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# PHYLOGENETIC ANALYSIS OF BEAN YELLOW MOSAIC VIRUS ISOLATES FROM GLADIOLI IN UKRAINE

***Bean yellow mosaic virus (BYMV) is the one of most prevalent and harmful viruses infecting gladiolus plants worldwide. The aim of the study was to perform phylogenetic analysis of two Ukrainian BYMV isolates from gladioli grown in different regions of Ukraine and determine phylogenetic relationships of the isolates to other BYMVs. Visual diagnostics, enzyme-linked immunosorbent assay in DAS-ELISA modification, total RNA extraction, RT-PCR, phylogenetic analysis and statistical data analysis were used in this research. The results of the studies showed that gladioli of variety Pamyat' (Poltava region, 2018) and var. Galyna Zelenobirska (Kyiv region, 2020) with symptoms of chlorotic stripes and mosaics on the leaves, flower color breaks are infected by BYMV. Fragments of the CP gene sequence of Ukrainian gladiolus isolates of bean yellow mosaic virus, named GIMP-18 (MK416160) and BYMV-GI-SV-20 (MZ286966) were sequenced and deposited to the NCBI GenBank. Nucleotide sequences of these isolates corresponding to 578 nt of the coat protein gene (CP) located at the position 8727–9305 of the viral genome and amino acid sequences were compared with 40 known BYMV isolates/strains. Phylogenetic analysis demonstrated that GIMP-18 and BYMV-GI-SV-20 have identity of nucleotide sequence 100 % and amino acid sequence 100 % with each other. Both Ukrainian isolates clustered with the Monocot group. The identity of the CP gene sequences of the two gladiolus isolates GIMP-18 and BYMV-GI-SV-20 from geographically remote regions of the country and in different years of selection indicates a common origin of isolates and probable their distribution of planting material.***

***Keywords: gladiolus, bean yellow mosaic virus, phylogenetic analysis.***

**Introduction.** Bean yellow mosaic virus (BYMV) is the one of the most widespread and harmful virus that infects gladiolus worldwide. BYMV belongs to the *Potyvirus* genus of *Potyviridae* family and can infect more than 200 plant species from 14 families. Infection with BYMV and CMV was reported to cause (i) reduction of the number of corms and cormels; (ii) shorter life of the stock; (iii) lower number of florets per spike; and (iv) deterioration of the flowers quality [1, 2].

There are two main classifications of BYMV isolates. One divides all isolates into seven phylogenetic groups (general, monocot, lupine, canna, W, pea, broad bean) according to the coat protein sequences [3]. Second divides all isolated into nine phylogenetic groups (I-IX) based on full genome sequences [4]. Within the host-specific groups of the first classification, all isolates originate from the domesticated species, and none were identified in the wild plants. On the contrary, isolates in the general group were identified in wild and domesticated hosts of monocots and dicots [3]. Some members of the monocot group can infect dicotyledonous species from three families*: Orchidaceae, Iridaceae, Gentianaceae* [5–9]. As phylogenetic relationships among BYMV isolates correlate with their natural hosts, Wylie et al. (2008) proposed seven BYMV phylogenetic groupings based on coat protein sequences and the original hosts of the isolates: (i) general group with a broad host range of monocots and dicots; (ii) broad bean group; (iii) canna group; (iv) lupine group; (v) monocot group; (vi) pea group; and (vii) one strain 'W' from *L. albus* [3]. An isolate PAC-1 from *Passiflora caerulea* did not cluster with any of seven host groups [10]. Interestingly, all gladiolus isolates fall into two different groups: monocot and general. Isolates belonging to the monocot group are of Europe and Asia origin from four hosts, which are all domesticated ornamental plants within two monocot (*Orchidaceae, Iridaceae*) and one dicot (*Gentianaceae*) family. All these host species are propagated vegetatively. International trade in infected corms of *G. hybrida* would explain such a wide geographic distribution of the BYMV isolates from the monocot group.

The aim of the study was to perform a phylogenetic analysis of two BYMV isolates from gladioli grown in different regions of Ukraine and determine phylogenetic relationships of the isolates to other BYMVs.

**Materials and methods.** *Samples collection and visual diagnostics.* Sampling was carried out in the summer–autumn period in 2018 and 2020 in the territories of Poltava region and Kyiv region. Visual diagnostics revealed symptoms of viral infection on leaves, flowers and corms. Samples of gladiolus with both virus-specific symptoms and visually healthy plants were selected.

