# Incidence of *Garlic common latent virus* in Argentina, and phylogenetic and recombination analyses of isolates

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Abstract – The objective of this work was to estimate the incidence and prevalence of *Garlic common latent virus* (GarCLV) in the main production regions of garlic (*Allium sativum*) in Argentina, and to perform phylogenetic and recombination analyses in isolates from these regions. Leaf samples (3,050) were taken from four garlic commercial types, in 13 departments of the four main garlic-producing provinces of Argentina, in a 1,175-ha sampling area. Virus infection was evaluated with DAS-Elisa test using specific antiserum, and the phylogenetic and recombination analyses were done with capsid protein (CP) nucleotide sequence of seven GarCLV isolates from the provinces. The incidence of GarCLV in the evaluated provinces varied between 6.7 and 22% of the samples, whereas the prevalence varied between 52.6 and 70%. In the analysis of garlic commercial types, Morado showed the highest incidence of the virus, in the province of San Juan, whereas Rosado Paraguayo had the lowest incidence, in the province of Cordoba. Nucleotide identity in the CP sequences ranged between 80.3 and 97.6%. The phylogenetic analysis shows the presence of two main groups of GarCLV and of a possible third group that would include only a German isolate. The recombination analysis between isolates from different parts of the world evidences the presence of recombinant isolates from Poland and Australia.

Index terms: *Allium sativum*, *Carlavirus*, GarCLV, prevalence, viral diversity.

## Incidência de *Garlic common latent virus* na Argentina, e análise filogenética e de recombinação de isolados

Resumo – O objetivo deste trabalho foi estimar a incidência e a prevalência de *Garlic common latent virus* (GarCLV) nas principais regiões produtoras de alho (*Allium sativum*) da Argentina, e realizar análises filogenética e de recombinação de isolados dessas regiões. Amostras foliares (3.050) foram coletadas de quatro tipos comerciais de alho, em 13 departamentos, nas quatro principais províncias produtoras de alho na Argentina, em uma área amostrada de 1.175 ha. A infecção viral foi avaliada pelo teste DAS-Elisa com antissoro específico, e as análises filogenética e de recombinação foram feitas com sequências de nucleotídeos da proteína capsidial (CP) de sete isolados das províncias. A incidência de GarCLV nas províncias avaliadas variou de 6,7 a 22% das amostras, enquanto a prevalência variou de 52,6 a 70%. Na avaliação dos tipos comerciais de alho, Morado apresentou as maiores incidências do vírus, na Província de San Juan, enquanto Rosado Paraguayo apresentou a menor incidência, na Província de Córdoba. A identidade de nucleotídeos nas sequências de CP variou entre 80,3 e 97,6%. A análise filogenética mostra a presença de dois grupos principais de GarCLV e de um possível terceiro grupo formado por apenas um isolado da Alemanha. A análise de recombinação entre isolados de diferentes partes do mundo evidencia a presença de isolados recombinantes da Polônia e da Austrália.

Termos para indexação: *Allium sativum*, *Carlavirus*, GarCLV, prevalência, diversidade viral.

## Introduction

More than ten different viruses have been reported causing mixed infections in garlic (*Allium sativum* L.). Although these infections do not kill the plants, they can cause important yield losses (Walkey & Antill, 1989; Conci, 1997; Canavelli et al., 1998; Conci et al., 2003; Lunello et al., 2007; Perotto et al., 2010). These viruses are widespread in garlic plants throughout the world due to the exclusively agamic propagation of the species. The rate of plants infected with garlic viruses can be reduced by using virus-free garlic plants.

Mite-borne allexiviruses, aphid-borne potyviruses, and carlaviruses have been reported in the major garlic production areas worldwide. The carlaviruses present in *Alliaceae* were misidentified for a long time. *Shallot*

*latent virus* (SLV) was originally detected in shallots and then in garlic by several authors (Bos et al., 1978; Van Dijk, 1993; Barg et al., 1994; Tsuneyoshi et al., 1998; Torrico et al., 2010), whereas *Garlic latent virus* (GLV) was found in garlic by Lee et al. (1979). However, biological and genomic comparisons of SLV and GLV showed that both viruses were in fact the same species (Van Dijk, 1993; Tsuneyoshi et al., 1998).

In Argentina, the presence of a new carlavirus was confirmed in garlic; the detected viral particles reacted strongly with antisera from different laboratories against *Carnation latent virus* (CLV), but they did not react with antisera against SLV (Conci et al., 1992). Therefore, this carlavirus was shown to be different from CLV from carnation and was named *Garlic common latent virus* (GarCLV) (Van Dijk, 1993; Barg et al., 1994; Barg et al., 1997).

GarCLV was mentioned as one of the most frequent viruses affecting garlic crop (Van Dijk, 1993; Tsuneyoshi et al., 1998), being reported in several countries of Asia (Barg et al., 1994; Tsuneyoshi et al., 1998; Pramesh & Baranwal, 2013), Europe (Bellardi et al., 1995; Tsuneyoshi et al., 1998; Dovas et al., 2001; Dovas & Vovlas, 2003; Klukácková et al., 2007; Shahraeen et al., 2008), Oceania, North America (Pappu et al., 2005; Pérez-Moreno et al., 2007), and South America (Conci, 1997; Fajardo et al., 2001; Nieto et al., 2005). A study of GarCLV incidence conducted in Greece showed that infection rates were variable, with up to 97.6% of infected plants in the province of Arcadia (Dovas et al., 2001). In Apulia, Italy, recorded values ranged between 23 and 28% (Dovas & Vovlas, 2003). In Mexico, incidence values of 32.3% were registered (Pérez-Moreno et al., 2007).

