Short Communication

VIRUSES INFECTING ORNAMENTAL *ALLIUM* SPECIES IN POLAND

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SUMMARY

The aim of the present work was to identify allexiviruses (GarMbFV, GarV-A, GarV-B, GarV-C, GarV-D, GarV-E, GarV-X and ShVX), carlaviruses (GarCLV, SLV) and potyviruses (LYSV and OYDV) in ornamental plants from the genus *Allium*. Two groups of tested plants were used. The first group included bulbs of thirteen ornamental species of *Allium* growing in the Polish Academy of Sciences Botanical Garden, at Powsin. The second group comprised seven other species of *Allium* as well as three *Allium* hybrids (cultivars) purchased from a Warsaw retail nursery. Bulb samples were tested by ELISA using antibodies specific for GarCLV, GarV-A, GarV-B, GarV-C, LYSV, OYDV, ShVX, and SLV. The identification of GarMbFV, GarV-D, GarV-E and GarV-X in bulbs was performed by RT-PCR. The RT-PCR confirmed the ELISA detection of GarV-B in *A. caeruleum* and ShVX in *A. vineale*. GarV-D was found in *A. fistulosum*. To the best of our knowledge, this is the first report of GarV-D in *A. fistulosum* and ShVX in *A. vineale.* Moreover, GarV-B was detected in *A. caeruleum* for the first time in Poland.

Keywords: ornamental *Allium* plants, *Allexivirus*, *Potyvirus*, *Carlavirus*, diagnosis, phylogenetic analysis.

The genus *Allium* comprises around 500-750 species. Apart from species used for human consumption, e.g. *Allium cepa* (onion), *A. sativum* (garlic) and, to a lesser extent, *A. ascalonicum* (shallot) and *A. ampeloprasum* (leek), there are eighty species grown as ornamentals. In recent years, most of the ornamental *Allium* species have become favorites in rock, heather and formal gardens. In addition, many of them are excellent as fresh cut and everlasting flowers and are becoming an increasingly important segment of the specialty cut flower market (Krzymińska, 2008). The ornamental *Allium* species are infected with various viruses of several genera, including *Allexivirus*, *Carlavirus*, *Potyvirus* as well as *Tospovirus* and *Tobravirus*. Allexivirus *Garlic virus A* (GarV-A) was detected in *A. senescens* by Ward *et al.* (2009), while *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E) and *Shallot virus X* (ShVX) were found in *A. caeruleum* and *A. sphaerocephalon* by Bampi *et al.* (2015). Shallot virus X was identified in *A. caeruleum* by Bereda and Paduch-Cichal (2016). According to results described by Ward *et al.* (2009) and Bampi *et al.* (2015), carlaviruses were detected in three ornamental *Allium* species. *A. murrayanum* and *A. senescens* were infected with *Garlic common latent virus* (GarCLV) and *Shallot latent virus* (SLV), whereas *A. moly* was infected with SLV. Viruses belonging to the genus *Potyvirus* were also detected in ornamental *Allium* species. *A. bulgaricum* and *A. atropurpureum* were infected with *Leek yellow stripe virus* (LYSV) (Bampi *et al.*, 2015). Srace *et al.* (2015) used reverse transcription polymerase chain reaction (RT-PCR) to test plant material from 32 *Allium* ornamental species from nurseries in the United Kingdom or the Netherlands. LYSV was detected in *A. ampeloprasum*, *Onion yellow dwarf virus* (OYDV) was detected in *A. amethystinum* and *Ornamental onion stripe mosaic virus* (OrOSMV) was detected in *A. caeruleum*. Moreover, *Turnip mosaic virus* (TuMV) was detected in A. *ampeloprasum*, which confirmed the results of previous research conducted in Japan by Noda and Inouye (1989) and in Israel by Gera *et al.* (1997). Besides allexi-, carla- and potyviruses, plants from the genus *Allium* may be infected with virus species belonging to other genera, e.g. *A. senescens* and *A. murrayanum*: *Iris yellow spot virus* (IYSV) (Ward *et al.*, 2009); *A. caeruleum*: *Pea early browning virus* (PEBV) or *Tobacco rattle virus* (TRV), both belonging to genus *Tobravirus* (Srace *et al.*, 2015). In addition to the data presented above, plants of *A. christophii*, *A. giganteum*, *A. unifolium* and *A. globemaster* were infected with Allium virus X, which is a putative member of the genus *Potexvirus* (Miglino *et al.*, 2011). Viruses are a particular problem in the ornamental *Allium* species, since vegetative propagation leads to their accumulation and dissemination through viruses in planting material (Cafrune *et al.*, 2006; Perotto *et al.*, 2010). Viruses are often transmitted between hosts by aphids (*Potyvirus*, *Carlavirus*) (Melhus et al., 1929; Bos *et al.*, 1978; Kumar *et al.*, 2011), while *Aceria tulipae* spreads allexiviruses (Van Dijk *et al.*, 1991), tospoviruses are vectored by thrips (Srinivasan *et al.*, 2012) and tobraviruses are carried by nematodes (Hernández *et al.*, 1997).

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Most ornamental *Allium* species infected by viruses show severe discoloration, stunting, and white or yellow streaking of the foliage. The flowers are often aborted (Armitage and De Hertgoh, 2000).

The aim of the conducted research was to examine the potential presence of twelve virus species belonging to the genera *Allexivirus* (GarMbFV, GarV-A, GarV-B, GarV-C, GarV-D, GarV-E, GarV-X, ShVX), *Potyvirus* (OYDV, LYSV) and *Carlavirus* (GarCLV, SLV) in ornamental *Allium* species using the ELISA (enzyme-linked immunosorbent assay) test as well as RT-PCR technique. Virus surveys were performed in twenty ornamental *Allium* species and three *Allium* hybrids (cultivars). Bulb tissue was collected from each *Allium* species and *Allium* cultivar and was further tested. Two groups of plants from the genus *Allium* were used for the study. The first group consisted of 13 botanical species growing in the Polish Academy of Sciences Botanical Garden, Center for Biological Diversity Conservation at Powsin, i.e. *A. aflatunense* (4 bulbs) *A. altaicum* (5 bulbs), *A. carinatum* subsp. *pulchellum* (7 bulbs), *A. fistulosum* (8 bulbs), *A. karataviense* (2 bulbs), *A. pskemense* (5 bulbs), *A. ramosum* (8 bulbs), *A. schoenoprasum* (8 bulbs), *A. senescens* subsp. *montanum* (7 bulbs), *A. stipitatum* (10 bulbs), *A. thunbergii* (8 bulbs), *A. tuberosum* (8 bulbs), *A. vineale* (6 bulbs). The other group comprising *A. atropurpureum* (10 bulbs), *A. caeruleum* (10 bulbs), *A. giganteum* (5 bulbs), *A. nigrum* (syn. *A. multibulbosum*) (10 bulbs), *A. rosenbachianum* (5 bulbs), *A. roseum* (10 bulbs), *A. schubertii* (5 bulbs) and *Allium* cultivars ('Gladiator' – 5 bulbs, 'Mount Everest' – 5 bulbs, 'Purple Sensation' – 10 bulbs) originating from the Netherlands were bought from a garden center in Warsaw.

Detection of GarCLV, GarV-A, GarV-B, GarV-C, LYSV, OYDV ShVX, and SLV was carried out using a commercial ELISA kit (Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Samples were prepared by grinding 0.5 g of fresh bulbs in phosphate buffer saline supplemented with 2% polyvinylpyrrolidone and 0.2% egg albumin in the ratio 1:10 (w:v) and tested according to the manufacturer's protocol. P-nitrophenyl phosphate substrate was utilized for detection and absorbance was measured at 405 nm. A test was considered positive if the A_{405} of the sample exceeded three times the mean of the negative controls. The positive and negative controls in each ELISA test were from the commercial ELISA kit (DSMZ, Braunschweig, Germany). Absorbance was analysed with an Infinite 200 Pro microplate reader (Tecan, Austria GmbH).

RT-PCR with total RNA and appropriate primers was used in order to confirm the ELISA results and to detect and identify isolates of GarMbFV, GarV-D, GarV-E, GarV-X in garlic plants. Total RNA was extracted from bulb tissues using the silica capture (SC) method described by Malinowski (1997). RNA extracts were subjected to amplification by RT-PCR using the Transcriptor One-Step RT-PCR Kit (Roche Applied Science, Germany) and primer pairs, designed by the authors, specific to the part of the coat protein gene derived from the coat protein gene of GarMbFV, GarV-A, GarV-B, GarV-C and GarV-D (Table 1). Primer pairs for detection of GarV-E, GarV-X and ShVX were designed in the open reading frame I (ORFI, replicase). Primer pairs for OYDV, GarCLV and SLV detection were designed in the whole coat protein gene. Primer pairs for detection of LYSV were designed by Parrano *et al.* (2012) in a part of the N-terminal domain of the coat protein gene of this virus (Table 1). Samples were subjected to reverse transcription for 30 min at 50°C, 2 min of denaturation at 94°C, followed by 35 cycles of 30 s of denaturation, 45 s annealing (see Table 1 from annealing temperature for each primer set), and 45 s elongation at 68°C with a final extension of 7 min at 68°C. The reaction products were resolved by electrophoresis in TBE buffer in 1.2% agarose gel.

The nucleotide sequences were determined using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence data were assembled using DNA Baser Sequence Assembler ver. 4 (Heracle BioSoft, Romania). Sequence alignments were constructed in MEGA ver. 5 (Tamura *et al*., 2011). Sequence similarity and identity analysis of the twelve viruses tested for in this study was performed in BioEdit (Hall, 1999).

Of the twelve viruses tested in this study, only three viruses were detected: GarV-B and ShVX, both using ELISA and RT-PCR, and GarV-D using RT-PCR. Amplicons of the expected size were obtained for three, one and four GarV-B, GarV-D and ShVX isolates, respectively. GarV-B was found in three out of ten tested bulbs of *A. caeruleum*. The presence of GarV-B in *A. caeruleum* is in agreement with Bampi *et al.* (2015), who also reported the presence of GarV-C, GarV-D, GarV-E and ShVX in this species. The occurrence of ShVX in *A. caeruleum* was also mentioned by Bereda and Paduch-Cichal (2016). GarV-D was found for the first time in one out of eight bulbs of *A. fistulosum* tested. During this study ShVX was identified for the first time in 4 out of 6 bulbs of *A. vineale* tested. GarCLV, GarMbFV, GarV-A, GarV-C, GarV-E, GarV-X, LYSV, OYDV and SLV and were not found in any of the samples tested. Likewise, Winiarczyk *et al.* (2014) were unable to detect GarCLV, LYSV or OYDV in *A. fistulosum*, *A. senescens* subsp. *montanum* and *A. tuberosum* plants. There is no evidence for the presence of these viruses in species of ornamental *Allium* in Poland.

The obtained PCR products were sequenced and submitted to GenBank with the following accession numbers: GarV-B (GarV-B149_AC accession No. KX588127, GarV-B150_AC accession No. KX588128, GarV-B151_AC accession No. KX588129), GarV-D (GarV-D73AF accession No. KX588130), ShVX (ShVX128_AV accession No. KX580719, ShVX129_AV accession No. KX580720, ShVX130_AV accession No. KX580721, ShVX131_AV accession No. KX580722).

Table 1. RT-PCR primer pairs specific to the segment of GarV-A, GarV-B, GarV-C, GarV-D, GarV-E, GarV-X, GarMbFV, ShVX, GarCLV, SLV, LYSV and OYDV RNA, product size and annealing temperature.

