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Molecular Analysis of Viruses Occurring in Garlic

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1. Previous Research

Garlic (*Allium sativum* L.) is not only a significant plant due to its culinary and medicinal uses but also because of its economic importance. As a cultivated vegetable, the market demand for garlic is generally constant, although cultivation is challenged by various factors. One of the biggest problems is viral diseases, which not only reduce yield but also significantly degrade the plant's nutritional content. Garlic's vegetative propagation makes it particularly susceptible to these infections, which is why viral decline in garlic is a well-known phenomenon.

In 2020, garlic was the 21st most produced crop globally, with a total production of 28,054,318 tons. The world's leading garlic producer in 2020 was China, which produced 20,712,087 tons on 825,302 hectares - this accounted for 73.8% of the world's total garlic production and 50.5% of the global garlic cultivation area. Spain was Europe's and the European Union's largest garlic producer in 2020, with approximately 269,090 tons produced. Hungary was the 56th largest garlic producer in the world in 2020, with 5,210 tons produced, according to FAOSTAT data, achieving this result with a yield of 6.29 tons per hectare. The Central Statistical Office (KSH) estimates the same year's production at 5,216 tons (KSH, 2020). This result is far below the global average of ~17.19 tons per hectare (FAOSTAT, 2020) and below the 12-16 tons per hectare that is realistically achievable in Hungary based on literature data (SZALAY, 2004).

Currently, it is known that onion-related species are infected by 36 species across 10 virus families. Of these, 24 species, to varying degrees, have been proven to infect garlic, with 13 species classified as dangerous in the literature. However, the presence of most of these species has not yet been documented in Hungary.

The relationship between the success of Hungarian onion cultivation and the threat posed by viruses infecting onions was first highlighted by János Szirmai in 1958 (SZIRMAI, 1958). The virus he identified, which causes onion yellow streaking, onion mosaic (*Marmor cepae* H.), and yellow dwarfing (*Yellow dwarf*), is known today as *Onion yellow dwarf virus* (OYDV), first described in 1929 in the U.S. state of Iowa (MELHUS et al., 1929). It is worth noting that among the symptoms caused by the disease, Szirmai mentioned the higher water content of infected onions, citing Bremer's 1937 article, which is now recognized as the first description of the *Leek yellow stripe virus* (LYSV) (BREMER, 1937). Szirmai already warned of the dangers of viral decline affecting the Makó region at that time, urging selective breeding for resistance in Makó onions. During their previous surveys in 1956, they examined seed-producing onion fields where they also found strikingly healthy specimens. These specimens

were characterized by a more robust, upright growth habit and thicker, "bluish-waxy" leaves. Szirmai considered the seemingly asymptomatic plants in the infected fields as suitable starting material for resistance breeding, although he did not clarify whether these were resistant, tolerant, or merely latent infected plants (SZIRMAI, 1958).

In 1987, in the book "Cultivation of Alliums" which also included practical advice, István Tóbiás, like Szirmai, primarily emphasized the importance of controlling vectors and using healthy propagation material (TÓBIÁS, 1987). He mentioned the LYSV virus as well, although its first proven description in Hungary has not yet been made.

In 1991, Antal Regős briefly reported on the status of garlic virus research in the journal "Magyar Mezőgazdaság." He touched on the significance of complex infections, yield losses caused by viruses, and the importance of virus elimination (REGŐS, 1991). He published his research findings in detail in 1992 in the journal "Kertgazdaság." Through ELISA tests, he was the first in Hungary to detect OYDV using serological methods and provided the first domestic description of the *Shallot latent virus* (SLV) and *Garlic latent virus* (GLV) (REGŐS, 1992).

Later, in an article published in the journal "Növényvédelem," detailing garlic protection, the authors continued to highlight the above-mentioned three pathogens as proven and harmful viruses in Hungary (BUDAI et al., 1999). The same issue also reported on newly identified garlic viruses spread by mite vectors in the Far East. Citing Korean research results, these viruses were then classified under the *Rymovirus* genus, including the *Garlic mite-borne mosaic virus* (GarMbMV), which is now identified as *Garlic virus* C (GarV-C), belonging to the *Allexivirus* genus (KOO, 1999; NCBI, 2019a).