Argentina is the second largest garlic exporter worldwide, exporting 60% of its production. Current commercial garlic types grown in the country are: Morado, Blanco, Colorado, and Rosado Paraguayo (Burba, 2008). The Morado type is adapted to all the geographical regions in Argentina, with a growing season that runs from April to September/October; Blanco and Colorado are cultivated in cold regions, from April to November/December; whereas Rosado Paraguayo is adapted to temperate areas, from April to September/October.

Garlic quality is a major concern in Argentina, and great efforts are made to obtain cultivars with good agricultural traits and high-quality bulbs. Therefore,

a virus-free garlic plant production system has been implemented, which produces mother plants in specialized laboratories (Conci & Nome, 1991; Conci et al., 2005).

It is important to evaluate the sanitary condition as to GarCLV infection in garlic-producing regions in order to plan adequate management and sanitary strategies in the future. The results from this kind of study can contribute to the knowledge of the intraspecific diversity of GarCLV, which can be determined with phylogenetic and recombination analyses based on the capsid protein (CP) gene. There are no reports on GarCLV incidence in Argentina, nor on the virus frequency distribution in the main producing regions.

The objective of this work was to estimate the incidence and prevalence of GarCLV in the main production regions of garlic in Argentina, and to perform phylogenetic and recombination analyses in isolates from these regions.

## Materials and Methods

A total of 3,050 leaf samples were taken from 13 departments, covering 1,175 ha, distributed in the four main garlic-producing regions of Argentina, in the provinces of: Mendoza, San Juan, Buenos Aires, and Córdoba, which represent 90% of the cultivated area in the country (Figure 1). The sample distribution in the evaluated areas was as follows: 1,290 samples from seven departments of San Juan Province (Pocito, 25 de Mayo, Caucete, San Martín, Angaco, 9 de Julio, and Calingasta); 1,140 samples from four departments of Mendoza Province (Guaymallén, San Carlos, San Martín, and Tupungato); 320 samples from one department of Córdoba Province (Cruz del Eje); and 300 samples from one department ("partido" in this case) of Buenos Aires Province (Villarino). Samples were taken from spring (September) to early summer (December), depending on the garlic commercial type.

The following commercial types of garlic (Burba, 2008) were evaluated: Morado (purple Asiatic), Blanco (white), Colorado (red), and Rosado Paraguayo (pink). A systematic sampling procedure was carried out in 99 crop fields, following a V-shaped design within each field. Samples from 15 plants were taken from each V arm, totaling 30 plants per field, except for Córdoba, where sampling included 40 plants per field. The sampled plants were equidistant from one another and separated by rows. The second youngest leaf was

removed from each sample, placed in a labeled plastic bag, and maintained at 4ºC until laboratory analyses. The sampled 99 fields represented between 5.3 and 38% of the cultivated area in each province.

Serological detection was performed using a direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-Elisa) (Clark & Adams, 1977), modified for garlic, following Conci et al. (2002). The antiserum used to detect GarCLV was purchased from Bioreba SRL Latin America (Mar del Plata, Argentina).

Incidence was determined as the percentage of infected plants in the analysed samples, per department and per province. Prevalence was calculated as the percentage of fields with at least one infected plant. The effects of province on the commercial type were included in the adjusted model. In this case, incidence was assessed using a generalized linear model under a binomial distribution and the logit link function. This analysis is recommended when binary-type variables (presence or absence of disease) are used. Multiple comparisons of the statistically-significant mean effects were performed with the DGC test (Di Rienzo et al., 2002). The statistical analysis was conducted using the InfoStat software, version 2013 (Universidad Nacional de Córdoba, Córdoba, Argentina).

Leaves of different garlic plants serologically positive for GarCLV were used for total RNA extraction with the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. Genomic RNA was used as the template for cDNA synthesis using the commercial kit M-MLV Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) and the Oligo (dT)-primer (Invitrogen Life Technologies, Carlsbad, CA, USA). A pair of oligonucleotides, Car-V3/PC-R4, was used to amplify a genomic segment of GarCLV, as described by Tsuneyoshi et al. (1998); these primers amplify a 1,465 nucleotide (nt) fragment including the CP region. The reaction mix consisted of 200 ng cDNA, 2.5 µL of each primer (2.5 µmol L$^{-1}$), 10 µL reaction buffer (5x), 0.5 µL *Taq* DNA polymerase, 5 U µL$^{-1}$, (Promega, Madison, WI, USA), a dNTP mix of 5.0 µL (2.5 µmol L$^{-1}$), and nuclease-free water to a final volume of 50 µL. The cycle program included: 2 min at 94ºC; 40 cycles of 94ºC for 30 s, 55ºC for 30 s, and 72ºC for 1 min 30 s; and a final extension at 72ºC for 15 min.

