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BIOLOGICAL CHARACTERIZATION OF AN UKRAINE ISOLATE OF ALFALFA MOSAIC VIRUS

Background. ***Alfalfa mosaic virus (AMV) is one of the most widespread viruses, affecting over 600 plant species, including economically important crops. This pathogen continuously evolves, adapting to different geographical regions and hosts, which complicates the development of effective control strategies and highlights the need for ongoing monitoring and research into the biological properties of AMV. The aim of this study was to investigate the biological properties of Alfalfa mosaic virus (AMV) found in potential reservoir plants collected from public green spaces in Kyiv.***

Methods. ***A Double Antibody Sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) was used for the identification of Alfalfa mosaic virus and to rule out mixed infections with other viruses, including cucumber mosaic virus (CMV), soybean mosaic virus (SMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tobacco rattle virus (TRV), turnip crinkle virus (TCV), turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV), Potato virus Y (PVY), and Potato virus X (PVX). Commercial test systems (Loewe Biochemica, Germany) were used for the ELISA. Biological testing was conducted on different indicator plants through mechanical inoculation.***

Results. ***Screening of potential plant reservoirs of alfalfa mosaic virus (AMV) was conducted in public green spaces of Kyiv, Ukraine. Among a wide range of tested plants, AMV was detected only in representatives of a single species. For the first time globally, natural infection of Ph. alkekengi plants with AMV was identified. It was established that the Ukrainian AMV isolate exhibits a broad range of host plants. Following artificial inoculation, the highest virus concentration was observed in representatives of the Fabaceae family (G. max and Ph. vulgaris), which, according to the literature, are natural hosts of this pathogen.***

Conclusions. ***The circulation of alfalfa mosaic virus in public green spaces of Kyiv was demonstrated. For the first time globally, natural infection of Ph. alkekengi plants with AMV was identified. The identified Ukrainian isolate stands out from those described in the literature in terms of its biological properties, specifically regarding the characteristics of certain indicator plants.***

Keywords: ***virus, detection, enzyme-linked immunosorbent assay, indicator plants.***

Background

Alfalfa mosaic virus (AMV) is one of the most widespread viruses, affecting over 600 plant species from 70 families, including economically important crops. AMV belongs to the genus *Alfamovirus* of the *Bromoviridae* family, and its virions typically have a bacilliform shape, with structures based on icosahedral symmetry (T = 1), with a constant diameter of 18 nm and a length ranging from 30 to 57 nm, depending on the size of the encapsidated nucleic acid (Kumar et al., 1997). AMV contains a tripartite single-stranded RNA genome (RNA1, RNA2, and RNA3) and subgenomic RNA4, which are separately encapsidated into bacilliform particles (Thole, Miglino, & Bol, 1998). Several studies provide detailed information on the morphology, physicochemical properties, and gene expression regulation of AMV (Kasteel et al., 1997; Sánchez-Navarro, & Bol, 2001; Sánchez-Navarro, Herranz, & Pallás, 2006).

The vectors of AMV are insects from the order Hemiptera, family *Aphididae*, non-persistently transmitted by at least fourteen aphid species. Known AMV vectors include *Myzus persicae* (Sulzer), *Acythosiphon pisum* (Harris), *Aphis gossypii* (Glover), *Therioaphis* (*Pterocallidium*) *trifolii* (Monell), and others (Barış et al., 2017). In addition to aphids, AMV can also be transmitted by pollen and seeds in nature (Frosheiser, 1974; Gallo, & Ciampor, 1977; Hemmati, & McLean, 1977; Li et al., 2024).

AMV is widespread in the United States (Abdalla, & Ali, 2012; Harveson, & Porter, 2023), China (Chen, Z., Wang, & Chen, F., 2024), Chile (Peña et al., 2024), Egypt (Amin et al., 2023), and Europe, particularly in the Czech Republic, Spain, Italy, France, and Croatia (Kvícala, 1975; Parrella et al., 2020; Mallor et al., 2002; Stankovic et al., 2014; García-Arenal, & Fraile, 2021).

