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Note

Survey of viruses belonging to different genera and species in noble garlic in Brazil

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ABSTRACT: Garlic (*Allium sativum* L.) is a host to several viruses, most commonly those belonging to the *Allexivirus*, *Carlavirus*, or *Potyvirus* genera. Nine species distributed among these three genera have been reported in Brazil: two species within carlaviruses, two within potyviruses, and five within allexiviruses. To quantify the prevalence of these viruses, young leaves from 520 plants (plants either symptomatic or asymptomatic) were collected from commercial fields grown in four Brazilian states and analyzed using universal and species-specific primers via the reverse transcription polymerase chain reaction (RT-PCR). Potyvirus presence was positive in 306 samples (81 %), 151 of them (38 %) in mixed infections with other viruses. The most frequent potyviruses were *Onion yellow dwarf virus* (OYDV, 56 %) and *Leek yellow stripe virus* (LYSV, 55 %). 187 samples (49 %) were positive for allexivirus, with 33 (9 %) showing single infections and 154 (41 %) showing mixed infections with *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), and species belonging to the *Carlavirus* and *Potyvirus* genera. The predominant species in which allexiviruses were found were GarV-A and GarV-D. Only 15 samples (4 %) were infected solely by a carlavirus, and 63 (17 %) showed mixed infections with viruses from different genera. The dominant species of carlavirus was *Garlic commom latent virus* (GarCLV). Carlaviruses and allexiviruses are frequently associated with mixed infections with potyviruses, whereas mixed infections with carlaviruses and allexiviruses are rare. About 70 % of the plants collected were positive for at least one species of virus.
Keywords: *Allexivirus, Allium sativum*, *Carlavirus*, *Potyvirus*, occurrence

Introduction

Garlic (*Allium sativum* L.) is propagated vegetatively. This procedure allows for the accumulation of pathogens (especially viruses), which are perpetuated by bulbs from one production cycle to the next. Typically, garlic is infected by several viruses belonging to the *Allexivirus*, *Carlavirus*, and *Potyvirus* genera (Barg et al., 1994; Sumi et al., 1993), which significantly reduce crop yield.

Potyvirus species are the most common and damage more garlic when plants are mix-infected with other genera of viruses (Salomon, 2002). They are represented by the *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) species (Chen et al., 2001; Maeso et al., 1997). As regards the *Carlavirus* genus, the most common species found is the *Garlic common latent virus* (GarCLV) (Fajardo et al., 2001), and *Shallot latent virus* (SLV) was recently reported in Brazil (Mituti et al., 2011).

Species in the *Allexivirus* genus include the *Garlic mite-borne filamentous virus* (GarMbFV), *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E), *Garlic virus X* (GarV-X), and *Shallot virus X* (ShVX) (Kanyuka et al., 1992; Sumi et al., 1993; Yamashita et al., 1996). Species reported in Brazil include the GarMbFV, GarV-A, GarV-B, GarV-C, GarV-D, and GarV-X (Melo Filho et al., 2004; Oliveira et al., 2013).

The mid-western and southeastern regions of Brazil are currently the most important areas which produce noble garlic in Brazil, and no prior surveys in commercial fields indicating the prevalence of viruses in these areas exist. Thus, the goal of the authors in this study was to evaluate the occurrence of viruses belonging to the *Allexivirus*, *Carlavirus*, and *Potyvirus* genera.

Materials and Methods

Sample collection

520 samples of garlic (both from symptomatic and asymptomatic plants) presenting mosaic and chlorotic streaking were collected in commercial fields approximately 70 days after planting in four Brazilian states and seven municipalities, including the following: Goiás (Campo Alegre 9° 46' 46'' latitude S, 36° 21′ 1″ longitude W, and Ipameri 17° 43′ 29″ latitude S, 48° 9′ 35″ longitude W) , Minas Gerais (Santa Juliana 19° 18′ 32″ latitude S, 47° 31′27″, longitude W and São Gotardo 19° 18' 27'' latitude S, 46° 3′ 22'' longitude W), Paraná (Bandeirantes 23° 06′ 36″ latitude S, 50° 22′ 0″ longitude W, Piraquara 25° 26′ 30″ latitude S, 49° 03' 48″ longitude W, and Guarapuava 25° 23′ 43″ latitude S, 51° 27′ 29″ longitude W), and São Paulo (São Manuel 22° 43′ 52″ latitude S, 48° 34′ 14″ longitude W). Collections were made from May 2007 – Oct 2011, for a total of 34 collections. Samples collected in Guarapuava were cultivated in a greenhouse, where samples that were free of the garlic virus were propagated.