The amplified fragments were cloned into pGEm-T Easy Vector (Promega, Madison, WI, USA), following the manufacturer's instructions. The recombinant plasmids obtained were tested with PCR using specific primers. The amplified products were visualized in 2% agarose gel, and the clones were purified using a Qiagen Plasmid Purification kit (Qiagen, Hilden, Germany). Three recombinant clones were selected from each sample and sequenced in both directions. A consensus sequence belonging to the complete encoding region for the viral CP was inferred from the obtained nucleotide sequences.

These sequences were analyzed using the Lasergene software, version 8.0.2 (DNAStar, Madison, WI, USA). The CP sequences obtained from Argentinean GarCLV isolates were compared with 42 sequences available from the GenBank database (National Center for Biotechnology Information, Bethesda, MD, USA). The phylogenetic analysis of available sequences was performed with the Mega software, version 5.10 (Tamura et al., 2011), using the maximum likelihood method, the general time-reversible (GTR) model with G+I (invariant sites and distributed range), and 2,000 replicates.
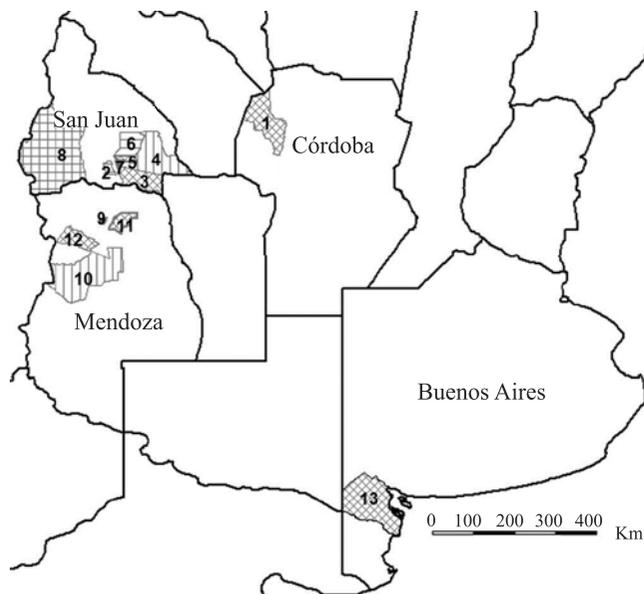


**Figure 1.** Garlic-producing departments sampled for evaluating *Garlic common latent virus* incidence and prevalence in Argentina. 1, Cruz del Eje; 2, Pocito; 3, 25 de Mayo; 4, Caucete; 5, San Martín; 6, Angaco; 7, 9 de Julio; 8, Calingasta; 9, Guaymallén; 10, San Carlos; 11, San Martín; 12, Tupungato; and 13, Villarino.

Identification of likely parental sequences and localization of possible recombination breakpoints were determined using the recombination detection RDP4 software, version 4.22 (Martin & Rybicki, 2000), with the RDP, GeneConv, Chimaera, MaxChi, BootScan, SiSan, and 3Seq methods. A multiple-comparison-corrected p-value cutoff of 0.01 was used throughout the analyses. Only recombination events that were detected by three or more of the seven methods implemented in RDP, version 4.22 (Martin & Rybicki, 2000), and that showed phylogenetic evidence were considered.

## Results and Discussion

This is the first epidemiological report of GarCLV in South America. Of the 3,050 analyzed samples, 476 had a positive reaction to GarCLV antiserum. The virus incidence in Argentina was 15.6%, and it was detected in all the sampled areas, with incidence values between 6.7 and 22%, depending on the province (Table 1). The sampled departments exhibited incidence values ranging between 2.5%, in San Carlos, in the province of Mendoza, and 33.6% in Pocito, in San Juan (Table 1).

The Rosado Paraguayo commercial type was cultivated only in the province of Cordoba; the Colorado type, in Buenos Aires and Mendoza; and the Blanco and Morado types, in San Juan, Cordoba, and Mendoza. The highest GarCLV incidence was detected in the Morado type, cultivated in San Juan (49.72%), which was significantly higher than those cultivated in Córdoba (13.75%) and Mendoza (13.89%). Furthermore, the incidence in the Blanco type, in San Juan (11.08%), was significantly lower than that observed in Blanco, in Córdoba (22.50%) and Mendoza (21.11%). The incidence in the Colorado type did not differ significantly between Mendoza and Buenos Aires. Rosado Paraguayo showed 0.63% of GarCLV incidence, which was significantly lower than in the other garlic types cultivated in the same province (22.5% in Blanco and 13.75% in Morado) or in other provinces (Figure 2).