In the 2000s, garlic virus research in Hungary declined, although the economic and technological challenges of cultivation remained in focus. Authors of that time primarily dealt with issues related to cultivation technology and variety selection (LACZKÓ, 2003a; LACZKÓ, 2003b; SOMOGYI, 2006).

In 2010, research on garlic viral diseases was revived at the University of Debrecen. An MSc thesis was developed from this research, focusing on samples of onion and garlic collected from the Makó region. The aim of the research was to detect the presence of *Iris yellow spot virus* (IYSV*), Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV), and *Garlic common latent virus* (GCLV) using the DAS-ELISA method. The results confirmed the presence of only GCLV (SZARVAS, 2010).

In 2011, a doctoral dissertation related to garlic cultivation was completed at the University of West Hungary, which briefly addressed the cultivation challenges posed by viral diseases, although it did not present any new virological findings (GOMBKÖTŐ, 2011).

In 2015, research on garlic viral infections began at the Department of Plant Pathology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences. This research, for the first time in Hungary, used nucleic acid-based molecular diagnostic methods to study the virus species from the three most significant genera (*Potyvirus, Carlavirus, Allexivirus*) mentioned in international literature as dangerous to garlic.

2. Objectives

In the course of our work, we have outlined the following objectives:

- Identification of virus species infecting garlic in Hungary.
- Genus- and species-specific identification of virus species belonging to the *Allexivirus* genus, which infect garlic, using both custom-designed and literature-based primers.
- Identification of the most important virus species belonging to the *Carlavirus* genus, which infect garlic, using both literature-based primers and primers designed by us.
- Identification of virus species belonging to the *Potyvirus* genus, which infect garlic, using both literature-based primers and primers designed by us.
- Determination of the sequence data of the LYSV isolate identified by us in Hungary, using a combination of our own primers, literature-based, and literature-derived but modified primer sets.
- Examination of the occurrence of complex infections and evaluation of their specific characteristics.
- Testing and evaluation of the transmission properties of the LYSV isolate identified by us in Hungary.
- Examination of the nucleotide sequence data of the isolates studied and determination of their phylogenetic relationships.

3. Materials and Methods

Origin of the Samples Used

The collected samples originated from China, Romania, Hungary, Austria, the Czech Republic, France, Portugal, the Netherlands, and Spain. In Hungary, our samples were obtained from various locations, including Hajdúnánás, Pusztaottlaka, Makó, Ferencszállás, Baja, Kalocsa, Tata, Jászjákóhalma, Nagykőrös, Szabadszállás, Nyársapát, and Csór. Our research incorporated both certified seed cloves, according to Hungarian regulations, as well as samples from home gardens and markets.

HaPoty, HaCarla, and HaAllexi Primer Sets and Their Design Considerations

For the species-specific identification of *Potyvirus* and *Carlavirus* genera, we designed sense primers (HaPoty, HaCarla) that target conserved genomic regions within the genus but are variable among species. These primers, along with the M4 antisense primer, produced PCR products of varying lengths, enabling the simultaneous detection of different species within a single PCR reaction. Due to the high number of species within the *Allexivirus* genus, only genus-specific detection was possible using the HaAllexi sense and M4 antisense primer pair. In addition to our designed primers, we also used M4-M4T primers published by Chinese researchers (CHEN et al., 2001b), which served as part of the universal Potyviridae primer set during cDNA synthesis. Alongside our designed sense primers, we utilized the M4-M4T primers published by Chinese researchers (CHEN et al., 2001b). The universal *Potyviridae* primer set's M4T antisense primer was used during cDNA synthesis, with its poly-T region hybridizing with the polyadenylated tail characteristic of the *Potyviridae* family, while the M4 primer served as the antisense primer during PCR.

Optimization of Gupta and Yoshida Primers

To obtain more detailed sequence data of the LYSV isolate detected in sample 23A, we searched for literature primers, but found that the primer set described in the literature was only marginally applicable to the full LYSV genomes available in the NCBI database. To increase primer binding specificity, we optimized the primers. Among the downloaded full LYSV genome sequences, the KP258216 reference isolate showed the highest genetic similarity to the LYSV isolate from sample 23A in the CP-coding region. We modified the original Gupta and Yoshida primers, designed for full LYSV genome detection, to better match the KP258216 isolate, replacing certain bases and designing degenerate primers where necessary.

Species-Specific Allexivirus Primers

For species-specific identification of allexiviruses, we used both literature-derived primers and primers designed by us. However, we found that during BLAST analysis, primers from the literature did not always target the intended organism. In many cases, these primers did not even align with the reference isolates published using the same primers, nor did they match the full genomes of other sequences within the same species. Therefore, when selecting literature-based, species-specific allexivirus primers, we chose those that returned the target organism during BLAST analysis and aligned with as many reference isolates as possible from the NCBI database. Ideally, the target species had already been described in a European country, and we had already detected it through sequence analysis, allowing us to use it as a positive control to confirm primer functionality.

In addition to using primers from the literature, we also designed our own primers, heavily relying on the reference isolates available in the NCBI database.

RT-PCR Protocol

We used traditional RT-PCR for the HaPoty, HaCarla, HaAllexi, and M4 primer sets, as well as for the species-specific allexivirus primers. For the Gupta and Yoshida primers, along with our own LYSV primers, we employed both traditional PCR and touchdown-PCR (td-PCR) techniques. The goal of td-PCR is to increase PCR reaction specificity by starting with a higher annealing temperature, which provides greater specificity. Through the applied td-PCR protocols, we aimed to maximize the acquisition of sequence data for the 23A LYSV isolate while working with three different protocols to improve specificity.

Artificial Infection with LYSV Isolate

For artificial infection with LYSV, we used leek (*Allium porrum* 'Lincoln') and garlic (*Allium sativum* 'Makói Tavaszi'). We first verified the plants' virus-free status with RT-PCR, then performed the infection using leaf tissue infected with LYSV from the 23A sample. The infection was facilitated by applying celite as an abrasive to the leaf surface along with the homogenized sample and inoculation buffer. To confirm successful infection, we collected samples again 14 days later and checked for the presence of the virus using the HaPoty and M4 primer pairs.

Mixed *Carlavirus* Infection Provocation Experiment

In the provocation experiment aimed at carlaviruses, our goal was to create a mixed infection, as we had not previously found samples infected with both GCLV and SLV carlaviruses. For the experiment, we propagated 40 garlic plants and selected virus-free samples based on preliminary tests. The infection was carried out using inoculums prepared from two samples already proven to be infected by GCLV and SLV. These samples were homogenized together in a single mortar to ensure the simultaneous presence of both viruses and achieve mixed infection. The inoculation was performed on two different leaves of each plant, and the success of the infection was checked using the HaCarla and M4 primer pairs.

Sequence Analysis, BLAST Analysis, and Phylogenetic Trees

The accuracy of sequence analysis results was verified using the Chromas Lite program, based on chromatograms provided by BaseClear. If the chromatogram was unclear, ambiguous sites in the sequence analysis were left unchanged. The corrected sequence data were then compared with the nucleotide sequences of isolates in the international database using the NCBI BLAST analysis service. Based on the resulting phylogenetic relationships, we selected reference isolates necessary for drawing the phylogenetic tree, which was created using the CLC Sequence Viewer 7.0 bioinformatics software, applying the UPGMA method, Jukes-Cantor technique, and a 1000-repetition bootstrap analysis.

4. Results

During my research, I examined 45 garlic samples from various locations for viral infections using RT-PCR to detect potyvirus, carlavirus, and allexivirus infections. For the samples deemed to be virus-infected, I performed sequence analysis 31 times, from which phylogenetic trees were also constructed.

The Hungarian 23A LYSV isolate was analyzed in greater detail to broaden the understanding of its genome. For this, I used a set of primers borrowed from the literature and optimized by me, as well as a set of primers I designed to study the entire genome. I employed several conventional PCR and td-PCR protocols for this purpose.

In the artificial infection experiment conducted with the Hungarian LYSV isolate, I also performed virological analysis on 8 garlic and 8 leek samples using the RT-PCR method. In the provocation experiment aimed at creating mixed infections between different carlavirus species, I examined an additional 20 garlic samples using the RT-PCR method.

A total of 81 different plant samples were virologically examined in this study. The LYSV, GCLV, SLV, GarV-B, GarV-C, GarV-D, and GarV-X virus species were detected using RT-PCR, and these results were further confirmed by sequence analysis.

During the research, 52 primers were used, of which 14 sense and 11 antisense primers were developed by me, 2 sense and 3 antisense primers were from the literature, and an additional 10 primer pairs were literature-derived but optimized by us.

Results with HaPoty, HaCarla, and HaAllexi Primer Sets

Based on the gel images obtained using the HaPoty and M4 primer pairs, it can be concluded that all known potyvirus species infecting garlic were successfully detected in the samples tested, with the presence of the LYSV species further confirmed by sequence data. Using the self-designed HaPoty sense primer in the presence of the M4 antisense primer, the species could be clearly distinguished based on the length of the PCR product.

Using the HaCarla and M4 primer pairs, both garlic-infecting species of the *Carlavirus* genus were detected using RT-PCR. The HaCarla sense primer we designed, in the presence of the M4 antisense primer, proved suitable for the simultaneous detection and differentiation of the two carlavirus species based on the different lengths of the PCR products. In Hungary, the GCLV species is more prevalent, with the SLV species detected only once.

Using the HaAllexi and M4 primer pairs, the presence of the *Allexivirus* genus was detected in 93% of the 41 samples tested, with only three negative samples identified. The

HaAllexi sense primer developed by us, in the presence of the literature-derived M4 antisense primer, did not allow species-level determination due to the high number of species within the genus, so the species were identified by sequence analysis.

Results with Modified Gupta and Yoshida Primer Sets

The Gupta and Yoshida primers were used in a total of five PCR protocols. In conventional PCR, an annealing temperature of 50 °C was applied for some primers, while 55 °C was used for others. Td-PCR was performed for each primer pair according to three different protocols using different annealing temperature intervals. PCR products that fell within the pre-determined size range of + / - 200 bp were considered of appropriate length. This was because the primers were designed based on the available sequence data of complete LYSV genomes; however, comprehensive preliminary sequence data were not available for the 23A LYSV isolate we examined. To filter out experimental errors caused by possible deletions or insertions in the genome of the LYSV isolate we studied, compared to the literature data, the theoretically established appropriate bp length range was handled with some margin in practice.

Results with Self-Developed LYSV Primers

In experiments with the self-developed LYSV primers, using PCR and td-PCR protocols with different annealing temperatures, we successfully detected the sequences encoding the HC-Pro-P3, CI, and CP proteins using the LYSV2 FOR/REV, LYSV4 FOR/REV, and LYSV8 FOR/REV primer pairs. With these primer pairs, we got back the PCR products with optimal amplicon size using both conventional and td-PCR techniques, showing the highest genetic similarity to the KP258216 and HQ918255 LYSV reference isolates. However, for some PCR products that fell within the appropriate length range, we did not obtain interpretable sequence data during BLAST analysis, indicating that although more appropriately sized DNA fragments were obtained with td-PCR, the number of nonspecific products was higher compared to conventional PCR.

Results with Species-Specific *Allexivirus* Primers

During the detection of different allexivirus species, several samples were tested using various primer pairs. In the case of GarV-X infection detection, neither the self-developed nor the literature-based primers produced appropriately sized PCR products, suggesting that the primers used were not optimal. The species was detected using the HaAllexi and M4 primer pair through sequence analysis. GarV-C infection was detected using the Bereda primer pair,

which was also confirmed by sequence analysis. GarV-B infection was similarly detected using the species-specific primer pair designed by us, and this was also confirmed by sequence analysis. Using the self-developed GarV-D primer pair, we also obtained appropriately sized PCR products in several cases.

Results of Artificial Infection with LYSV Isolate

In the artificial infection experiments investigating the host preference of the LYSV isolate, the 23A LYSV isolate showed significant infectivity in the 'Makói Tavaszi' garlic, successfully infecting 87.5% of the plants, as confirmed by the 1000-1200 bp PCR products. In contrast, in the 'Lincoln' leek, the isolate was only able to infect to a limited extent, with infection observed in only 25% of the plants, and lower virus concentrations were detected here. The results suggest that the LYSV isolate has a stronger host preference for garlic and replicates less effectively in leek.

Results of Provocation Infection Experiment with *Carlavirus* Isolate

In the provocation experiment aimed at creating a mixed carlavirus infection, a total of 11 infections developed in the 'Makói Tavaszi' garlic samples: 5 cases of GCLV and 6 cases of SLV infection. The GCLV-infected samples produced PCR products of approximately 800 bp, while the SLV-infected samples produced PCR products of approximately 700 bp. However, the experiment failed to create a mixed infection, despite inoculating the samples with plant tissues containing GCLV and SLV. Thus, the experiment showed that the two carlavirus species successfully infected separately, but a mixed infection did not develop.

5. Conclusions

Using the HaPoty sense primer we designed, we successfully detected the LYSV potyvirus species that infects garlic. The viral infections found in Chinese and Spanish isolates paint a concerning picture of the introduction of virus species, as these two countries are not only among the largest garlic producers globally, but China was the world's largest and Spain the second-largest garlic exporter in 2020 (FAOSTAT, 2020).

It is worth mentioning that the HaPoty sense primer also has potential uses as a potentially universal potyvirus primer. This primer sequence has proven to be effective in detecting the Bean yellow mosaic virus in another study conducted by the Department of Plant Pathology when used together with the M4 antisense primer.

Our optimization process with the Gupta and Yoshida primer sets has yielded mixed results. On the one hand, we achieved results with three of the nine primer pairs, with two of the Gupta primer pairs undergoing actual optimization. In this sense, the effectiveness of the optimization can be considered low, as we obtained positive results in only two cases.

On the other hand, the sequence data obtained using the Gupta and Yoshida primers and our own primer pairs indicate that the fundamental idea of optimizing primers based on the closest relationship of the full genome according to the coat protein region was a valid approach. In all nine phylogenetic trees we created, we observed that the gene segment in question always showed the closest relationship with the KP258216 reference isolate. This may suggest that our prognosis of relatedness based on the coat protein region was confirmed by the phylogenetic analysis of other gene segments, though this requires further research and a larger sample size for validation.

When mapping the genome of an isolate with an unknown nucleotide sequence, primer optimization may be a necessary step, as the efficiency of literature-derived primers cannot always be confirmed in silico based on available reference isolates. However, the optimization method still needs improvement to increase its effectiveness.

The necessity of optimization, particularly when using literature-derived primers, is further highlighted by the fact that we achieved significantly better results with our own LYSV primers, created entirely based on reference isolates available in the NCBI database, compared to their literature-derived counterparts, which showed less homology with the reference isolates.

The investigation of LYSV host preference yielded an interesting result: the domestic isolate we identified appeared to be either unable or only minimally capable of infecting its

namesake host species. While the literature mentions a garlic-infecting strain of LYSV, LYSV-G (LOT et al., 1998), the NCBI database does not contain reference isolates under this specific name, so mapping the relationship between our isolate and various LYSV strains is not currently possible.

Our results with carlaviruses revealed the absence of samples co-infected with both species of the genus. Before arriving at this conclusion, we verified the reliability of the HaCarla primer and its ability to differentiate between GCLV and SLV viruses through sequence analysis and phylogenetic tree construction. We then conducted a provocation infection test with inocula containing both virus species, during which we were unable to create a mixed infection in a single plant under artificial conditions. The literature contains results where the two viruses were not present simultaneously (DOVAS and VOVLAS, 2003), but there are also findings where both species were detected in the same plant material (PAUZI et al., 2018). Further investigation may be necessary, as the literature suggests that different species within the genus should not cause cross-protection against each other in a host plant (KING et al., 2011).

The absence of mixed infections is further complicated by the fact that samples infected with individual species exhibit certain geographical patterns; we detected only the GCLV species in samples from France, only the SLV species in samples from China and Romania, and neither species in samples from Spain. Within Hungary, regional differences are also apparent; samples from Makó were infected with GCLV, those from Hajdúnánás with SLV, while samples from Pusztaottlaka contained neither species. Although our sample size was too small to determine the statistical significance of the correlation between geographical location and the infectious species, this phenomenon warrants further attention in future research.

Following the BLAST analysis of sequence data from the SLV isolates we studied, we found that these isolates showed the greatest genetic similarity to the GLV species. Due to the confusion in nomenclature and the renaming of virus species, it is unclear whether the virus isolates referred to as GLV are more closely related to the GCLV or SLV samples. During taxonomic reclassifications, GLV was identified as a strain of SLV that infects garlic (SLV-G), raising the question of whether the isolates referred to as GLV actually represent a distinct lineage within the SLV species, and whether the isolates we detected could indeed belong to the SLV-G strain.

Our phylogenetic tree analysis revealed that the GCLV and SLV isolates are sharply separated from each other, but the GLV and SLV isolates are not. This suggests that although we can distinguish between GCLV and SLV species using the HaCarla sense primer we

developed, the phylogenetic tree analysis does not confirm the BLAST analysis finding that the isolates we detected belong to the SLV-G lineage.

The functionality of our HaAllexi sense primer in the presence of the M4 antisense primer has been confirmed multiple times through sequence analysis. However, due to the high number of species within the *Allexivirus* genus, species-specific identification is not possible with this primer pair. We also confirmed the functionality of our literature-derived and species-specific primers, but we were unable to detect GarV-X infection using either our own or the literature-derived primer pair, despite the fact that the 49A sample used in the experiment had previously been confirmed to be infected with GarV-X. The reason for this is unknown.

The names of the *Garlic virus* B, C, D, and X isolates we detected may be subject to taxonomic changes in the future. Some research suggests that GarV-B and GarV-X may represent two different lineages of the same virus species, just as the GarV-A, GarV-E, and GarV-D species could potentially be combined (CELLI et al., 2018).

In addition to the potential decrease in the number of species, there is also the possibility of an increase in species numbers due to reclassification. One of the *Garlic virus* B isolates we detected showed the greatest genetic similarity to a hypothetical *Garlic virus* G (NCBI, 2019c) isolate in BLAST analysis. If the ICTV officially recognizes the existence of *Garlic virus* G, the status of the GarV-B isolate we detected from the 23A sample may be revised as the first Hungarian description of GarV-G.

Another point of interest regarding the species-specific detection of allexiviruses is that although the 43A and 43B samples represent virus isolates from two different cloves of the same garlic bulb, the GarV-D isolates infecting them showed high genetic similarity with different reference isolates in BLAST analysis, and they did not cluster together on the phylogenetic tree. This raises the possibility that different strains of the same virus species could simultaneously infect a single garlic plant. This suggests a new axis of infection events in garlic that has not yet been studied: the possibility of complex infection or recombination between strains within the same species, in addition to complex infections between genera and species. This could open new research avenues in studying synergistic and antagonistic interactions between genera and species, as well as the crop losses caused by these interactions.

It may be worthwhile to extend the investigations toward the GarV-A, GarV-E, and ShVX species in the future, as these species have already been described in Poland (CHODORSKA et al., 2014).

In our studies, we did not identify any virus-free garlic plants, meaning that all the samples we examined were infected with at least one species from the three genera. In Hungary,

certified garlic seed cloves are typically screened for the OYDV potyvirus species (FVM 50/2004. (IV. 22.)), but the certified seed cloves we examined, produced in Hungary, were almost always infected with carlavirus and allexivirus species. In cultivation practice, this means that in the event of potyvirus infection, which is likely to occur in the field (CONCI et al., 2003), the virus does not appear as a single infection but immediately manifests as a virus complex with the potential for significant economic damage (LOT et al., 1998). If the grower does not purchase seed cloves but plants them back from the previous year's harvest, the likelihood of developing a virus complex and the potential yield loss further increase (CONCI et al., 2003). Due to vegetative propagation, not only certified seed cloves but also garlic sold for consumption can serve as potential home garden propagation material, which, in the long term, facilitates the propagation of viruses. In increasingly globalized markets, garlic imports could be another potential source of infection.

The high infection rate of the Makó samples is striking. This not only threatens garlic cultivation but the entire onion production sector, as some of these virus species can also infect other members of the *Allium* genus. As a result, in *Allium* species such as onions or leeks, which can reproduce generatively, vegetatively propagated garlic could act as a reservoir plant. In the future, we recommend not only testing garlic cultivation and propagation material production for OYDV but also supplementing this with comprehensive virological testing. It may also be worth investigating the reservoir role of the surrounding weed flora, as well as the vector role of cultivation practices in addition to animal vectors.

6. New Scientific Results

- We developed new, effective primers for detecting virus genera and species within the *Potyvirus*, *Carlavirus*, and *Allexivirus* genera that infect garlic.
- We achieved the first domestic detection of *Leek yellow stripe virus*, *Garlic virus B*, *Garlic virus C*, *Garlic virus D*, and *Garlic virus X*.
- We uncovered the phylogenetic relationships of the isolates based on sequence data.
- We characterized the host-specific and virus transmission properties of the domestic LYSV isolate.
- We characterized the complex infection properties of carlaviruses that infect garlic.

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