*Enzyme-linked immunosorbent assay.* To determine the presence of viral antigens, the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used. The analysis was performed using commercial antibodies against BYMV manufactured by Loewe (Germany) in three replicates. Samples of healthy gladioli were used as negative controls. Commercial BYMV preparations (Loewe, Germany) were used for positive controls. The reaction results were recorded on the Termo Labsystems Opsis MR (USA) reader with Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive [11].

*RNA extraction, RT-PCR and sequencing.* GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, USA) was used to extract total RNA from gladiolus leaves. Samples of healthy gladioli were used as negative control. cDNA synthesis was performed using RevertAid Reverse Transcriptase (Thermo Scientific, USA) and BYMV-specific oligonucleotide primers for amplifying a fragment of BYMV coat protein gene were used: BYMV-CP-5, 5′-GAACTGTTGGAACGTTTTCAATTCC-3′; and BYMV-CP-3, 5′-TCTGTTCCAA CATTGCCATCAAG-3′ [12]. Amplification steps using Dream Taq Green PCR Master Mix (Thermo Scientific, USA) were performed using a Genetic Research Instrumentation LTD thermocycler (UK). The amplification reactions were set up as follows: initial denaturation for 3 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 62 °C for 30 s and 72 °C for 45 s. The final extension was at 72 °C for 5 min. The primers are expected to amplify DNA product sections of the BYMV capsid protein gene of 590 bp. PCR products were separated on a 1.5 % agarose gel with DNA markers



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CSL-MDNA-100 bp (Cleaver Scientific, UK), and visualized under UV light. The PCR products were purified from the agarose gel using Zymoclean Gel DNA Recovery Kit (Zymo Research, USA). Sanger dideoxy direct sequencing was performed on the 3130 Genetic analyzer (Applied Biosystems-HITACHI) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and primers used for cDNA synthesis and amplification. Sample analysis was performed using Sequencing Analysis Software v5.2.0.

*Phylogenetic analysis.* The obtained sequences of the *CP* gene of two Ukrainian BYMV isolates from gladioli were compared with the sequences available in the NCBI GenBank database using the BLAST program. Forty BYMV isolates from different countries were used for the analysis. Nucleotide and amino acid sequences were aligned using Clustal W. Phylogenetic trees for the 578 nt fragment of *CP* gene of BYMV isolates were constructed in MEGA X by the Neighbor Joining method [13] using the Jukes–Cantor model with 1000 bootstrap replicates to estimate the statistical significance of each node. Clover yellow vein virus (Ac No NC_003536) was taken as an outgroup for the tree [14]. The pairwise nucleotide sequence identity scores between isolates were determined using MEGA X.

**Results and discussion**

During the summer–autumn period of 2018 and 2020, samples of gladioli variety Pamyat' and var. Galyna Zelenobirska were collected in Poltava and Kyiv region, respectively. Virus-specific symptoms of chlorotic stripes and mosaics on the leaves, colour breaks on the flowers were observed (Fig. 1).

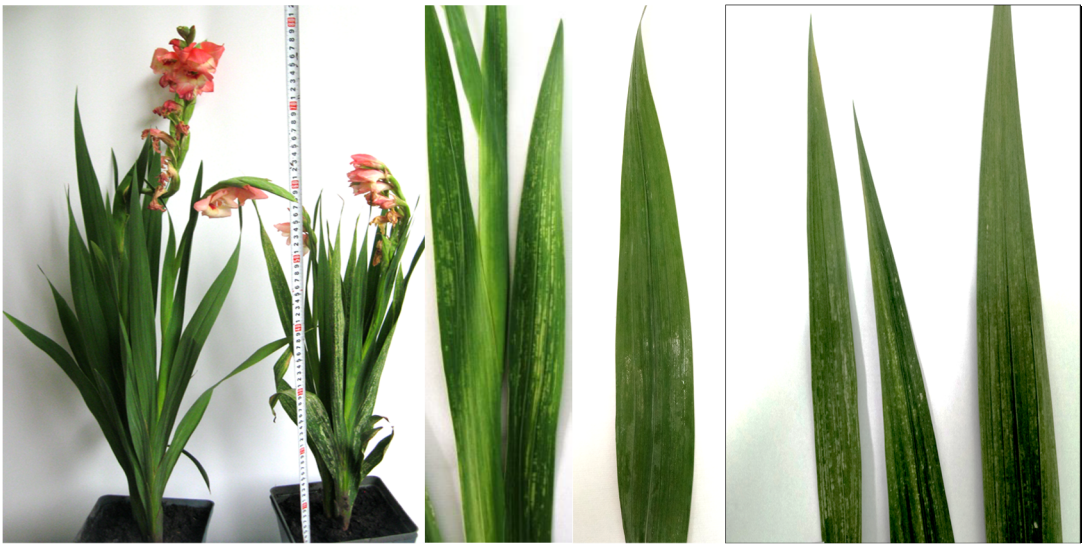


**Fig. 1. Symptoms of BYMV infection on gladiolus plants: a) – healthy, var. Pamyat', b, c – chlorotic stripes var. Pamyat', d – healthy var. Galyna Zelenobirska; e – chlorotic mosaics and stripes, var. Galyna Zelenobirska**