GarCLV prevalence in Argentina, expressed as the percentage of fields that had at least one positive sample, was 57.6%, ranging from 52.6%, in the province of Mendoza, to 70% in Buenos Aires (Table 1). The department of Cruz del Eje, in Córdoba, showed a prevalence of 62.5%. In San Juan, prevalence

**Table 1.** Incidence and prevalence of *Garlic common latent virus* in garlic (*Allium sativum*) production departments and provinces in Argentina.

| Department | Hectares sampled (%)[1] | Number of | | | Incidence (%)[2] | | Prevalence (%)[3] | |
|---|---|---|---|---|---|---|---|---|
| | | Fields | Samples | Positive samples | Department | Province | Department | Province |
| Province of Córdoba | | | | | | | | |
| Cruz del Eje | 38.0 (38/100) | 8 | 320 | 30 | 9.4 | 9.4 | 62.5 | 62.5 |
| Province of San Juan | | | | | | | | |
| Pocito | | 15 | 450 | 151 | 33.6 | | 73.3 | |
| 25 de Mayo | | 4 | 120 | 13 | 10.8 | | 50.0 | |
| Caucete | | 3 | 90 | 19 | 21.1 | | 33.3 | |
| San Martín | 22.4 (448/2,000) | 3 | 90 | 22 | 24.4 | 22.0 | 66.7 | 58.1 |
| Angaco | | 7 | 210 | 44 | 21.0 | | 57.1 | |
| 9 de Julio | | 3 | 90 | 8 | 8.9 | | 66.7 | |
| Calingasta | | 8 | 240 | 26 | 10.8 | | 37.5 | |
| Province of Mendoza | | | | | | | | |
| Guaymallén | | 5 | 150 | 39 | 26.0 | | 60.0 | |
| San Carlos | 5.3 (552/10,366) | 8 | 240 | 6 | 2.5 | 12.5 | 25.0 | 52.6 |
| San Martín | | 9 | 270 | 43 | 15.9 | | 66.7 | |
| Tupungato | | 16 | 480 | 55 | 11.5 | | 56.2 | |
| Province of Buenos Aires | | | | | | | | |
| Villarino | 15.5 (137/885) | 10 | 300 | 20 | 6.7 | 6.7 | 70.0 | 70.0 |
| Total | | 99 | 3,050 | 476 | - | - | - | - |

[1]Hectare sampled per hectare cultivated with garlic in each province. [2]Incidence per province was calculated as the percentage of infected plants in all samples analyzed in the province. [3]Prevalence per province was calculated as the percentage of departments containing at least one infected sample.

values ranged between 33.3%, in Caucete, and 73.3% in Pocito. In Mendoza, values ranged between 25%, in San Carlos, and 66.7% in San Martín. In Villarino, in Buenos Aires, a prevalence of 70% was verified (Table 1).

The primers used in the present study allowed to amplify the expected genomic segments of 1,465 nt, which included the CP-encoding gene. The CP reading frame contains a start and a stop codon corresponding to the triplets ATG and TGA or TAG, respectively. The consensus sequences of the CP nucleotide obtained from seven isolates were 960-nt long, including the stop codon; 319 deduced amino acid (aa) long; and had an estimated molecular weight of 35.5 kDa.

The highest variability was found in the N-terminal of the CP gene. The conserved AAFD motif of filamentous plant viruses (Koonin et al., 1993) and the conserved TGGXXG motif of carlaviruses (Hataya et al., 2000) were found at the C-terminal of all the sequences. Comparative analyses of the seven GarCLV sequences obtained in the present study with 42 GarCLV sequences from the GenBank database (National Center for Biotechnology Information, Bethesda, MD, USA) showed 80.4–96.6% nt identity for Blanco 1; 80.5–96.6% nt identity for Blanco 2; 80.3–96.8% nt identity for Blanco 3; 80.3–96.9% nt identity for Blanco 4; 81.4–95.0% nt identity for

Morado 1; 81.5–97.6% nt identity for Morado 2; and 80.9–97.0% nt identity for Colorado (Table 2).

According to the species demarcation criteria of the International Committee on Taxonomy of Viruses (ICTV) (Adams et al., 2012), carlavirus isolates of different species should have less than 72% shared nt identity, or less than 80% aa identity, in their CP or polymerase genes to be considered a different species. According to these criteria, the isolates analyzed in the present study might be identified as GarCLV.

The lowest identity percentages (80.3–81.5% nt) were observed with a GarCLV German isolate (accession number X81138). However, all isolates from Argentina showed the highest nt identity with a South Korean isolate (accession number DQ520092). Phylogenetic relationships of 49 GarCLV sequences selected according to their geographical origin revealed that Argentinean isolates were positioned among sequences from other countries. Two defined clusters were observed in the phylogenetic tree (Figure 3): one includes the studied Argentinean sequences among others from Australia, Brazil, South Korea, Netherlands, India, and one from Germany (accession number AB004805); whereas the other group includes the isolates from USA, Poland, China, and a sequence from Taiwan (accession number AB004566).

A sequence from Germany (accession number X81138), which had the lowest identity values with Argentinean isolates, was clearly separated from these groups. This isolate forms a third cluster, which is well differentiated from the others.

