biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2023) 67: 67-74 DOI: 10.32615/bp.2023.006

Special Issue "Plant-Microbe Interactions"

Cucumber mosaic virus and turnip mosaic virus occurrence in garlic mustard in Ukraine

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Abstract

Garlic mustard (*Alliaria petiolata*) is an herbaceous biennial plant native to Europe. In Ukraine, in addition to becoming a serious invader, garlic mustard can serve as a host to several viruses, which may affect agricultural crops. In view of this, the purpose of the study was to identify the virome of garlic mustard growing in Ukraine. Plant samples collected in Kyiv regions were tested for the presence of cucumber mosaic virus (CMV), turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV), watermelon mosaic virus II (WMV), and turnip crinkle virus (TCV) by serological and/or molecular methods. According to the results found in the present study, symptomatic *A. petiolata* obtained in 2021 were infected with CMV (60%), TuMV (20%), or co-infected with CMV + TuMV (20%). TYMV, WMV II, and TCV were not detected in any of the collected samples. The cDNA fragments encoded the coat protein (CP) gene of CMV and TuMV were sequenced and named as CMV-Ap and TuMV-Ap, respectively. In phylogenetic analysis, the CMV-Ap closely resembled the German isolate MW582807 (*Sarracenia* sp.), with 99.8% nucleotide identity and belongs to subgroup II of CMV. In the phylogenetic tree, TuMV-Ap clustered with isolates AP017803, AP017764, AP017791, and JQ073722, and represented the highest identity (98.6%) to Iranian isolate IRNTRa9 (AP017803) from *Rapistrum rugosum* and Turkish isolate TUR49 (AP017872) from *Raphanus raphanistrum*. The sequences of CMV-Ap and TuMV-Ap were deposited in the GenBank under Accession Numbers MZ540213 and OM799323, respectively. The results obtained in the study indicate the important role of infected garlic mustard as alternative host and natural reservoir of CMV and TuMV from which these economically important viruses can spread to other wild and cultivated plants. This is the first molecular evidence of TuMV infection in *A. petiolata* from Ukraine.

Keywords: *Alliaria petiolata*, mixed infection, plant virus reservoirs.

Introduction

Alliaria petiolata (*Alliaria petiolata*, (M. Bieb.) Cavara & Grande, named also *Alliaria officinalis* Andrz. *ex*. Bieb.), known as "Alliaria" or garlic mustard is a biennial herbaceous plant belonging to the mustard family (*Brassicaceae*). The genus *Alliaria*, in addition to garlic mustard includes two other species – *Alliaria auriculata* Kom. and *Alliaria brachycarpa* M. Bieb. (http://www.theplantlist.org/tpl1.1/search?q=Alliaria). *A. petiolata* is

Received 4 September 2022, *last revision* 2 March 2023, *accepted* 6 March 2023.
Abbreviations: ACP-ELISA - antigen coated plate ELISA; CMV - cucumber mosaic virus; CP - coat protein; CMV-Ap - CMV isolate from *Alliaria petiolata*; DAS-ELISA - double-antibody sandwich ELISA; ELISA - enzyme-linked immunosorbent assays; NJ - neighbor-joining; RT-PCR - reverse transcription polymerase chain reaction; TCV - turnip crinkle virus; TuMV - turnip mosaic virus; TuMV-Ap - TuMV isolate from *Alliaria petiolata*; TYMV - turnip yellow mosaic virus; WMV - watermelon mosaic virus.
Acknowledgements: This work was carried out with funds supplied by the National Academy of Sciences of Ukraine (governmental registration number of work is 0120U000221) and was partly funded by the Ministry of Education and Science of Ukraine (project 0121U109863). The authors are very grateful to Dr. M. Kharchuk (Institute of Microbiology and Virology of NASU, Ukraine) for help in conducting electron microscopy studies.
Conflict of interest: The authors declare no conflict of interest.

the only species of the genus *Alliaria* widespread in Ukraine. Plants grow throughout the country in forest habitats, forest edges, along roadsides and disturbed lands as well as in parks, orchards, and in field margins, often surrounding croplands (Vasilchenko and Pidotti 1975, Dobrochaeva *et al*. 1987).

Garlic mustard has a three-stage life cycle: seeds, rosettes, and adults. Seedlings of the plant appear in early spring and develop into rosettes during the first growing season, typically by mid-summer. Rosettes overwinter and then become adult plants in the spring. Adult plants flower, set seed, and subsequently die in midsummer. Once mature plants have produced fruit they senesce and die (Pardini *et al.* 2009). A single plant produces hundreds of seeds, most of which fall nearby but may be carried further by wind, water, wildlife, and people. Despite the fact that garlic mustard contains ascorbic acid and carotene, flavonoids, nitrogen-containing compounds, higher fatty acids, and oil (about 30% in seeds), plants has been little used in herbal medicine (Stuart 1990, Kornievsky *et al*. 2009). In some countries (USA, North America, and Canada) *A. petiolata* is considered as one of the most notorious invasive plants that actively displace local plants and, forming dense monocultures, reduce the biodiversity and aesthetic value of natural areas. The impact of plants on ecosystems is long-lasting, as plants produce secondary metabolites that can exert negative effects on native plants, insects, and microbes causing changes in species composition and ecosystem processes (Wolfe *et al*. 2008). In addition, the environmental impact of the species is determined of its ability to serve as a host plant to several viruses – cucumber mosaic virus (CMV, *Bromoviridae*; *Cucumovirus*) (Brčák and Polák 1963, Boehm and Nameth 2000, Jerković-Mujkić *et al*. 2017), turnip mosaic virus (TuMV, *Potyviridae*; *Potyvirus*) (Horvath *et al*. 1975, Lisa and Lovisolo 1976, Stobbs and Van Schagen 1987, Lockhart 2012), white clover mosaic virus (WClMV, *Alphaflexiviridae*; *Potexvirus*) (Polak 1985, Zhao *et al*. 2016), turnip vein-clearing virus (TVCV, *Virgaviridae*; *Tobamovirus*) (Lockhart *et al*. 2014, Tóth *et al*. 2019), turnip crinkle virus (TCV, *Tombusviridae*; *Betacarmovirus*) (Gaafar *et al*. 2019), and turnip yellow mosaic virus (TYMV, *Tymoviridae*; *Tymovirus*) (Pelikánová 1990, Pelikánová *et al*. 1990), which may affect horticultural plants and a wide range of agricultural crops.

Since the role of *A. petiolata* as virus reservoir in Ukraine has not yet been studied, the purpose of this work was to identify the virome of garlic mustard growing in Ukraine. Turnip mosaic virus, white clover mosaic virus, and turnip vein-clearing virus are currently known to be found in garlic mustard in the USA, while cucumber mosaic virus, turnip crinkle virus, and turnip yellow mosaic virus have been found in Europe. In the present study, we have focused our attention on two cosmopolitan and important viruses infecting field-grown vegetable crops and weeds – cucumber mosaic virus and turnip mosaic virus. In addition, the plants have also been tested for TCV and TYMV, common on garlic mustard in Europe. Although watermelon mosaic virus II (WMV II, *Potyviridae*; *Potyvirus*) has not been reported to infect garlic mustard, samples were also tested for this virus, given that WMV II has a wide range of host plants, including more than 170 plant species from 27 different families (Hajizadeh and Mohammadi 2016), and is widely distributed in various crops growing in Ukraine (Shevchenko *et al.* 2021).

Materials and methods

Sample source, data collection: During a survey in May and June of 2020 - 2021 growing seasons, symptomatic plants of the weed *A. petiolata* showing vein banding, mosaic, and leaf deformation were sampled from ruderal sites of five Kyiv regions for subsequent virus screening (Fig. 1). In 2020, we sampled only one location (number 1 on the sampling map), while in 2021 we repeated a targeted sampling of plants from this location and also collected 5 samples (4 from plants showing virus-like symptoms and 1 from healthy, asymptomatic plants) from each of the additional 4 locations. Thus, in site 1, we sampled the same plants of the first and second years of vegetation (5 in 2020, and 5 in 2021). A total of 30 samples were collected in 2020 - 2021 from symptomatic and healthy plants. Sap from infected and healthy plants was extracted in 0.01 M potassium phosphate buffer (pH 7.0) and used for infectivity assays and electron microscopy.

Transmission electron microscopy (TEM): Droplet of sap from infected *A. petiolata* was mounted on formvar coated electron microscope grid (200 mesh), stabilized with evaporated carbon film for 30 s. After washing, the grids were negatively stained with 1% (m/v) uranyl

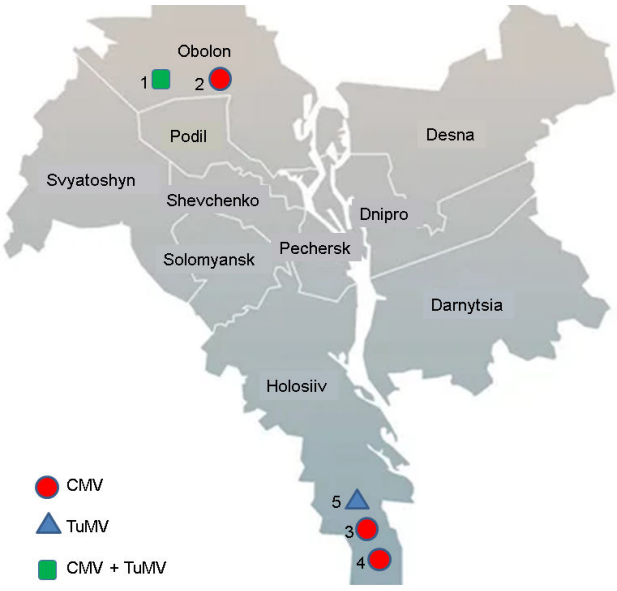

Fig. 1. Sampling locations in Kyiv where symptomatic plants were collected and confirmed positive for CMV (*red dots*), TuMV (*blue triangle*), or mixed infection of CMV+TuMV (*green square*).

acetate and examined with an electron microscope (*JEM 1400*, *JEOL*, Tokyo, Japan) (Milne 1993). Transmission electron microscopy studies were conducted at the Center for Collective Use of Scientific Equipment of National Academy of Sciences of Ukraine.

Experimental host range assays: For host-range studies, infected leaf extract was inoculated mechanically to different plant species, namely *Cucumis sativus*, *Cucurbita pepo*, *Capsicum anuum*, *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *botrytis* (cauliflower cv. Early Harvest), *B. napus* cv. Redstone, *Nicotiana tabacum* cv. Samsun NN, *Nicotiana benthamiana*, *Nicotiana rustica*, *Phaseolus vulgaris* cv. Top Crop, *Chenopodium amaranticolor*, and *Chenopodium quinoa*. Eight plants from each plant species were inoculated and kept under greenhouse conditions (at 22°C; natural daylight). Plants were regularly inspected for symptom development for up to 30 d post inoculation.

Enzyme-linked immunosorbent assays (ELISA): The preliminary screening was performed by antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA), using wide-spectrum monoclonal antibodies as described by Liu *et al*. (2017). ACP-ELISA kits (*RT-0573/1*, *DSMZ*, Braunschweig, Germany) were used for the generic serological detection of potyviruses (73 species of the genus *Potyvirus*) in infected plant tissues. Leaf samples were also tested by double-antibody sandwich ELISA (DAS-ELISA) for CMV, TYMV, TuMV, and WMV II infection using the phytodiagnostic ELISA kits (*Loewe Biochemica*, Sauerlach, Germany). ACP- and DAS-ELISA were performed according to the manufacturers' guidelines as described by Clark and Adams (1977), Ward *et al.* (2004), and Liu *et al*. (2017). Absorbance at 405 nm were measured using a *Termo Labsystems Opsis MR* microtitre plate reader (*Thermo Fisher Scientific*, Waltham, MA, USA) with *Dynex Revelation Quicklink* software (*DYNEX*, Zelienople, USA). Absorbance values, greater than three times those of the negative controls were considered positive. The samples scored as positive by DAS-ELISA used for the subsequent analysis by reverse transcription polymerase chain reaction (RT-PCR).

RT-PCR and sequencing: Nucleic acid extracts were tested by RT-PCR for the presence of virus RNA using the three sets of universal primers for detection of CMV (CMV-F/R5'-TATGATAAGAAGCTTGTTTCGCGCA-3'; 5'-TTTTAGCCGTAAGCTGGATGGACAACCC-3') (Bariana *et al*. 1994), TCV (HZ632/HZ633 5'-AAAGGCAAAAFCTGGGTGGGA-3';5'-TAAAGTTT GCGGCTAGGGG-3') (Gaafar *et al*. 2019), and TuMV (CP-F/R5'-TATACACGCCGGAGCAGACG-3'; 5'-CGCAGTGCTGCTGCTTTCAT-3') (Lockhart 2012). Total RNA was extracted from 0.5 g of symptomatic or healthy leaf tissues using *AmpliSens Ribo-Sorb DNA/RNA extraction kit* (*AmpliSens*, Moscow, Russia). The RT was performed with *AmpliSens Reverta-L-100* (*AmpliSens*), according to the manufacturer's instruction. For PCR, 10 - 50 ng of the template genomic DNA was amplified in total volume of 20 mm^3 containing 1× reaction buffer with 1.5 mM $MgCl_2$, 5 pmol of each primer, 0.2 mM dNTPs, 0.5 U Taq polymerase, and nuclease-free water. Reactions were performed under the following conditions: initial denaturation at 95°C for 3 min, thermal cycling for 35 cycles (1 min at 94°C, 1 min at temperature optimal for each primer pairs, and 1 min 30 s at 72°C), ending with the final extension at 72°C for 5 min. PCR products of amplification were visualized in 1.7% (m/v) agarose gels with Tris-borate-EDTA (TBE) buffer and ethidium bromide (0.5 mg cm^{-3}). The resulting PCR products of CMV and TuMV (500 bp and 463 bp fragments, respectively) were sequenced by *Sanger dideoxy* sequencing method using the *BigDye Terminator Cycle Sequencing* kit (*Applied Biosystems*, Waltham, Massachusetts, USA). The samples were run on the *ABI Prism 3130 Genetic Analyzer* (*Applied Biosystems*).

Phylogenetic analyses: The sequence homology was checked by NCBI *BLASTn* tool (http://www.ncbi.nlm.nih.gov/) with CMV and TuMV sequences available in GeneBank. Isolates/strains associated with subgroup I (A/B) and II of CMV were also used for differentiating of CMV isolate. Pairwise sequence comparisons along with nucleotide identities of selected sequences were calculated by *CLUSTAL W* (Thompson *et al*. 1994). The phylograms were constructed in the *MEGA X* software package (Kumar *et al*. 2018) using the Jukes-Cantor model for calculation of evolutionary distances and the Neighbor-joining (NJ) method with 1 000 bootstrap replicates.

Results

In the year 2020 severe virus-like mosaic symptoms – vein banding, mosaic, and leaf deformation were observed on several *A. petiolata* plants growing along road-side in one of the districts of Kyiv (location 1 on the sampling map, Fig. 1). During 2021 growing season, field surveys were conducted at this and an additional four ruderal sites of Kyiv regions, and symptomatic *A. petiolata* plants showing conspicuous virus-like symptoms were collected. Leaf tissue extracts obtained from individual diseased plants (n = 4 from location 1 in 2020 and n = 20 from locations 1 - 5 in 2021) as well as from asymptomatic ones (n = 1 from location 1 in 2020 and n = 5 from locations 1 - 5 in 2021) were used for subsequent virus screening. Sampling sites are numbered in the article from 1 to 5; sampling site 1 was the same in 2020 and in 2021 (Table 1).

The plants collected in the surveyed sites in 2021 differed in the symptoms of the disease; therefore, they were divided into two groups. Plants of one group collected in four of the five study areas, exhibited symptoms of interveinal chlorosis, mosaic, bleaching, and green vein-banding. No crinkling symptoms were observed and no strong deformity, although the leaf blistering and waving of the edge were in some leaves very pronounced (Fig. 2*A-D*). While the plants of the other group showed symptoms of the leaf blistering, leaf malformation, and rugosity (Fig. 2*E*,*F*). The stems of second-year infected plants of both groups were small, with short internodes,

Table 1. Serological tests of *Alliaria petiolata* samples with virus-like symptoms (Mo - mosaic, Chl - chlorosis, LB - leaf blistering, GVB - green vein-banding; NCS - no crinkling symptoms, LM - leaf malformation. Sampling site 1 in 2020 is the same as in 2021.

Year/site	Samples examined	Symptoms	Positive by ACP-ELISA for *Potyvirus* genus	Positive by DAS-ELISA for TuMV	WMV II	CMV	TYMV
2020/1	4	Mo, Chl, LB, GVB	4	4	0	4	0
2021/1	4	Mo, Chl, LB, GVB	4	4	0	4	0
2021/2	4	Mo, Chl, GVB, NCS	0	0	0	4	0
2021/3	4	Mo, Chl, GVB, NCS	0	0	0	4	0
2021/4	4	Mo, Chl, GVB, NCS	0	0	0	4	0
2021/5	4	LB, LM	4	4	0	0	0
2021/total	20		8 (40%)	8 (40%)	0	16 (80%)	0

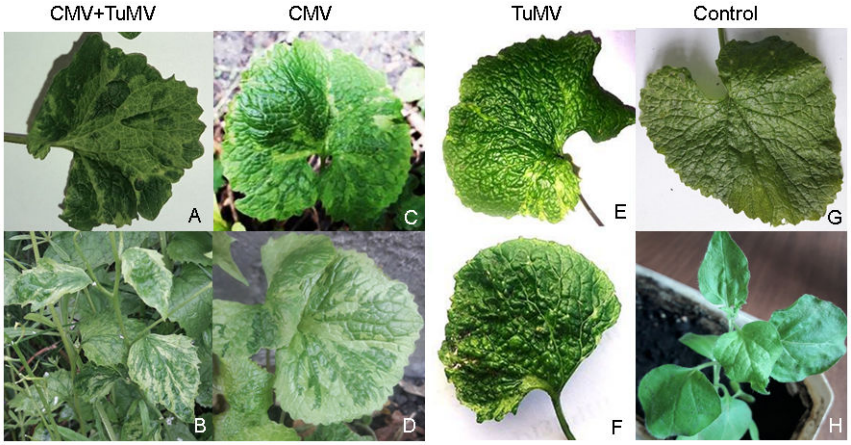

Fig. 2. Disease symptoms on *Alliaria petiolata* (*A*-*G*) and *Nicotiana benthamiana* (*H*) plants used in this study: *A*,*B* - mixed infection of CMV+TuMV (*A* - first-year plants observed in 2020, *B* - second-year plants observed in 2021); *C*,*D* - first-year plants infected by CMV; *E*,*F* - first-year plants infected by TuMV; *G* - leaf of symptomless plant; *H* - symptomless *Nicotiana benthamiana*, artificially infected by CMV.

and in general the plants looked noticeably stunted. Whereas the symptoms of the disease differ in plants of two groups, it can be assumed that garlic mustard infection is caused by different virus types.

Preliminary screening was conducted by ACP-ELISA using wide spectrum monoclonal antibodies for serological detection of potyviruses. It was shown that 40% of the samples collected in 2021 and all samples collected in 2020 were potyvirus-positive (Table 1). After prescreening with ACP-ELISA, these samples were tested by DAS-ELISA specifically for TuMV and WMV II. All specimens were also tested by DAS-ELISA for CMV/TYMV and by RT-PCR for TCV. According to the results of serological tests, all potyvirus-positive samples of *A. petiolata* collected in years 2020 and 2021 were infected by TuMV whereas watermelon mosaic virus II and turnip yellow mosaic virus were not identified (Table 1).

Results of PCR and ELISA obtained in 2021 indicated that out of 20 symptomatic *A. petiolata*, 12 (60%) were CMV positive, whereas 4 (20%) were TuMV positive. Co-infection of CMV and TuMV was diagnosed in 20% of samples. Moreover, co-infected mustard plants showed an enhanced accumulation of CMV in DAS-ELISA-treated samples: the absorbance value (A_{405}) was higher in these plants compared to plants infected only with CMV. Thus, CMV was detected in 80% of the analyzed samples, 60% of which were monoinfected. Asymptomatic garlic mustard plants were negative in ELISA for CMV, TuMV, TYMV, WMV II, and TCV viruses.

By a transmission electron microscopy, icosahedral virions of 28 - 30 nm in diameter, characteristic of viral particles belonging to *Cucumovirus*, *Betacarmovirus*, and *Tymovirus* genera were observed in crude plant sap preparations obtained from garlic mustard of the first group (growing in four out of five sampling sites) (the data not provided) (King *et al*. 2012). Plants of this group, as noted above, were scored as positive in DAS-ELISA using CMV-specific antibodies and negative for TYMV infection. Turnip crinkle virus was not detected in examined samples by RT-PCR using a pair of primers HZ632/HZ633 (Gaafar *et al*. 2019). Flexuous rod-shaped virus particles about 750 nm in length typical of the family *Potyviridae* were observed in negatively stained infected tissue of the second plant group (growing in location 1 and 5)

(the data not provided). All symptomatic plants of this group were positive for TuMV by DAS-ELISA.

The host range studies were conducted using test plants selected mainly from known host ranges of CMV and TuMV viruses. CMV detected in plant of the first group was successfully transmitted to plants of *Cucurbitaceae* family as well as to *Nicotiana tabacum* cv. Samsun NN, *N. rustica*, and *Phaseolus vulgaris* cv. Top Crop. All indicator plants, excluding *N. benthamiana*, developed CMV-specific symptoms at 12 - 14 d post inoculation. Although *N. benthamiana* showed no symptoms, productive CMV infection for all inoculated plants was revealed by DAS-ELISA. These results demonstrate that CMV isolated from *A. petiolata* has broad host range similar to that reported for other CMV isolates with the exception of *N. benthamiana* plants, where infection was latent, without any clear symptoms. After mechanical inoculation of *Brassica rapa* and *Brassica oleracea* var. *botrytis* by the sap of TuMV-infected plants systemic mosaic or systemic black ringspot symptoms, respectively, appeared in each tested species. Necrotic lesions or chlorotic spots were observed on *Chenopodium quinoa* and *Chenopodium amaranticolor* 8 d after inoculation with TuMV.

The results of serological and host range studies subsequently were confirmed by RT-PCR with CMV- and TuMV-specific primer pairs (Bariana *et al*. 1994, Lockhart 2012). Separation of obtained PCR products in agarose gel showed fragments at ~463 bp and ~500 bp, characteristic for TuMV and CMV, respectively. In this way, it was shown that at least two viruses TuMV and CMV infected garlic mustard in Ukraine (Fig. 3). No amplification was observed in the samples obtained from asymptomatic plants.

To determine the genetic relationships among viruses

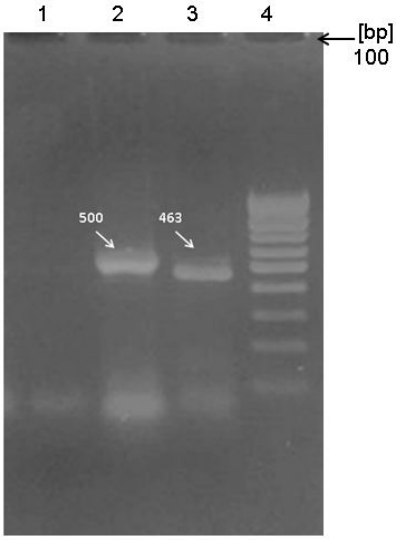

Fig. 3. RT-PCR detection of TuMV and CMV in *Alliaria petiolata*: DNA fragments of a 1 – healthy plant (K^-); 4 – 100 bp ladder length marker (*SybEnzime*). *Arrows* show the: 2 – CMV (500 bp) and 3 – TuMV (463 bp) partial CP gene products obtained from the PCR amplification.

obtained in the study, phylogenetic trees have been constructed on the base of partial CP nucleotide sequences for each virus. The sequence of CMV was named as *CMV-Ap* and TuMV sequence as *TuMV-Ap*. Based on an alignment of 500 nucleotides of the partial *CP* gene with other *CMV* sequences available in the GeneBank using *BLASTn* and *MEGA X* software, it was shown that *CMV-Ap* (GenBank accession No. MZ540213) has the greatest similarity to the isolates from Germany (MW582807), Slovakia (MN792886), and Japan (AB006813) (99.8, 99.6, 99.6% pairwise identity, respectively) (Fig. 4*A*). Minor nt differences noted between analyzed sequences of the part of *CP* gene of CMV, however, were synonymous mutations and did not lead to any expected amino acid changes in the final protein product. Phylogenetic analysis of 28 different CMV isolates selected worldwide revealed that *CMV-Ap* clustered with isolate MW582807 obtained from *Sarracenia* sp. in Germany.

To determine the subgroup of *CMV-Ap* isolate obtained, a phylogenetic analysis was done on CMV strains representing the CMV subgroups IA, IB, and II. For phylogenetic analyses we used the sequences of the RNA 3 ORFs of 15 CMV strains/isolates and the partial CP gene of *CMV-Ap* (Roossinck 2002). It was found that in phylogenetic tree *CMV-Ap* clustered with CMV subgroup-II that supports that *CMV-Ap* is a member of CMV subgroup II (Fig. 5).

The phylogenetic analysis revealed that Ukrainian TuMV-Ap isolate clustered with Iranian isolates infecting different plants in the cabbage family: bastard cabbage *Rapistrum rugosum* (AP017803), broccoli *Brassica oleracea* var. *italica* (P017764), small tumbleweed mustard *Sisymbrium loeselii* (AP017791), and rapeseed *Brassica napus* (JQ073722) (Fig. 4*B*). TuMV-Ap is closely related to the Iranian isolate IRNTRa9 (AP017803) and Turkish isolate TUR49 (AP017872), and shared from 95.9 to 98.6% similarity in nucleotides among 100 sequences found in *BLAST* search. The sequences generated in this work have been submitted to GenBank under the following accession numbers: MZ540213 for *CMV-Ap* and OM799323 for *TuMV-Ap*.

Discussion

Cucumber mosaic virus is one of the most widespread plant viruses. Besides a large number of horticultural crops, CMV infects many weed species. In Ukraine, CMV has been detected on a various host plants, and as our previous study showed, the virus can infect naturally garlic mustard as well (Kyrychenko *et al*. 2022). The natural occurrence of TuMV infection in *A. petiolata* has been confirmed in Europe (Horvath *et al*. 1975, Lisa and Lovisolo 1976) and Canada (Stobbs and Van Schagen 1987) since 1975. In Ukraine researches exploring the occurrence of viruses in wild plants and, in particular, in garlic mustard, have not been conducted. According to the results of biological assay, serological, and molecular detection presented here (Table 1, Fig. 3) CMV is widespread in central Ukraine (Kyiv region) with 80% incidence among symptomatic

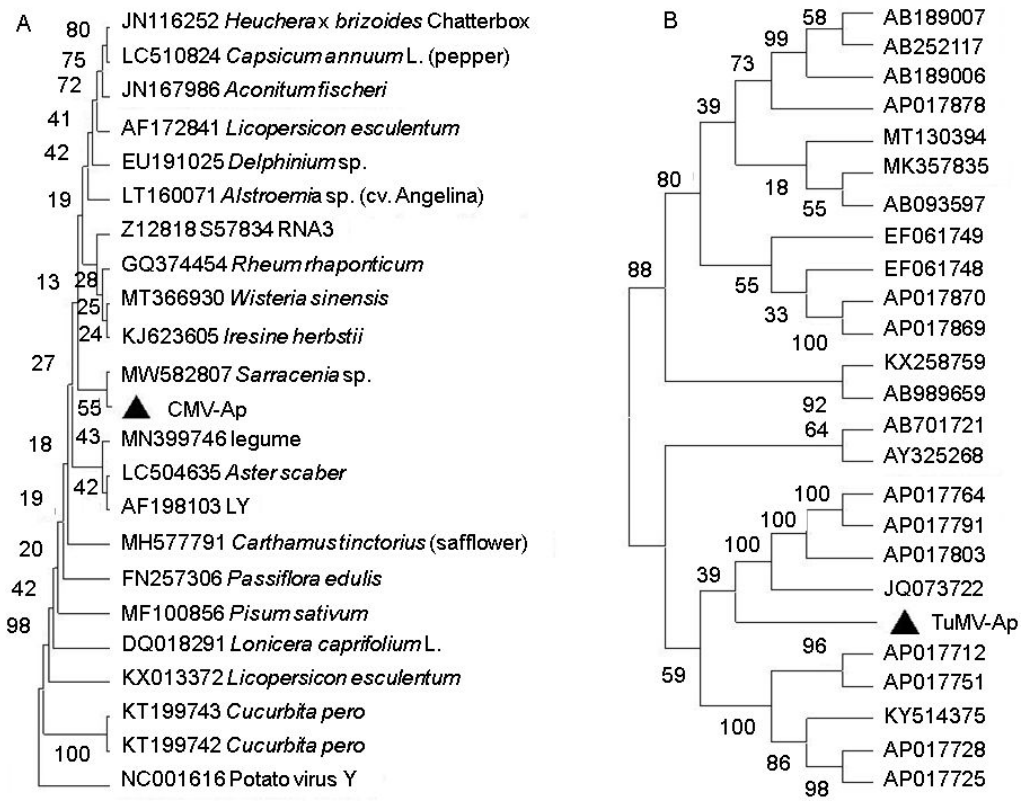

Fig. 4. Phylogenetic trees based on nucleotide sequences of CP domain of CMV (*A*) and TuMV (*B*) from Ukraine along with reported CMV or TuMV isolates, respectively. The analyses were conducted using the *MEGAX* software, the Jukes-Cantor evolutionary model and the NJ method with 1 000 bootstrap replicates.

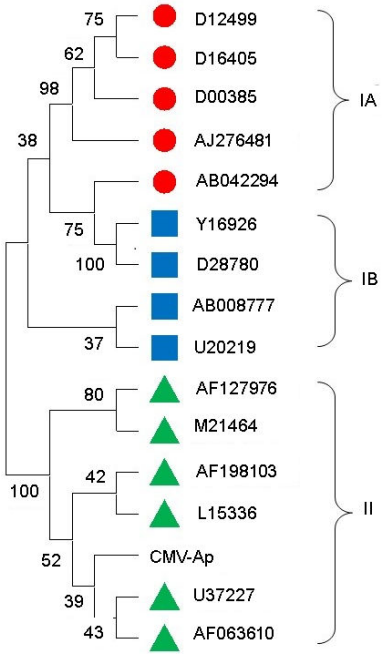

Fig. 5. Phylogenetic relationships of partial CP sequences of CMV-Ap isolate with 15 RNA 3 sequences of CMV isolates, determined by *MEGAX* software package using the Jukes-Cantor evolutionary model and the NJ method with 1 000 bootstrap replicates.

plants surveyed in the study. This is consistent with our previous result on the detection of CMV in *A. petiolata* in Ukraine (Kyrychenko *et al*. 2022). It is noteworthy that 20% of CMV-positive samples were co-infected with TuMV. The incidence of TuMV among the studied samples was 40%, and half of these TuMV-positive samples were also infected with CMV. To our knowledge, this is the first report of TuMV isolate naturally infecting garlic mustard in Ukraine. None of the other analyzed viruses (TYMV, WMV, TCV) was detected in any sample under study.

CMV and TuMV are naturally transmitted by aphids (*Hemiptera*, *Aphidoidea*) in a non-persistent manner. At least 89 species of aphids are efficient vectors for TuMV and 80 species for CMV (Sevik 2017, Lu *et al*. 2022). TuMV is transmitted at a higher rate both by green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*). The main transmission mediators of CMV are *Myzus persicae* and cotton aphid *Aphis gossypii* although *Brevicoryne brassicae* can also be an active vector of the virus (Palukaitis and García-Arenal 2003, Garzo *et al*. 2004, Sevik 2017). It has been shown that aphids of two species, *Myzus persicae* and *Aphis gossypii*, can transmit either a single virus (TuMV or CMV) or both viruses (TuMV and CMV) simultaneously (Fujisawa 1985). All three species (green peach, cotton, and cabbage aphids) are widespread throughout the world, including Ukraine, and can be effective vectors of these viruses in the field.

Despite the fact that previous reports on the detection and characterization of the CMV showed the prevalence in Ukraine viruses of subgroup I (Shevchenko *et al*. 2015, Sovinska *et al*. 2022), the present study showed that the CMV-Ap belongs to subgroup II strains. Thus, the CMV isolates distributed in Ukraine belong to subgroups that have a worldwide distribution (IA or II) as well as to subgroup restricted mainly to Asia (IB) (Miftakhurohmah and Wahyuno 2017).

In Ukraine, TuMV has been detected in *Brassica oleracea* var. *capitata*, *Raphanus sativus*, *Sinapis alba*, *Brassica juncea*, *Camelina sativa*, and *Bunias orientalis* (Shevchenko *et al*. 2016, 2018) and this study shows for the first time the occurrence of TuMV in *A. petiolata*. Based on phylogenetic analyses, *TuMV-Ap* isolate was clustered along with isolates AP017803, P017764, AP017791, and JQ073722 from Iran. At the same time, Ukrainian isolates LC537585, LC537586, LC537587, LC537588, LC537589, and LC537590 of TuMV fell into distinct clade. Ukrainian isolate UKR9, previously reported in the Kyiv region (Shevchenko *et al*. 2018), have relation to Turkish isolates, while the *TuMV-Ap* is very closely related to Iranian isolates. UKR9 biologically was classified into BR host type, isolates of which infect both *Brassica* and *Raphanus* plants systemically with mosaic symptoms (Nguyen *et al*. 2013). Unfortunately, we have not established the host infecting type of TuMV-Ap although the question of the genetic diversity of TuMV populations in Ukraine is very important for understanding evolutionary history of this virus. Nevertheless, our results may provide evidence that more than one TuMV lineage circulates in Ukraine.

Mixed infection of CMV and potyviruses has been studied by many scientists (Wang *et al*. 2002, Fattouh 2003, Mascia *et al*. 2010, Fukuzawa *et al*. 2010, Barbosa *et al*. 2016, Arogundade *et al*. 2018, Salehzadeh 2018). As a rule, researchers reported about synergistic effect on different hosts. Accumulating of CMV to a considerably higher amount and reduction of potyvirus accumulation in mixed infection has also been reported by other authors (Poolpol and Inouye 1986, Sano and Kojima 1989). The results obtained in the study also showed an increase in CMV accumulation in doubly infected mustard plants. Obviously, the synergistic interactions of potyviruses with CMV as well as with viruses belonging to other genera in *Alliaria* sp. are the subject of a more detailed study.

Turnip crinkle virus is not widespread in the world. Based on the information available in the literature, the virus is distributed in the former Yugoslavia, Germany (on garlic mustard), and the United Kingdom (https://www.cabi.org/isc/datasheet/54292). To our knowledge, a survey of distribution of TCV in Ukraine has not been conducted by the time. Though we did not find the virus in garlic mustard, it is obvious that the monitoring of the virus should be carried out in both cultivated plants and weeds, including garlic mustard.

Thus, the results presented in this paper indicate that biennial *Alliaria petiolata* plants are affected by CMV and TuMV in mono- and co-infection and therefore can play an important role in survival of these viruses acting as potential pathogen reservoirs for virus transmission onto crop fields by vectors. Since these plants can be important sources of primary virus inoculum, management of the CMV and TuMV diseases may be feasible through weed control.

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