In nature, AMV infects approximately 150 species from 22 families, but when including experimental susceptible hosts, the number of species increases to over 600 from 70 families. Although most of these are herbaceous plants, such as alfalfa (*Medicago sativa* L.), pepper (*Capsicum annum* L.), beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), celery (*Apium graveolens* L.), lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), and others (Moed, & Veldstra, 1969; Xu, & Nie, 2006; Abdalla, & Ali, 2012), AMV can also infect woody plant species. For example, Chinese wisteria (*Wisteria sinensis* Sims), a tree infected with AMV in Iran (Moradi, & Mehrvar, 2022). AMV infects plants from the *Fabaceae*, *Solanaceae*, *Apiaceae*, *Asteraceae*, *Amaranthaceae*, *Boraginaceae*, and *Lamiaceae* families (Mallor et al., 2002; Parrella et al., 2012; García-Arenal, & Fraile, 2021). AMV can cause biomass loss in alfalfa up to 30% (Han et al., 2019). AMV symptoms in alfalfa can vary depending on the host genotype, presenting as mosaic patterns, spotting, and various developmental defects, although in some cases, AMV infection may be asymptomatic (Fath-Allah, 2000). Some weeds serve as natural hosts of alfalfa mosaic virus, including *Stachys annua* L., which showed yellow-white streaks and spots on leaf blades; *Prunella vulgaris* L., which exhibited bright yellow spots near the leaf margins; *Atriplex patula* L., which showed bright yellow spots almost

across the entire leaf blade (Kvícala, 1975). The wide variety of AMV host plants confirms the virus's significant ability to overcome natural plant defense mechanisms (Parrella et al., 2000). This pathogen continually evolves, adapting to different geographical regions and hosts, which complicates the development of effective control strategies and highlights the need for continuous monitoring and research into the biological properties of AMV. Especially favorable conditions for the adaptation of a pathogen to a new host are found in the green spaces of large cities, as the dense collection of woody, shrub, and herbaceous plants in a specific area facilitates virus transmission through various methods.

The aim of this study was to investigate the biological properties of Alfalfa mosaic virus (AMV) found in potential reservoir plants collected from public green spaces in Kyiv.

Methods

Sample collection. In 2023–2024, plants from urban green spaces in Kyiv were surveyed, focusing on representatives from the families *Fabaceae*, *Solanaceae*, *Apiaceae*, *Asteraceae*, *Amaranthaceae*, *Boraginaceae*, *Brassicaceae*, *Caryophyllaceae*, *Lamiaceae*, and *Urticaceae*. Plants exhibiting typical viral symptoms, such as mosaic, mottling, leaf deformation, and various developmental abnormalities, were collected.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Collected symptomatic samples were tested for the presence of AMV. To rule out mixed infections, samples were also tested for other viruses, including cucumber mosaic virus (CMV), soybean mosaic virus (SMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tobacco rattle virus (TRV), turnip crinkle virus (TCV), turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV), Potato virus Y (PVY), and Potato virus X (PVX) using commercial ELISA kits (Loewe Biochemica GmbH, Sauerlach, Germany). Additionally, artificially inoculated indicator plants were tested for AMV presence. A 300 mg sample of each plant was homogenized in a mortar with sample buffer (pH 7.4) at a 1:10 (m/v) ratio. The resulting homogenate was centrifuged at 5000 rpm for 20 minutes at 4˚C to remove tissue components. The supernatant was then used for viral pathogen detection. The assay was performed according to standard guidelines (Clark, & Adams, 1977; Ward et al., 2004) and the manufacturers' instructions. Alkaline phosphatase-conjugated antibodies and p-nitrophenyl phosphate substrate (Loewe Biochemica GmbH, Sauerlach, Germany) were used. Absorbance was measured at 405 nm with a µQuantTM (BioTek Instruments, Inc., USA). Samples were considered positive if the absorbance value was more than three times that of the negative control.