Based on the data set of 49 CP sequences, recombination detection analysis yielded seven potential recombination events (PREs), through three or more methods with p-value cutoff of 0.01, but none in GarCLV isolates from Argentina. Of these seven events, five were significant recombination events, and two events may not be considered significant because the phylogenetic trees constructed with the recombinant region showed no correlation between the major parent and the presumed recombinant. With the exception of one isolate from Poland (accession number KF862701), whose both parents were from Poland (major parent KF862695 and minor parent KF862692), all significant events showed recombination between Polish isolates (accession numbers KF862695 and KF862692), as the major parents, and one Australian isolate (accession number JQ899445) as the minor
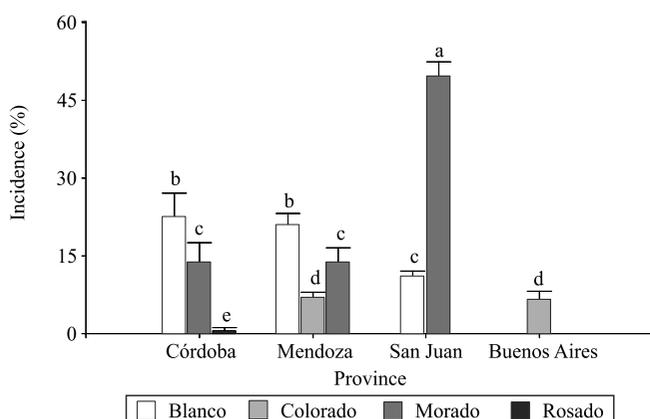


**Figure 2.** Incidence of *Garlic common latent virus* in the garlic (*Allium sativum*) commercial types evaluated in each province. Means followed by equal letters do not differ according to the generalized linear model under a binomial distribution and the logit link function, at 5% probability. Morado (purple Asiatic), Blanco (white), Colorado (red), and Rosado Paraguayo (pink).

**Table 2.** Identity of nucleotide sequences (%) of the capsid protein gene from isolates of *Garlic common latent virus* from different countries obtained from the GeneBank database.

| Sequence | Garlic type | | | | | | |
|---|---|---|---|---|---|---|---|
| | Blanco 1 (KJ124845) | Blanco 2 (KJ124846) | Blanco 3 (KJ801305) | Blanco 4 (KJ801306) | Morado 1 (KJ124847) | Morado 2 (KJ124848) | Colorado (KJ801307) |
| | Isolates from Argentina (n=7) | | | | | | |
| KJ124845 | - | 99.8 | 96.5 | 96.6 | 94.8 | 98.3 | 97.2 |
| KJ124846 | | - | 96.5 | 96.6 | 94.8 | 98.3 | 97.2 |
| KJ801305 | | | - | 99.3 | 94.4 | 97.9 | 96.8 |
| KJ801306 | | | | - | 94.5 | 98.0 | 96.9 |
| KJ124847 | | | | | - | 96.5 | 95.3 |
| KJ124848 | | | | | | - | 98.4 |
| KJ801307 | | | | | | | - |
| | Isolates from Australia (n=2) | | | | | | |
| JF320810 | 95.1 | 95.1 | 94.4 | 94.5 | 93.9 | 96.0 | 95.3 |
| JQ899445 | 94.1 | 93.9 | 93.0 | 93.1 | 92.4 | 94.9 | 93.8 |
| | Isolates from Brazil (n=1) | | | | | | |
| AF228416 | 95.1 | 95.1 | 94.8 | 94.9 | 93.8 | 96.7 | 95.5 |
| | Isolates from China (n=7) | | | | | | |
| HQ873853 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| HQ873854 | 89.5 | 89.3 | 89.0 | 88.9 | 89.4 | 90.1 | 90.0 |
| HQ873855 | 89.3 | 89.1 | 88.8 | 88.7 | 89.2 | 89.9 | 89.8 |
| HQ873857 | 89.3 | 89.1 | 88.8 | 88.7 | 89.2 | 89.9 | 89.8 |
| HQ873858 | 89.5 | 89.3 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| HQ873859 | 89.4 | 89.2 | 89.0 | 88.9 | 89.4 | 90.1 | 90.0 |
| HQ873861 | 89.2 | 89.0 | 88.7 | 88.6 | 89.1 | 89.8 | 89.7 |
| | Isolates from Germany (n=2) | | | | | | |
| AB004805 | 94.9 | 94.7 | 94.2 | 94.3 | 93.9 | 95.8 | 94.9 |
| X81138 | 80.4 | 80.5 | 80.3 | 80.3 | 81.4 | 81.5 | 80.9 |
| | Isolates from India (n=5) | | | | | | |
| JQ818255 | 95.5 | 95.5 | 95.8 | 95.9 | 94.0 | 96.7 | 96.1 |
| JQ818256 | 96.0 | 96.0 | 96.1 | 96.3 | 93.9 | 97.0 | 96.9 |
| JQ818257 | 96.1 | 96.1 | 96.5 | 96.4 | 94.0 | 97.1 | 97.0 |
| JQ818258 | 96.0 | 96.0 | 96.4 | 96.3 | 94.5 | 97.0 | 96.9 |
| JQ818259 | 95.5 | 95.5 | 95.8 | 95.9 | 94.0 | 96.7 | 96.1 |
| | Isolates from Netherlands (n=1) | | | | | | |
| AB004804 | 94.7 | 94.5 | 94.0 | 93.9 | 93.4 | 95.8 | 94.7 |
| | Isolates from Poland (n=12) | | | | | | |
| KF862692 | 86.7 | 86.5 | 85.7 | 85.6 | 86.1 | 86.9 | 86.8 |
| KF862693 | 92.0 | 91.8 | 91.2 | 90.9 | 90.8 | 92.4 | 92.0 |
| KF862694 | 91.5 | 91.3 | 90.5 | 90.5 | 90.2 | 92.2 | 91.2 |
| KF862695 | 89.7 | 89.5 | 89.2 | 89.0 | 90.1 | 90.5 | 89.9 |
| KF862696 | 89.9 | 89.7 | 89.5 | 89.3 | 90.5 | 90.7 | 90.1 |
| KF862697 | 89.4 | 89.2 | 89.0 | 88.8 | 89.8 | 90.2 | 89.6 |
| KF862698 | 89.9 | 89.7 | 89.4 | 89.2 | 89.9 | 90.4 | 89.8 |
| KF862699 | 89.0 | 88.8 | 88.4 | 88.2 | 89.4 | 89.8 | 89.2 |
| KF862700 | 88.6 | 88.4 | 88.2 | 87.8 | 88.7 | 89.1 | 88.7 |
| KF862701 | 88.2 | 88.0 | 87.8 | 87.6 | 88.2 | 89.1 | 88.6 |
| KF862702 | 89.3 | 89.1 | 88.7 | 88.4 | 88.0 | 89.9 | 89.4 |
| KF862703 | 90.7 | 90.5 | 90.4 | 90.2 | 89.5 | 91.6 | 90.8 |
| | Isolates from South Korea (n=1) | | | | | | |
| DQ520092 | 96.6 | 96.6 | 96.8 | 96.9 | 95.0 | 97.6 | 96.9 |
| | Isolates from Taiwan (n=1) | | | | | | |
| AB004566 | 89.9 | 89.9 | 89.0 | 89.1 | 89.0 | 90.6 | 90.0 |
| | Isolates from the USA (n=10) | | | | | | |
| GQ475419 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| GQ475420 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| GQ475421 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| GQ475422 | 89.2 | 89.0 | 88.7 | 88.6 | 89.1 | 89.8 | 89.7 |
| GQ475423 | 89.3 | 89.1 | 88.7 | 88.6 | 89.1 | 89.8 | 89.7 |
| HQ873852 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| HQ873856 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| HQ873860 | 89.3 | 89.1 | 88.7 | 88.7 | 89.1 | 89.8 | 89.7 |
| HQ873862 | 89.4 | 89.2 | 88.9 | 88.8 | 89.3 | 90.0 | 89.9 |
| HQ873863 | 89.2 | 89.0 | 88.7 | 88.6 | 89.1 | 89.8 | 89.7 |

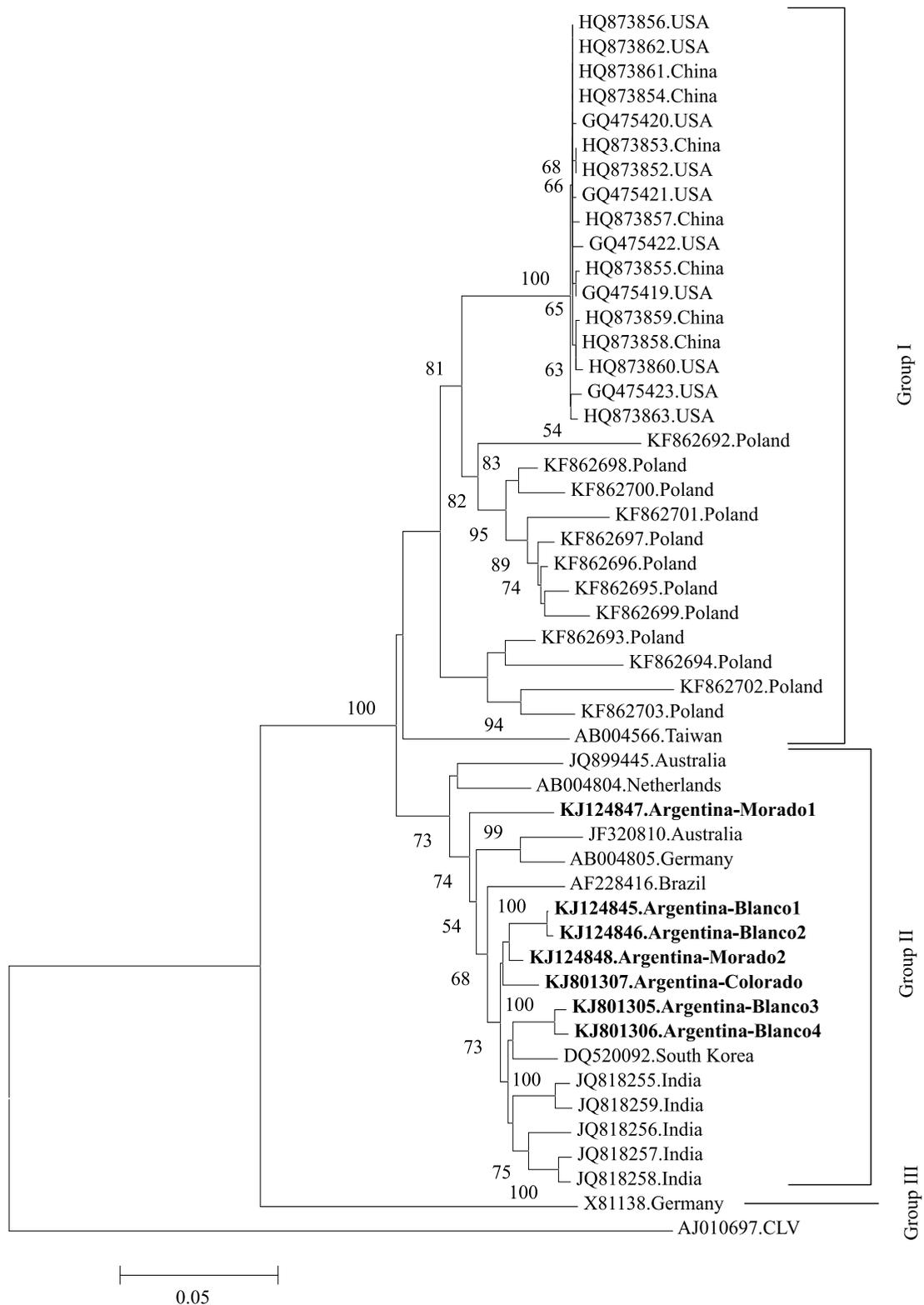Morado (purple Asiatic), Blanco (white), and Colorado (red).

**Figure 3.** Consensus phylogenetic tree constructed using the Mega software, version 5.10 (Tamura et al., 2011), the maximum likelihood method, the general time-reversible (GTR) model with G+I (invariant sites and distributed range), and 1,000 replicates based on the alignment of nucleotide sequences encoding the capsid protein of *Garlic common latent virus* (GarCLV) and *Carnation latent virus* (CLV). Values in the nodes indicate the bootstrap values (%) with 2,000 replicates.

parent. A total of five recombinants from Poland were identified among GarCLV isolates (accession numbers KF862693, KF862694, KF862701, KF862702, and KF862703). All recombination events showed undetermined beginning breakpoint, suggesting that it may be located in another gene. The KF862695 isolate had its N-terminal replaced with the corresponding region of the Australian isolate (accession number JQ899445) (Table 3). The five recombinant isolates were grouped together with American, Polish, and Chinese isolates, but the minor parent for most of them was from Australia, located in the other group.

GarCLV was reported in Argentina in 2004 (Nieto et al., 2005). Studies of GarCLV incidence conducted in different countries showed a variable presence of the virus. Iran GarCLV was detected only in one of the ten analyzed regions, with 15.8% positive samples (Shahraeen et al., 2008). In Greece, incidence values were very different among sampled regions. Indeed, 98% of infected plants were detected in Arcadia and 18% in Evia, with low or nil virus presence in Evros and Thessaloniki (Dovas et al., 2001). In Czech Republic, high incidence values were detected in Czech cultivars, varying from 98.9 to 100%, and variable percentages in imported materials, with 0% in garlic material from China and 85.7% in cultivars from Spain (Klukácková et al., 2007). In contrast, in Argentina, the virus was detected in all the sampled provinces and departments, with relatively low incidence percentages in all places, which ranged between 6.7 and 22% among provinces, and between 2.5 and 33.6% among departments (Table 1). GarCLV was also found in all garlic commercial types analyzed, including the Morado type, which is a selection from Chinese garlic.

Blanco and Morado garlic susceptibility was apparently influenced by environment, since differences in incidence percentage on both genotypes were recorded among San Juan, Córdoba, and Mendoza, suggesting a possible host-pathogen-environment relationship. However, incidence in Colorado garlic showed no dependence on location, since the percentages of both Buenos Aires and Mendoza were statistically equal (Figure 2).

The GarCLV incidence of 15.6% reported in the present study for Argentina was lower than the incidence of two potyviruses previously identified in garlic in the country (Conci, 1997; Conci et al., 2002). *Onion yellow dwarf virus* and *Leek yellow stripe virus*, respectively, were reported by those authors in the main garlic-producing regions, with incidence percentages ranging from 33 to 100% and from 34 to 98%. These results disagree with those found in other works (Van Dijk, 1993; Tsuneyoshi et al., 1998; Klukácková et al., 2007), which indicated that GarCLV is the most common virus infecting garlic.

Since all these viruses are transmitted by aphids in a non-persistent manner, similar incidence percentages should be expected between them; however, differences are noticeable. These differences could be due to different factors, such as resistance or tolerance of some garlic genotypes to GarCLV, or a lower aphid transmission efficiency of these viruses (Van Dijk, 1993). Considering that garlic is a species of exclusively agamic propagation, it is assumed that infected plants transmit the virus to successive generations, that the viruses will persist, and that the number of infected plants will increase gradually. This mechanism is markedly enhanced by the natural vector transmission to healthy plants. After many years of commercial garlic production and permanent distribution of the material among different producing regions worldwide, all plants might be expected to be

**Table 3.** Recombination events detected in the capsid protein gene of *Garlic common latent virus* isolates using RDP methods showing the parental and recombinant sequences.

| Recombinant isolate | Parental isolate | | Recombination breakpoint | | Recombination detection[1] | |
|---|---|---|---|---|---|---|
| | Major | Minor | Start | End | Method[2] | P-value |
| KF862693 | KF862695 | JQ899445 | Undetermined | 281 | B, M, C, S, 3S | $3.799 \times 10^{-6}$ |
| KF862694 | KF862695 | JQ899445 | Undetermined | 458 | B, M, C, S, 3S | $3.799 \times 10^{-6}$ |
| KF862701 | KF862695 | KF862692 | Undetermined | 365 | M, C, S | $2.919 \times 10^{-4}$ |
| KF862702 | KF862692 | JQ899445 | Undetermined | 217 | R, G, B, M, C, S | $8.053 \times 10^{-3}$ |
| KF862703 | KF862695 | JQ899445 | Undetermined | 230 | B, M, C, S, 3S | $3.799 \times 10^{-6}$ |

[1]The underlined methods are the ones with the p-values shown in the other column. [2]B, BootScan; M, MaxChi; C, Chimaera; S, SiScan; 3S, 3Seq; R, RDP; and G, Geneconv.

infected. However, this does not seem to be the real situation. These differences and the low infection of GarCLV in garlic crop in some countries or regions suggest that GarCLV might have some difficulty to multiply in garlic plants or in genotypes that show low occurrence. The unfavorable climatic conditions for virus multiplication could also explain the low incidence of GarCLV in some regions. Another aspect that might be explored is the population of aphid vector species present in each region or country, which may have influenced the spread of the virus. It is widely known that different species of vectors may show differences in the efficiency of virus transmission (Hull, 2002). This, coupled with the use of virus-free garlic plants, directly influences incidence values. Argentina has a very important program for virus-free garlic plant production and for distribution of healthy plants, in the most important garlic-growing areas (Conci, 1997; Conci et al., 2005).

Further studies should be conducted to explain differences in virus infection and incidence in different provinces, focusing on the interaction among viruses, vectors, hosts, and environment.

The analysis of GarCLV sequences showed that the greatest variability occurred in the N-terminal of the CP gene, which is in agreement with the findings of Tsuneyoshi et al. (1998) and Pramesh & Baranwal (2013), who found that the N- and C-terminal of the CP are more variable than the central region of the virus sequences.

The phylogenetic analysis of GarCLV sequences available in the GenBank (National Center for Biotechnology Information, Bethesda, MD, USA) plus the ones reported in the present study allowed the confirmation of the existence of two groups of virus (Figure 3), which is consistent with the results obtained by Pramesh & Baranwal (2013). However, a clustered sequence from Germany (access number X81138), separated from the other sequences, could suggest the existence of a third group. Tsuneyoshi et al. (1998) also found that the German GarCLV isolate was separated from the others.

Fajardo et al. (2001) suggested that the GarCLV present in Brazil could be from China; however, the phylogenetic analysis of the present study showed that the isolates from Brazil and Argentina are very close. They were grouped with sequences from Australia, Netherlands, and Germany, within the same cluster and distant from isolates from India and South Korea,

although all of them belong to the same group. The South Korean isolate (accession number DQ520092) exhibited the highest sequence identity with the seven Argentinean isolates (95.0–97.6% nt). The other group, which is separated from the isolates mentioned above, comprises all the isolates from USA, China, and Poland, including one from Taiwan, suggesting that the virus present in the USA and Poland are from China, and those present in South America (Brazil and Argentina) are probably from Australia, Netherlands, Germany, India, and South Korea.

It is noteworthy to point out that the viruses from Australia, Netherlands, Germany, and India grouped together, despite the geographic distances of the countries, and this deserves further research. One of the possible reasons is the continuous exchange of garlic between countries.

Recombination events in *Carlavirus* genus have been frequently reported. Singh et al. (2007) found the first evidence of homologous recombination of *Chrysanthemum virus B* isolates from India infecting *Chrysanthemum morifolium*. A recombinant *Lily symptomless virus* from Korea showed recombination with isolates from other countries (India, China, and Netherlands), and this event was associated with exchange of *Lilium longiflorum* germplasm (Singh et al., 2008). Duarte et al. (2012) detected a recombinant *Potato virus* S (PVS) from Czech Republic that showed parental evidence with different strains of PVS: an ordinary strain from Germany and an Indian strain from Brazil. A *Cowpea mild mottle virus* isolate from Brazil that infected soybean showed recombinant evidence with another isolate from the same location (Zanardo et al., 2014). Pramesh & Baranwal (2013) analyzed 28 GarCLV sequences and found an Indian recombinant isolate from two other Indian isolates. In the present work, this recombination event was not detected when the new database of GarCLV (49 sequences) was used. However, the recombination study conducted in the present study revealed the existence of five recombinant isolates from Poland, four of which had the major parent from Poland and the minor parent from Australia (Table 3). These five isolates are located in different groups, suggesting that the observed genetic changes were possible due to the movement of garlic bulbs among the countries.

## Conclusions

1. In Argentina, *Garlic common latent virus* (GarCLV) is present in all the major producing regions of the country, regardless of the garlic commercial type evaluated.

2. GarCLV incidence varies among garlic commercial types, in the different provinces.

3. Two main groups of GarCLV isolates are present in Argentina, besides a putative third group, represented by a German isolate (accession number X81138).

4. Recombinant isolates between viruses from Poland and Australia are present in the evaluated areas.

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