Genera and species-specific primer development and analysis

Multiple alignments of the main species described in garlic around the world were done using the MEGA 4.0 (Tamura et al., 2007) program. Universal and specific primers were synthesized to detect genera and different species and to partially amplify the region of coat protein (CP) except for LYSV, of which the primers amplified the partial region of the P1 gene (Table 1).

Total RNA was extracted (Bertheau et al., 1998) and used in a one-step reverse transcription polymerase chain reaction (RT-PCR) assay. A total volume of 25 µL, 12.5 µL of 2X PCR master mix, 1 µM of each primer, one unit of AMV (*Avian Mieloblastosis virus*) reverse transcriptase, 2.5 µL of total RNA, and nuclease-free water were used. The reaction consisted of the following: 30 min at 42 °C; 1 min at 95 °C; 40 cycles of 94 °C for 40 s, 50 °C for 60 s, and 72 °C for 60 s; and a final extension for 10 min at 72 °C. The same reaction cycle was used to detect the species of the *Carlavirus* genus, GarCLV, OYDV, and LYSV. For the other primers, the reaction cycle was performed in accordance with the bibliographic references cited in Table 1. Amplicons of five samples of each genus and species were sequenced to evaluate the efficiency of primers.

Results and Discussion

Potyvirus was the most common genus identified in 306 samples (81 %), of which 155 (41 %) were infected only by potyviruses (Table 2). Mixed infections between OYDV and LYSV were found in 61 samples (16 %). 94 samples (25 %) had potyviruses and allexiviruses; 23 (6 %) were infected with potyviruses plus carlaviruses, and 34 (9 %) were infected with potyviruses, allexiviruses, and carlaviruses, indicating that approximately half of the collected samples had mixed infections of viruses belonging to the three different genera tested.

LYSV and OYDV were found with the same frequency in noble garlic from Brazil. Our results differ from previous studies by Fayad-Andre et al. (2011), which showed LYSV to be predominant compared to OYDV. In this study, only noble garlic was analyzed, while Fayad-Andre et al. (2011) collected garlic from different production systems. Noble garlic has the higher commercial price compared to tropical garlic, which produces lower quality and smaller bulbs over a shorter cycle (Filgueira, 2007).

Followed by potyviruses, allexiviruses were found with high incidence, being detected in 187 samples collected (49 %). GarV-D and GarV-A can be considered the predominant species, with 109 (29 %) and 107 (28 %) positive samples, respectively. 41 samples (11 %) were positive for GarV-C, while GarV-B (the rarest) was found only in 18 samples (5 %).

The transmission of allexiviruses can easily occur because the mite is a pest commonly found in bulbs, which facilitates the transmission of viruses (Cafrune et al., 2006). Our results also differed from those obtained by Fayad-Andre et al. (2011), because GarV-C was seen

Table 1 – Primer sequences used to detect various viruses in garlic.

Genus or species	Sequence	Reference
Carlavirus	5'-GGNTKKGAAWCTGGGAGDCC-3' 5'-CATKTMATTCCAAACAACNGGYGC-3'	Designed for this work
Potyvirus	5'-GAT TTA GGT GAC ACT ATA GT_{16}-3' 5'-ATG GTT TGG TGY ATY GAR AAT-3'	Gibbs et al., 1997 Mota et al., 2004
Allexivirus	5' CTACCACAATGGTTCCTC 3' 5' GATTTCTTTAACGCAGTG 3'	Oliveira et al., 2013
OYDV	5' CRCCARTTCTGGATAAYGC 3' 5' CTCCGTGTCCTCATCCG 3'	Designed for this work
LYSV	5' CTTCMTCRCASTCATGKTCC 3' 5' AATCTCAACACAACTTATRC 3'	Designed for this work Yoshida et al., 2011
SLV	5'-CTTTTGGTTCACTTTAGG-3' 5'-GCACGCAATAGTCTACGG-3'	Mituti et al., 2011
GarCLV	5'-GGSTTTGARACTGGGAGGCC-3' 5'-CATKTMATTCCAAACAACNGGYGC-3'	Designed for this work
GarV-A	5'-CCCAAGCTTACTGGAAGGGTGAATTAGAT-3' 5'-CCCAAGCTTAGGATATTAAAGTCTTGAGG-3'	Melo Filho et al., 2004
GarV-B	5' GCAGAATAARCCCCCYTC 3' 5' RAAGGGTTTATTCTGTTG 3'	Oliveira et al., 2013
GarV-C	5'-CCCAAGCTTCATCTACAACAACAAAGGCG-3' 5'-CCCAAGCTTATAAGGGTGCATGATTGTGG-3'	Melo Filho et al., 2004
GarV-D	5'-CCAAGCTTAAGCAAGTGAAGAGTGTAAG-3' 5'-CCAAGCTTTTTGGAAGAGGAGGTTGAGA-3'	Melo Filho et al., 2004