Experimental host range assays. The host range of the virus isolate was determined by mechanically inoculating tissue extracts onto various plant species: *Glycine max* L. var. Abelina, *Phaseolus vulgaris* L. var. Lastivka, *Nicotiana tabacum* cv. Samsun NN, *Nicotiana benthamiana* L., *Nicotiana rustica* L., *Datura stramonium* L., *Chenopodium album* L., *Tetragonia tetragonioides* (Pall.) Kuntze, *Galinsoga parviflora* Cav. As infectious material, the sap of infected plants was used. A plant material sample (5 g) was crushed and ground in a mortar with the addition of 0.1 M phosphate buffer (pH 7.4) at a 1:10 ratio. Mechanical inoculation was performed on young indicator plants at the two-true-leaf stage. The infectious material was applied to a leaf blade lightly dusted with carborundum, carefully rubbing it across the leaf surface. After 30 minutes, the viral material and carborundum were rinsed off with distilled water, and the plants were placed in a dark, unlit environment for one day. Biological testing was conducted during the summer-autumn period under greenhouse conditions, with a photoperiod of 16 hours and a temperature range of 20–24 °C. Results were recorded between 3 and 18 days following mechanical inoculation.

Results

In 2023–2024, we conducted a visual survey of the green spaces in the city of Kyiv. We focused on representatives from various plant families, namely: Fabaceae (Vicia faba L.), Solanaceae (Physalis alkekengi L., Petunia Juss), Apiaceae (Apium L.), Asteraceae (Erigeron annuus L., Taraxacum sp., Heliopsis helianthoides L.), Amaranthaceae (Chenopodium album L.), Boraginaceae (Cynoglossum L.), Brassicaceae (Alliaria petiolata M.Bieb.), Caryophyllaceae (Silene latifolia Poir.), Lamiaceae (Lamium L.), Urticaceae (Urtica L.). During the visual inspection, plants showing symptoms such as various mosaics, spotting, leaf blade deformations, and other developmental abnormalities were selected. After conducting serological diagnostics for viral infections of the selected samples, it was confirmed that we identified alfalfa mosaic virus (AMV) only in Physalis alkekengi plants, which showed mosaic symptoms and deformations of the leaf blades (Fig. 1). It should be noted that this is the first report of natural infection of common ground cherry (Physalis alkekengi) by AMV. Previously, Ph. alkekengi, after artificial inoculation with AMV, exhibited mosaic and chlorotic-necrotic rings (Horváth, 1996).

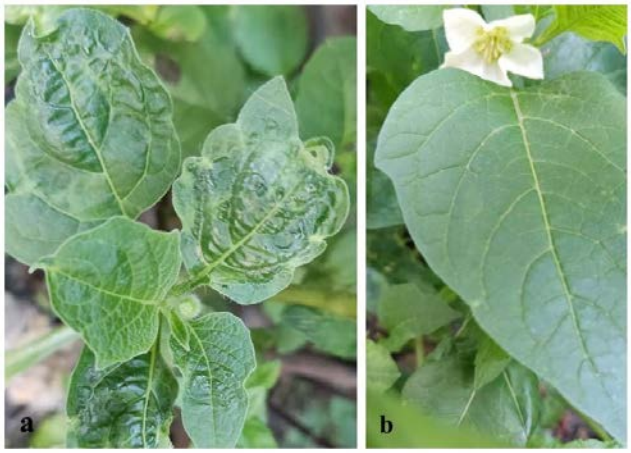

Fig. 1. Symptoms on Physalis alkekengi plants naturally infected with AMV, Kyiv region (a), control (b)

The monoinfection of AMV in *Physalis alkekengi* plants was confirmed by negative ELISA tests for CMV, SMV, TMV, ToMV, TRV, TCV, TuMV, TYMV, PVY, and PVX.

To study the biological properties of the identified AMV isolate, an extract from infected *Ph. alkekengi* plants was used for inoculating the leaves of three test indicator plants of each species. Symptoms were recorded on the infected plants 6–14 days after inoculation, and the presence of AMV in these plants was confirmed by ELISA (Fig. 2, Table 1). For statistical reliability, each test sample was analyzed in triplicate during the ELISA assay, and the average values are presented in the table.