Virus	Primer sequence		Product size (bp)	Annealing temperature (°C)
GarV-A[1]	F	5'-ATGTCGAATCCAACTCAGTCG- 3'	444	52
	R	5'-AGACCATGTTGGTGGCGCG- 3'		
GarV-B[1]	F	5'-TGACGGGCAAACAGCAGAATAA- 3'	576	49
	R	5'-ATATAGCTTAGCGGGTCCTTC- 3'		
GarV-C[1]	F	5'-TTGCTACCACAATGGTTCCTC- 3'	679	51
	R	5'-TACTGGCACGAGTTGGGAAT- 3'		
GarV-D[1]	F	5'-AAGGAGCTACACCGAAGGAC-3'	456	50
	R	5'-TAAAGTCGTGTGGATGCATCAGA-3'		
GarV-E[1]	F	5'-TTGCTAGACCACCTCAGTATTGAGAA-3'	458	55
	R	5'-TAT TGG GCG TAC ATC GGT GACTGT-3'		
GarV-X[1]	F	5'-GCGGTAATATCTGACACGCTCCA- 3'	286	55
	R	5'-ACGTTAGCTTCACTGGGGTAGAATAT- 3'		
GarMbFV[1]	F	5'-ATGAACGACCCTGTTGACC-3'	461	49
	R	5'-TCAGAACGTAATCATGGGAG-3'		
ShVX[1]	F	5'-ACCGAAATCACAGTTAACTCCTTTGG-3'	800	54
	R	5'-TCTACGGTTGTCGATTTTGTGCGT-3'		
GarCLV[1]	F	5'-TTATAGGGACGGCACAAAATCAATCA-3'	1417	54
	R	5'- AATAGCACTCCTAGAACAACCATT-3'		
SLV[1]	F	5'-AATYATTTACAATCGTCCAGCTA-3'	1303	48
	R	5'-ATAATATCAATCAAATMCACACAATT-3'		
LYSV[2]	F	5'- ACAAGTAAGAAACAGAAGGACAGC-3'	363	63
	R	5'- GAGGTTCCATTTTCAATGCACCAC-3'		
OYDV[1]	F	5'-TAGGGTTGGATTATGATTTCTCGA-3'	1278	50
	R	5'-TAGTGGTACACCACATTTCGT-3'		

[1]Primer pairs designed by the authors.
[2]Primer pair designed by Parrano *et al.* (2012).

In all three GarV-B isolates, the length of the obtained nucleotide sequences was 538 nucleotides (nts), and the isolates shared nt and amino acid (aa) identities of 99-100%. The Polish GarV-B isolates obtained from *A. caeruleum* plants showed 93% nucleotide sequence identity with the Argentinean GarV-B isolate Messi 13 (KM379144.1) from garlic (*A. sativum*), 92% nucleotide sequence identity with a large group of isolates from Japan, the Czech Republic, Spain and Australia, and 90-91% with the rest of the GenBank-deposited sequences of isolates from the Czech Republic, Poland, Iran, Sudan, China, Spain, Japan and Brazil.

In the four ShVX isolates the length of the obtained nucleotide sequences was 780 nts and the isolates shared 99-100% nt and 100% aa identity. The Polish ShVX isolates from *A. vineale* showed 99 to 100% nucleotide sequence identity with the Polish isolates from *A. caeruleum*, 97% with the Italian ShVX isolate P10 (KT898125) from shallot and 95% with a Russian ShVX isolate (JX310755) from shallot.

The length of the amplicon sequence of the Polish GarV-D isolate from *A. fistulosum* was 464 nts. The Polish GarV-D isolate showed the highest nucleotide sequences identity (99%) with Polish (KF446187.1) and Korean (AF519572.1) GarV-D garlic isolates. High sequence identity (96-98%) was also identified between isolate GarV-D73AF and 13 isolates from Poland and one isolate from Brazil. The remaining isolates from GenBank shared 88-91% nucleotide sequence identity with the isolate GarV-D73AF.

The information concerning the occurrence of allexiviruses, in particular in ornamental *Allium* plants, is important for virus indexing for the production of virus-free propagative material. Infected *A. caeruleum*, *A. fistulosum* and *A. vineale* plants may constitute a potential source of allexiviruses for garlic crops. *Aceria tulipae* is commonly known to participate in the transmission of allexiviruses. Despite their slow walking, minute eriophyid mites can disperse for long distances on air currents or specific animal carriers. Dispersal by wind was reported most frequently, and this was clearly dominant over any other mode of dispersal. Two other modes, i.e. by carriers and ambulatory, occurred rarely when compared to dispersal by wind, whereas dispersal by rain had the lowest frequency (Michalska *et al.*, 2010).

This report presents the results of the detection and identification of GarV-B, GarV-D and ShVX in some species of ornamental *Allium* plants that might act as sources of viral dispersal to the cultivated species.

Currently, ornamental *Allium* plants are becoming more and more popular. They are plants with highly ornamental flowers grown increasingly frequently in flower gardens. Moreover, they have major esthetic qualities and are an important addition to winter bouquets. Therefore, they can be a source of different viruses, not only allexiviruses. Thus, further research into ornamental *Allium* plant health is needed.

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