-A on the basis of sequence comparisons (22) and immunological evidence. Amino acid sequence comparisons demonstrated 91% homology between the CPs of pCC6 and GarV-A, while similar comparisons with GarV-B, -C, and -D showed homology values of 63, 61, and 76%, respectively. A lower degree of homology (75%) between pCC6 and GarV-A was found in the case of the 15 kDa product. In particular, the pCC6 sequence lacked three amino acids at the C-terminus, as compared with the sequence reported by Sumi et al. (22). This difference was ascribed to the genomic heterogeneity that is usually present in wild viral populations. On the other hand, nucleotide sequence comparisons showed much higher sequence homology between the 3' non-coding regions, which is commonly observed among different strains of the same virus (10).

The antigenic properties of the C6CP polypeptide and the specificity of the C6CP antiserum provided further support to the notion that GarV-type and garlic mite-borne viruses are closely related. Thus, while the C6CP polypeptide was recognized by anti-GMbFV antibodies, it was not detected by antibodies against OYDV, LYSV, SLV, GCLV, or CLV. On the other hand, the C6CP antiserum recognized a protein of about 30 kDa in virion preparations obtained from plants infected with garlic mite-borne viruses, but it failed to react with OYDV, LYSV, and CLV preparations. Similar evidence confirming this relationship was previously postulated in the case of other GarV-type and mite-borne viruses. For example, an anti-GarV-C antibody was able to recognize garlic mite-borne mosaic virus (GMbMV; 23), and GarV-D showed considerable sequence similarity with a novel garlic mite-borne virus recently isolated in England (23).

On the basis of our results, we cannot totally discard the possibility that the C6CP antiserum might recognize more than one GarV sub-type. In this regard, it should be noted that the anti-GarV-C antibodies obtained by Tsuneyoshi et al. (23) cross-reacted with GarV-B, -C, and -D, indicating that these viruses share common antigenic determinants. However, in mixtures including several garlic mite-borne viruses, the C6CP antiserum decorated a subpopulation of viral particles exhibiting a narrow range of size distribution, suggesting the recognition of a particular GarV-type component. Moreover, it was observed in several IEM-D assays that

anti-C6CP decorated a lower percentage of viral particles than anti-GMbFV, which also implies a high degree of specificity. Therefore, though the C6CP antiserum might eventually detect other GarV-type viruses, we concluded that it has practical diagnostic value and could be advantageously incorporated with the tests that are currently performed for indexing of propagative materials. Preliminary studies using the C6CP antiserum in DAS-ELISA showed that GarV-A is present in all major garlic cultivars grown in Argentina, with frequencies ranging from 17 to 60%. Additional determinations in a larger scale will be performed to precisely establish the incidence of this virus in different production areas.

Finally, tissue print immunoassays revealed that GarV-A is abundantly present in cloves, suggesting that this technique could be easily adapted as a semi-quantitative method for the certification of virus-free materials. In chronically infected bulbs of cultivar Blanco Mendocino, all cloves seem to be equally affected, implying that printing of a single clove could be used as a practical method to detect diseased seed-bulbs or to perform epidemiological studies with the C6CP antiserum. Nevertheless, more studies including larger number of bulbs, additional garlic varieties, and different plant growth stages must be carried out before implementing this proposal. Independently of the sampling method ultimately adopted, it is worth noticing that tissue printing could advantageously compete with DAS-ELISA due to its relatively low cost and high detection sensitivity (5).

Fig. 4. Immunochemical detection of garlic virus A (GarV-A) by tissue printing immunoassays using the C6CP antiserum. (A) tissue printing of cloves sampled from different infected and healthy garlic plants. H = cloves from healthy plants; I = cloves from infected plants. (B) Tissue printing of a single bulb obtained from a chronically infected garlic plant.

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