DAS-ELISA showed the presence of BYMV antigens in gladioli with described symptoms (Fig. 2). In asymptomatic gladiolus samples BYMV antigens were not detected. The results of RT-PCR are consistent with the data obtained by DAS-ELISA and demonstrate the presence of BYMV in the studied samples of gladiolus leaves.

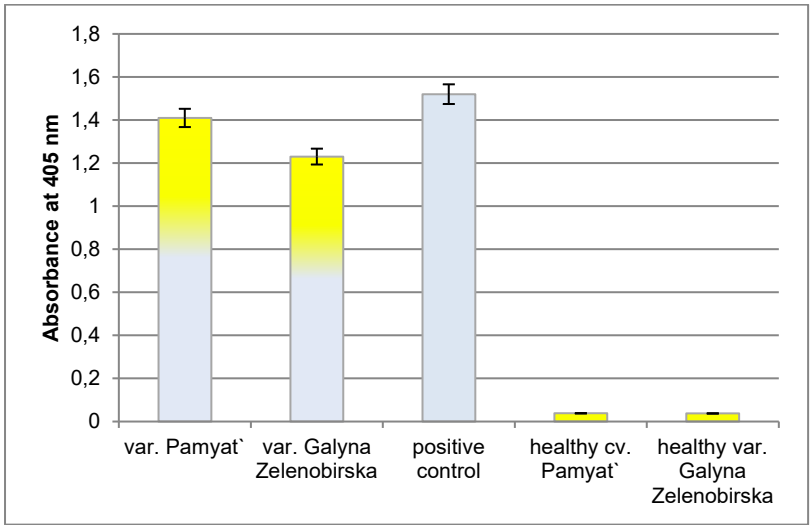


**Fig. 2. Detection of BYMV in gladioli leaves by DAS-ELISA**

Fragments of the *CP* gene sequence of Ukrainian gladiolus isolates of bean yellow mosaic virus, named GIMP-18 (MK416160) and BYMV-GI-SV-20 (MZ286966) were sequenced and deposited to the NCBI GenBank. Nucleotide sequences of these isolates corresponding to 578 nt of the coat protein gene (*CP*) located at the position 8727–9305 of the viral genome were compared with 40 known BYMV isolates/strains.

This experiment demonstrated that GIMP-18 and BYMV-GI-SV-20 are very similar to each other with the identity of nucleotide sequence 100 % and amino acid sequence 100 %. Both Ukrainian isolates clustered with the Monocot group (Fig. 3) and shared 96.6–99.4 % nucleotide sequence and 95.9–100 % amino acid identity with the members of this clade (Table 1).

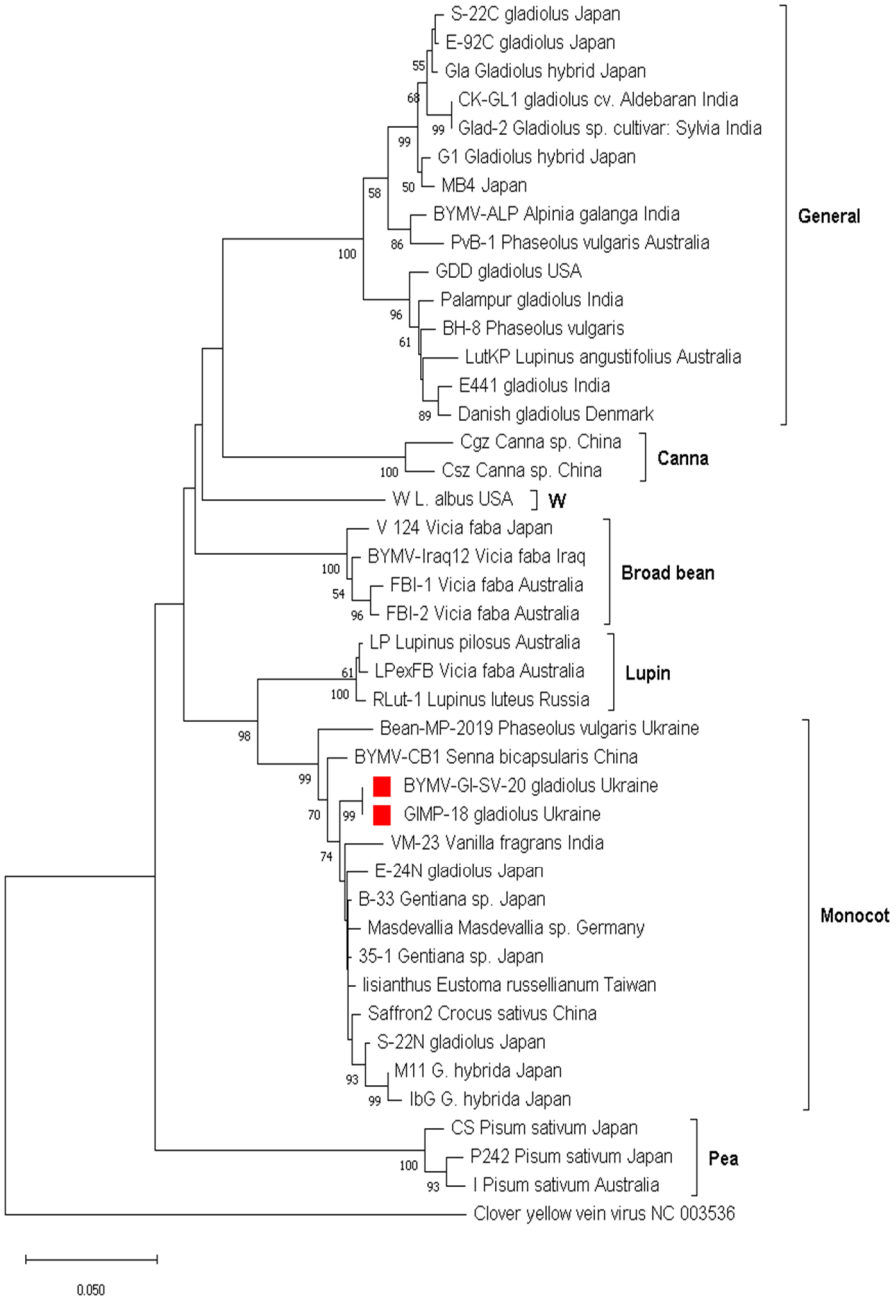


**Fig. 3. Neighbor-joining tree based on nucleotide sequences of 578 nt *CP* gene fragment of BYMV isolates. Jukes-Cantor model was performed. The scale bar shows the number of substitutions per base. Clover yellow vein virus (Ac No NC_003536) used as an outgroup. The studied Ukrainian gladioli isolates are marked with red squares**

Phylogenetic analysis of GIMP-18 and BYMV-GI-SV-20 showed the highest percentage of identity with Japanese isolates from *Gentiana* sp. 35-1 (AB097090), B-33 (AB097089), which is 98.6–99.4 % by nucleotide sequence and 98.5–100 % by amino acid sequence, as well as with Taiwanese isolate Lisanthus from *Eustoma russellianum* (AM884180), Chinese isolate Saffron-2 from *Crocus sativus* (MG002647) – 98.2 % nt and 100 % aa; German isolate Masdevallia from *Masdevallia* sp. (AF185961), Japanese gladiolus isolate E-24N (AB029438) – 98.2 % nt and 99.5 % aa sequences, respectively (Table 1).

Comparative analysis of identity with other BYMV groups showed that isolates GIMP-18 and BYMV-GI-SV-20 have an identity in the range of 92 % – 92.7 % nt, 98.9 % – 99.5 % aa with the Lupin group; with isolates from the Broad bean group – 85.5 % – 85.8 % nt, 97.9 % – 98.9 % aa; with General – 82.3 % – 83.6 % nt, 93.7 % – 96.3 % aa; with the Canna group 83.0 % – 83.6 % nt, 94.3 % – 94.8 % aa; with a representative from group W – 85.5 % nt, 95.3 % aa and with representatives of Pea group share identity of 80.1 % – 81.2 % nt and 92.2 % – 92.7 % aa (Table 1).

***Table* 1. Nucleotide and amino acid sequence identity of the coat protein gene fragments of the Ukrainian BYMV isolates with isolates from the GenBank (%)**

| No | Ac No в GenBank | Isolate | Host | Country | Group | GIMP-18 (MK416160) | | BYMV-GI-SV-20 (MZ286966) | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | nt | aa | nt | aa | |
| 1 | AB097090 | 35-1 | *Gentiana sp.* | Japan | Monocot | 99.4 | 100 | 99.4 | 100 | [7] |
| 2 | AB097089 | B-33 | *Gentiana sp.* | