Transmission of *Garlic virus B*, *Garlic virus C*, *Garlic virus D* and *Garlic virus X* by *Aceria tulipae* (Keifer) in leek

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**Abstract** Viruses belonging to genus *Allexivirus* infest garlic and are spread via propagation material and through a vector, the eriophyid mite *Aceria tulipae* (Keifer). The research material was garlic bulbs originating from Poland, available commercially on the Polish retail market. The aim of this study was to assess the possibility of transmission of Garlic virus B (GarV-B), Garlic virus C (GarV-C), Garlic virus D (GarV-D) and Garlic virus X (GarV-X) from garlic bulbs to leek plants by its vector, *A. tulipae*. These allexiviruses were detected in garlic bulbs and in leek leaves on which transferred mites fed. There was a high similarity of the genetic structure in the isolates of GarV-B, GarV-C, GarV-D and GarV-X collected from garlic bulbs and the isolates collected from the leek plants. The results of the study showed for the first time the potential of GarV-B, GarV-C, GarV-D and GarV-X to infect leek plants and constitutes the first attempt to examine the ability of *A. tulipae* to transmit these viruses from garlic to leeks.

**Keywords** Allexiviruses · *Aceria tulipae* · Garlic · RT-PCR technique · Virus transmission

**Main text**

Garlic (*Allium sativum* L.) is an important culinary plant that is cultivated throughout the world (Krzymińska 2008). The most severe threat to garlic crops is posed by viruses. The majority of garlic cultivars do not produce seeds, therefore garlic reproduction is only vegetative, i.e. by cloves and aerial bulblets (Etoh 1985; Simon and Jenderek 2003). It is commonly believed that plant species with vegetative reproduction are particularly threatened by viral diseases. Moreover, during cultivation or garlic bulb storage viruses can be transmitted by vectors such as insects (aphids) or arachnids (mites) (King et al. 2012). In garlic plants different virus species of the following genera were detected: *Potyvirus*, *Carlavirus*, *Cucumovirus* (Stefanac 1980), *Tobravirus* (Van Dijk 1993), *Tobamovirus* (Sako et al. 1991), *Cytorhabdovirus* (Sward 1990), *Nepovirus* (Van Dijk 1993), *Tospovirus* (Bag et al. 2009) and *Macluravirus* (Walkey 1990).

One of the most significant threats to garlic crops are virus species in the genus *Allexivirus*. A total of eight species have been described in this genus: *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) (The ICTV Online (10th) Report on Virus Taxonomy 2016). The genome of allexiviruses is composed of a single molecule of (+)-strand ssRNA. The size of the genome depends on the virus species and varies from 8000 to 9000 nucleotides (nt) (Chen and...
Chen 2002; Adams et al. 2004). In the allexivirus genome six open reading frames (ORF) have been identified (Adams et al. 2004).

Allexiviruses have been detected in garlic plants in different parts of the world: Africa (Jemal et al. 2015), North America (Gieck et al. 2009), South America (Cafrune et al. 2006; Mituti et al. 2015), Australia (Takaichi et al. 2001; Wylie et al. 2012; Singh et al. 2014), Europe (Lanzoni et al. 2006; Khukáková et al. 2007; Chodorska et al. 2014) and New Zealand (Ward et al. 2009). These viruses infect plants of the Allium species (A. caeruleum, A. cepa, A. fistulosum, A. sativum, A. ascalonicum, A. porrum and A. vineale) (Van Dijk et al. 1991; Van Dijk and Van der Vlugt 1994; Shahraeen et al. 2008; Bereda and Paduch-Cichal 2016). The occurrence of allexiviruses in garlic crops results in the worsening of yield quality, causing decreases in bulb diameter and bulb weight. It has also been reported that the negative effect on yield quality increased when the plant was infected by other viruses (Cafrune et al. 2006; Perotto et al. 2010). It is relatively rare for garlic plants to be infected by a single allexivirus, with mixed infections involving from two to six different species being the most frequent (Fujisawa 1989; Conci et al. 1992; Van Dijk 1993; Chodorska et al. 2014). Allexiviruses are spread with propagation material and transmitted by mites during the vegetation period, especially during the storage of garlic bulbs (Van Dijk et al. 1991; Yamashita et al. 1996; Koo et al. 1998; Kang et al. 2007; Granda et al. 2017).