As shown in Fig. 2, Table 1, only *Glycine max* plants reacted with a systemic response to the Ukrainian AMV isolate obtained from *Ph. alkekengi*. All other indicator plants, except *Nicotiana rustica*, exhibited necrosis of various sizes as a local reaction. No symptoms of viral

infection were observed on *N. rustica*, and AMV was not identified through DAS-ELISA. Biological testing indicates a wide range of hosts for the Ukrainian AMV isolate. It should be noted that the virus content varied between different hosts. In plants that, according to the literature, are potential natural hosts for AMV, particularly representatives of the *Fabaceae* family (*G. max* and *Ph. vulgaris*), the virus content was significantly higher. The lowest AMV concentration was found in members of the *Solanaceae* family (*N. tabacum* and *N. benthamiana*), the *Amaranthaceae* family (*Ch. album*), and the *Aizoaceae* family (*T. tetragonioides*).

Fig. 2. Symptoms on indicator plants infected with the Ukrainian AMV isolate:
Glycine max var. Abelina (a), *Phaseolus vulgaris* var. Lastivka (b), *Nicotiana tabacum* cv. Samsun (c), *Nicotiana benthamiana* (d), *Datura stramonium* (e), *Chenopodium album* (f), *Tetragonia tetragonioides* (g), *Galinsoga parviflora* (h); k – healthy control corresponding to each plant species

It should be noted that young *N. benthamiana* plants plants were completely necrotic 7–8 days after mechanical inoculation with AMV. To test the effect of AMV on adult *N. benthamiana* plants, we infected only one branch of the plant. As a result, this branch completely necrotized after 8 days, while the uninfected part of the plant remained healthy (Fig. 3).

Table 1

Host range study of the Ukrainian isolate of alfalfa mosaic virus

Indicator plants		Response to AMV infection	ELISA, O.D. at 405/630 nm*
spesies	family		
Glycine max var. Abelina	*Fabaceae*	systemic	**3,199±0,221**
Phaseolus vulgaris var. Lastivka	*Fabaceae*	local	**2,733±0,035**
Nicotiana tabacum cv. Samsun	*Solanaceae*	local	**0,643±0,183**
Nicotiana benthamiana	*Solanaceae*	local	**0,694±0,171**
Nicotiana rustica	*Solanaceae*	absent	0,171±0,022
Datura stramonium	*Solanaceae*	local	**1,452±0,353**
Chenopodium album	*Amaranthaceae*	local	**0,873±0,189**
Tetragonia tetragonioides	*Aizoaceae*	local	**0,666±0,211**
Galinsoga parviflora	*Asteraceae*	local	**1,378±0,250**
controls ELISA:			
k + – positive control			1,954±0,153
k- 1 – negative control, sap of virus-free plants			0,132±0,013
k- 2 – negative control, buffer			0,064±0,013

Fig. 3. Complete necrotization of young plants (left) and an individual branch (right) of *Nicotiana benthamiana* due to AMV infection

Thus, we detected and identified a Ukrainian isolate of alfalfa mosaic virus from *Physalis alkekengi* plants collected in public green spaces in Kyiv and studied its biological properties using indicator plants from various families.

Discussion and conclusions

Alfalfa mosaic virus (AMV) is one of the most widespread plant viruses. In addition to a wide range of agricultural crops, many species of weeds and ornamental plants can act as carriers of this pathogen, potentially leading to significant economic losses due to reduced yield and product quality. AMV is prevalent worldwide, highlighting its importance as a global pathogen. Monitoring AMV will help develop effective strategies for controlling this pathogen.

Summarizing the results of serological diagnostics, we can confirm that we are the first to report the natural infection of *Ph. alkekengi* plants with alfalfa mosaic virus. We conducted biological testing of the Ukrainian AMV isolate and established that this isolate has a wide range of host plants. Interestingly, representatives of the *Fabaceae* family, which, according to literature, are natural hosts of AMV, showed different responses to infection: *G. max* exhibited a systemic reaction in the form of mosaic, while *Ph. vulgaris* showed a local reaction, with small necroses. Moed and Veldstra (1969) described a natural systemic infection of AMV on *Ph. vulgaris* (Moed, & Veldstra, 1969), whereas the Ukrainian isolate caused a local reaction. Additionally, we also reported the detection of AMV on bean plants exhibiting mosaic symptoms in the Vinnytsia region (Burba, 2024), which suggests the circulation of different strains/isolates of this virus in Ukraine.

Chen et al. (2024) published the results of a study on the effects of the Chinese AMV isolate in mono-infection and co-infection with white clover mosaic virus on chloroplasts and photosynthetic characteristics in *N. benthamiana* (Chen et al., 2024). According to their findings, the Chinese AMV isolate only caused alternating yellow-green leaf discoloration and chlorosis in *N. benthamiana*, whereas the Ukrainian isolate caused

complete necrosis of young plants and infected branches of adult plants. This suggests the variability of AMV, indicating that it evolves and adapts to different geographical regions and hosts.

In Ukraine, AMV was identified only in the agrocenoses of the Vinnytsia region, in *Medicago sativa* and *Glycine max* plants in 2011 (Sherepitko, Boyko, & Sherepitko, 2011) and in our previous studies on *Solanum lycopersicum* and *Phaseolus vulgaris* (Burba, Snihur, & Budzanivska, 2024).

Thus, alfalfa mosaic virus poses a potential threat to agricultural crops in Ukraine, while the public green spaces in Kyiv serve as reservoirs for relatively dangerous viral pathogens, including AMV.

Authors' contribution: Halyna Snihur designed the study; Pavlo Burba and Halyna Snihur, prepared material for the experiments and collected data; All authors performed the experiments and wrote the paper, discussed the results, commented and approved on the manuscript.

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БІОЛОГІЧНА ХАРАКТЕРИСТИКА УКРАЇНСЬКОГО ІЗОЛЯТУ ВІРУСУ МОЗАЇКИ ЛЮЦЕРНИ

В с т у п . *Alfalfa mosaic virus (AMV) є одним із найпоширеніших вірусів, що уражає понад 600 видів рослин, у тому числі економічно важливі культури. Даний збудник постійно еволюціонує, адаптуючись до різних географічних регіонів і хазяїв, що ускладнює розробку ефективних стратегій контролю та підкреслює необхідність постійного моніторингу і дослідження біологічних властивостей AMV. Метою даного дослідження було дослідити біологічні властивості Alfalfa mosaic virus, виявленого в потенційних рослинах-резерваторах, відібраних у зелених насадженнях загального користування міста Києва.*

М е т о д и . *Імуноферментний аналіз у модифікації сендвіч (DAS-ELISA) використовували для ідентифікації Alfalfa mosaic virus та для запобігання змішаної інфекції з іншими вірусами, зокрема, cucumber mosaic virus (CMV), soybean mosaic virus (SMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tobacco rattle virus (TRV), turnip crinkle virus (TCV), turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV), Potato virus Y (PVY), and Potato virus X (PVX). Для ІФА використовували комерційні тест-системи (Loewe Biochemica, Німеччина). Біологічне тестування проводили на різних рослинах-індикаторах шляхом механічної інокуляції.*

Р е з у л ь т а т и . *Проведено скринінг потенційних рослин-резерваторів Alfalfa mosaic virus у зелених насадженнях загального користування міста Києва. Із широкого діапазону тестованих рослин AMV виявлено лише на представниках одного виду. Вперше у світі встановили природнє ураження рослин Ph. alkekengi AMV. Визначено, що український ізолят AMV має широкий діапазон рослин-хазяїв. У результаті штучної інокуляції найвища концентрація вірусу виявлена у представниках родина Fabaceae (G. max і Ph. vulgaris), що за літературними даними є хазяями даного збудника в природі.*

В и с н о в к и . *Показано циркуляцію Alfalfa mosaic virus у зелених насадженнях загального користування міста Києва. Вперше у світі виявлено природнє ураження рослин Ph. alkekengi AMV. За біологічними властивостями ідентифікований український ізолят відрізняється від описаних у літературі за симптомами на деяких рослинах-індикаторах.*

К л ю ч о в і с л о в а : *вірус, виявлення, імуноферментний аналіз, рослини-індикатори.*

Автори заявляють про відсутність конфлікту інтересів. Спонсори не брали участі в розробленні дослідження; у зборі, аналізі чи інтерпретації даних; у написанні рукопису; в рішенні про публікацію результатів.

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