OYDV= *Onion yellow dwarf virus*; LYSV= *Leek yellow stripe virus* ; SLV= *Shallot latent virus*; GarCLV= *Garlic commom latent virus*; GarV-A= *Garlic virus A*; GarV-B= *Garlic virus B*; GarV-C= *Garlic virus C*; GarV-D = *Garlic virus D*.

to be the prevalent species in all production systems, while GarV-D was limited to the Cerrado region.

Carlaviruses were detected in 78 samples (21 %), indicating relatively low prevalence, with GarCLV being the predominant species. SLV was detected only in 11 samples (3 %). In Brazil, the occurrence of carlaviruses is low, with GarCLV being the prevalent species. This genus can cause crop losses which are somewhat limited, but it can cause significant yield losses when plants are co-infected with potyviruses as a result of synergistic effects (Takaichi et al., 1998).

Potyviruses, allexiviruses, and carlaviruses were found in most regions, except for the state of Goiás, where GarV-B, GarV-C, and GarV-D were not detected. Also, SLV was not found in Paraná (Table 3). 141 samples were negative for the three genera. Most of them were found in Paraná (34 %), where garlic seed free of viruses is maintained in greenhouses, and Minas Gerais (27 %), where the same seed is multiplied in fields (Table 3).

Infected bulbs seem to be common in commercial fields, and the occurrence of two or more viruses of different taxonomic groups is also common. There was no correlation between the occurrence of species with producing regions of the country, probably as a result of the exchange of garlic seed in the southern, southeastern, and mid-western regions of Brazil.

Table 2 – Detection of genera and species of *Allexivirus*, *Carlavirus* and *Potyvirus* by the reverse transcription polymerase chain reaction (RT-PCR), using universal and specific primers.

Genus (no. positive samples)	No. of species	No. of samples (%)
Potyvirus only (155)	One species	94 (25 %)
	Two species	61 (16 %)
Carlavirus only (15)	One species	13 (3 %)
	Two species	2 (1 %)
Allexivirus only (53)	One species	33 (9 %)
	Two species	13 (3 %)
	More than three species	7 (2 %)
Potyvirus + Carlavirus (23)	Two species	11 (3 %)
	More than three species	12 (3 %)
Potyvirus + Allexivirus (94)	Two species	54 (14 %)
	More than three species	40 (11 %)
Carlavirus + Allexivirus (6)	Two species	6 (2 %)
Potyvirus + Allexivirus+ Carlavirus (34)	Three species	4 (1 %)
	Four species	9 (2 %)
	Five species	16 (4 %)
	Six species	5 (1 %)
Total of positive samples	380	

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Table 3 – Occurrence of species of Potyvirus (*Leek yellow stripe virus* - LYSV and *Onion yellow dwarf virus* - OYDV), Carlavirus (*Garlic common latent virus* - GarCLV and *Shallot latent virus* - SLV) and Allexivirus (*Garlic virus A* - GarV-A; *Garlic virus B* - GarV-B; *Garlic virus C* - GarV-C and *Garlic virus D* - GarV-D) in Brazil, analyzed from April 2007 to Oct 2011.

Genera	Species	Collection Site (number of collected samples)			
		Minas Gerais (228 samples)	Goiás (49 samples)	Paraná (149 samples)	São Paulo (94 samples)
Potyvirus	LYSV	98 (43 %)	21 (43 %)	42 (28 %)	47 (50 %)
	OYDV	91 (40 %)	24 (49 %)	47 (32 %)	49 (52 %)
Carlavirus	GarCLV	33 (14 %)	5 (10 %)	3 (2 %)	37 (39 %)
	SLV	5 (2 %)	1 (2 %)	0	5 (5 %)
Allexivirus	GarV-A	45 (20 %)	2 (4 %)	20 (13 %)	40 (43 %)
	GarV-B	3 (1 %)	0	7 (5 %)	8 (9 %)
	GarV-C	9 (4 %)	0	5 (3 %)	27 (29 %)
	GarV-D	40 (2 %)	0	39 (26 %)	30 (32 %)
Virus Free	-	62 (27 %)	9 (18 %)	50 (34 %)	19 (20 %)

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