One of the most dangerous pests of garlic and an important vector of allexiviruses is Aceria tulipae (Keifer) (Acariformes: Eriophyoidea). This mite species is commonly detected on young garlic leaves, but it can also feed and develop under the scales and on plump cloves on which withering and matt brown spots may appear (Jeppson et al. 1975; Keifer et al. 1982). This eriophyid mite species can cause severe loss of garlic bulbs, reducing yields by up to 23%. Most damage caused by A. tulipae occurs during the storage of garlic bulbs (Larrain 1986). Aceria tulipae was detected in garlic plants in Africa (Hassan et al. 1986), North America (Flechtmann and Davis 1971), South America (Rossetto 1972; González et al. 1973; Almaguel et al. 1986), Asia (Charanasri et al. 1984; Bala et al. 2015), Europe (Liro and Roivainen 1951; Boczek and Chyczewski 1974; Knaub and Buslawa 1975; del Estal et al. 1985; Courtin et al. 2000; Sapáková et al. 2012) and New Zealand (Manson 1970). According to the existing literature, only a few researchers have attempted to describe the mechanism of virus transmission by eriophyid mites. Wheat streak mosaic virus (WSMV) is transmitted by larvae, nymphae and adults of Aceria tosichella Keifer, but not through eggs. However, the adults transmit WSMV only if they acquired it during their immature stages; they cannot acquire the virus as adults and then transmit it (Orlob 1966). Paliwal (1980) detected particles of WSMV in haemocoel and salivary glands, and suggested that WSMV may be transmitted by A. tosichella in a semi-persistent manner. Proeseler (1969, 1972) reported that Fig mosaic virus (FMV) was transmitted in a persistent manner. According to the results obtained by Kulkarni et al. (2002), Pigeon pea sterility mosaic virus (PPSMV) was transmitted in a semi-persistent mode by Aceria cajani ChannaBaswanna. Studies of Peach mosaic virus (PMV) and Ryegrass mosaic virus (RMV) and other mite-transmitted viruses also indicated a semi-persistent mode of transmission (Slykhuis and Paliwal, 1972; Gispert et al. 1998). The mechanism of acquisition and transmission of viruses belonging to the Allexivirus genus by eriophyid mites has not been described yet.

The aim of the research was to examine the transmission of GarV-B, GarV-C, GarV-D and GarV-X from garlic bulbs to leek plants by A. tulipae. Leek plants were used as test subjects for several reasons. First, leek plants are a very common host plant of A. tulipae (Kiedrowicz et al. 2017). Second, the use of seedling leek plants ensures that the material has not been infected by allexiviruses, as they are not transmitted by the seeds (Bereda and Paduch-Cichal 2016). Finally, previous studies conducted by Shahraeen et al. (2008) have confirmed the presence of GarV-D, GarV-B and GarV-C in leek plants grown in Iran.

The study on the transmission of GarV-B, GarV-C, GarV-D and GarV-X by mites was preceded by the search for garlic bulbs infected by the aforementioned viruses. To perform the study, several garlic bulbs were collected from retail stores in Warsaw, Central Poland. Following observation with a stereomicroscope, three bulbs inhabited by more than 50 specimens of A. tulipae were selected. These bulbs were tested for the presence of allexiviruses using the reverse-transcription polymerase chain reaction (RT-PCR) technique. In all of these garlic samples GarV-B, GarV-D and GarV-X were detected, and one of them was also infected with GarV-C (Table 1). No evidence of GarV-A, GarV-E, ShVX, and GarMbFV was found in any of the bulbs.
Cloves of these bulbs were separated from the bulbs and external scales were removed. Then the cloves were placed in petri dishes with an 8 cm diameter. The petri dishes were placed in three separate transparent plastic containers covered with a transparent thick-weaved material and tied with a rubber band. The containers were stored for 14 days inside a Sanyo MRL-350H climate chamber (Sanyo Electric, Moriguchi, Japan) at 22 °C with 50–60% RH, under a 4200 lx light intensity and with a 16-h photoperiod.

To establish *A. tulipae* populations on leek plants, adult forms of the mites were transferred from each of the aforementioned three populations detected on garlic bulbs onto five alleivirus-free leek plants, 20 specimens for each plant. The plants were grown in 5-cm pots in a potting soil mixture. Healthiness of these leek plants was confirmed by RT-PCR. The transfer of mites was conducted using a preparation needle with an attached eyelash according to the procedure described by de Lillo et al. (2010). There were three transmission sets (which will be referred to as T1, T2 and T3). In each set

| No. Transmission | Sample | Viruses          |
|------------------|--------|------------------|
|                  |        | Garv-B | Garv-C | Garv-D | Garv-X |
| T1               | Garlic bulb | Mπ1 |  +  | -  | +  | +  |
|                  | Leek plants | P1  |  -  | -  | -  | +  |
|                  |         | P2  |  +  | -  | +  | +  |
|                  |         | P3  |  +  | -  | +  | +  |
|                  |         | P4  |  -  | -  | +  | +  |
|                  |         | P5  |  +  | -  | +  | +  |
| T2               | Garlic bulb | Mπ2 |  +  | -  | +  | +  |
|                  | Leek plants | P6  |  +  | -  | +  | +  |
|                  |         | P7  |  +  | -  | -  | +  |
|                  |         | P8  |  +  | -  | +  | +  |
|                  |         | P9  |  +  | -  | +  | +  |
|                  |         | P10 |  +  | -  | -  | -  |
| T3               | Garlic bulb | Mπ3 |  +  | -  | +  | +  |
|                  | Leek plants | P11 |  +  | +  | +  | +  |
|                  |         | P12 |  +  | +  | +  | +  |
|                  |         | P13 |  +  | +  | +  | +  |
|                  |         | P14 |  +  | +  | +  | +  |
|                  |         | P15 |  +  | -  | +  | +  |

| Total (number of plants transmitted/inoculated) | 13/15 | 5/5 | 13/15 | 14/15 |
| % of positive results                          | 86.7  | 100 | 86.7  | 93.3  |

*a* Pn – number of leek plant samples after the feeding of mites  
*b* + – virus detected  
*c* - – no virus detected  

cells highlighted in grey – sequenced and submitted to the GenBank samples
five different leek plants were used, with the total number of plants used in the study amounting to 15. Each consecutive transmission occurring a week after the previous one. Plants were placed in isolators made from bolting cloth in 40 μm mesh size. The plants were kept in the Sanyo MRL-350H climate chamber for two weeks and watered every 24 h. Plants were grown at 22 °C temperature, 50–60% RH and at a light intensity of 4200 lx with 16-h photoperiod. After two weeks following each transmission, leek plant tissues were tested for the presence of all known allexiviruses using the RT-PCR technique.

To detect and identify the isolates of GarV-A, GarV-B, GarV-C, GarV-D, GarV-E, GarV-X, ShVX and GarMbFV in the three garlic bulbs and leek plants, RT-PCR with total RNA and appropriate primers was applied. Total RNA was extracted from garlic bulbs and leek tissues using the silica capture (SC) method described originally by Boom et al. (1990) and next adapted to the detection of plant viruses by Malinowski (1997). RNA extracts were subjected to amplification by RT-PCR using the Transcriptor One-Step RT-PCR Kit (Roche Applied Science, Germany). RNA obtained from garlic bulb BP3 was used as a positive control and nuclease-free water was used as a negative control.

Primer pairs designed by Bereda et al. (2017), amplifying the coat protein gene (CP) and the nucleic acid binding protein (NABP) of GarMbFV, GarV-A, GarV-B and GarV-D were used. Primers for ShVX were specific to the part of the CP and also designed by Bereda et al. (2017). Other primer pairs were designed by Bereda (2015). Primer pairs for GarV-X detection was specific to the 420 nt part of replicase protein gene (RdRp). Primer pairs applied for detection of GarV-E and GarV-C were designed in the part of hypothetical protein (HP) or in the part of CP and NABP, respectively.

The 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 at 94 °C, 45 s of annealing (the temperature for each virus was different according to Bereda (2015) and Bereda et al. (2017)) and 45 s elongation at 68 °C with the final extension of 7 min at 68 °C. The reaction products were resolved by electrophoresis in the TBE buffer in 1.2% agarose gel. The nucleotide sequences of GarV-B, GarV-C, GarV-D and GarV-X detected in garlic bulbs and leek plants were determined using ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence data were assembled using DNA Baser Sequence Assembler ver. 5.15 (Heracle BioSoft SRL, Romania). Sequence alignments were constructed in MEGA ver. 5 (Tamura et al. 2011). Sequence similarity and identity of the studied parts of the genome of the Polish isolates of GarV-B, GarV-C, GarV-D and GarV-X and of a reference isolate for each virus from GenBank was performed in BioEdit (Hall 1999).

The results of the research on transmission of GarV-B, GarV-C, GarV-D and GarV-X from garlic bulbs to leek plants by A. tulipae are presented in Table 1. GarV-B was detected in three of five leek plants from the first transmission set and in all leek plants from the second and third transmission sets. In total, 86.7% of leek samples tested positive for GarV-B. GarV-C was detected in all leek samples from only one transmission set. However, GarV-C was not found in garlic bulbs from which adults of A. tulipae were transferred to leek plants in the remaining two sets. GarV-D was detected in four of five leek plant samples from transmission sets one and two and in five of five leek plant samples from transmission set three. As with GarV-B, 86.7% of leek samples tested positive for GarV-D. GarV-X was detected in five of five leek plant samples in transmission sets one and three and in four of five leek samples in transmission set two. In total, 93.3% of leek plant samples tested positive for GarV-X. GarV-A, GarV-E, GarMbFV and ShVX were not detected in any of the leek plants. However, these four viruses were not present in garlic bulbs on which populations of A. tulipae were held before mites were transferred to leek plants.

Isolates of each detected virus were sequenced and the sequences were deposited in GenBank and assigned accession numbers: MN175534–MN175538 for GarV-B isolates, MN175539 and MN175540 for GarV-C isolates, MN167132–MN167137 for GarV-D isolates and MN175541–MN175546 for GarV-X isolates. The comparative molecular analyses were performed based on the sequences of the CP and NABP of the six Polish GarV-B isolates and the one Argentinian isolate retrieved from GenBank (NC_025789.1); on the sequences of the CP and NABP of the two Polish GarV-C isolates and the one Japanese isolate retrieved from GenBank (NC_003376.1); on the sequences of CP and NABP of the six Polish GarV-D isolates and the one Australian isolate retrieved from GenBank (NC_022961.1) and on the sequences of the part of replicase protein of the six Polish isolates of GarV-X and the one Korean isolate retrieved from GenBank (NC_001800.1). In all six Polish GarV-B isolates, the
length of the obtained nucleotide sequences was 1155 nucleotides (nt). Each had two coding fragments: CP (735 nt) and NABP (384 nt) with a small intron between them (17 nt). The isolates shared an nt identity of 99.8% in the CP region and 99.7–100% nt in the NABP region. The comparison of the amino acid (aa) sequences of the CP and the NABP of the Polish virus isolates indicated an identity of 100%. The Polish GarV-B isolates and the Argentinian isolate (NC_003376.1) shared a 91.7–94.8% nt and a 95–97.9% aa in the CP region identity. The nucleotide and the amino acid identity in the NABP region equaled 90.6–94% nt and 89.7–97.6% aa. The length of the amplicon sequence of two Polish GarV-C isolates was 627 nt. Both sequences included part of the CP coding sequence (426 nt), a small intron (15 nt) and part of the NABP coding sequence (186 nt). The Polish GarV-C isolates showed a 99.2% nt and a 98.5% aa identity in the part of the CP. Nucleotide and amino acid identity of the two Polish GarV-C isolates was 100% in the partial NABP sequences. The Polish GarV-C isolates and the Japanese isolate (NC_003376.1) shared a 90.6–90.8% nt and a 96.4–97.1% aa in the CP region identity. The nucleotide and the amino acid identity in the NABP region were 90.8% nt and 95.1% aa. In the six Polish GarV-D isolates, the length of the nucleotide sequences obtained was 1139 nt (with 753 nt coding CP region and 387 nt coding NABP region). The isolates shared an identity of 98.6–99% nt and 99.2–100% aa in the CP region, and 99.4–99.7% nt and 100% aa in the NABP region. The isolates from Poland and the Australian isolate (NC_022961.1) shared a 93.3–95.6% nt and a 96.4–99.2% aa sequence identity in the CP region. In the NABP region the nucleotide and the amino acid identity of the Polish GarV-D isolates and the Australian isolate equaled 93.2–96.3% nt and 92.9–95.3% aa. The length of the nucleotide sequences of the six Polish GarV-X isolates was 420 nt and the they shared a 99.5% nt and a 100% aa identity in the part of the RdRp. The Polish GarV-X isolates and the Korean isolate (NC_001800.1) shared 92.1–97.6% nt and a 96.4–97.8% aa in the part of the RdRp identity. The conducted nt- and aa-based comparative molecular analyses of the selected genome parts of GarV-B, GarV-C, GarV-D, GarV-X and four reference sequences retrieved from GenBank (one for each virus) confirmed the pathogens detected in garlic bulbs and leek plants to be GarV-B, GarV-C, GarV-D and GarV-X.

According to previous reports, the existence of numerous A. tulipae populations on garlic plants showing chlorotic streaking and leaf curling has been associated with the ability of the mites to transmit allexiviruses (Yamashita et al. 1996; Koo et al. 1998; Kang et al. 2007; Granda et al. 2017). In the case of GarMbFV, it was proven that the colonization of healthy garlic plants by A. tulipae obtained from infected bulbs resulted in the presence of the virus in the bulbs tested (Yamashita et al. 1996; Koo et al. 1998), which corresponds with the results obtained by Kang et al. (2007) for GarV-B. According to Granda et al. (2017), the incidence of numerous mite populations in garlic crops in the Alausí area (Chimborazo Province, Ecuador) may indicate that A. tulipae is a vector of ShVX. However, our studies revealed that GarV-B, GarV-C, GarV-D and GarV-X were transmitted by A. tulipae to leek plants, on which populations of the vector achieved the highest growth rate (Kiedrowicz et al. 2017). After infection, yellow-stripe mosaic patterns and distortions were observed on the leaves. These four viruses were detected in leek tissues on which populations of A. tulipae developed from adults transferred from infected garlic bulbs. These findings clearly show that the adults of the species had the ability to transmit GarV-B, GarV-C, GarV-D and GarV-X to healthy leek plants.

The confirmation of the ability of A. tulipae to transmit GarV-B, GarV-C, GarV-D and GarV-X is important for understanding the epidemiology of these pathogens. It was proven that A. tulipae has the ability to transmit GarV-B, GarV-C, GarV-D and GarV-X, which has been previously reported by Van Dijk et al. (1991), Yamashita et al. (1996), Koo et al. (1998) and Kang et al. (2007).

One of the most important properties of plant viruses is their infectiveness, i.e. their ability to infect plants and move from plants already infected to virus-free plants. The infection of new plants results in an increase in the number of diseased plants, i.e. a rise in disease incidence. It should be noted that plant diseases are noticed and gain significance when they affect a large portion of the plant population. This occurs as a result of virus transmission from one plant to another or from one crop to another. Viruses are unable to actively move to new plants, therefore many of them are transmitted by vectors (Bragard et al. 2013). Despite their slow walking, minute eriophyid mites are known to disperse for long distances on air currents (Michalska et al. 2010). Such a mode of dispersal of A. tulipae increases the risk of the transmission of GarV-B, GarV-C, GarV-D and GarV-X from garlic plants to leek plants, which may constitute a threat to the leek crops (Shahraeen et al. 2008).
The results presented by the authors of this study constitute the first report of the ability of *A. tulipae* to transmit GarV-B, GarV-C, GarV-D and GarV-X from garlic bulbs to leek plants. Demonstration that *A. tulipae* can transmit allexviruses from garlic to leeks indicates a need for additional studies designed to determine the mechanism of transmission.

Compliance with ethical standards

The research does not involve any human participants and/or animals. The materials in the article have not been published in whole or in part elsewhere and not currently being considered for publication in another journal. All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content. The authors bear all the ethical responsibilities of this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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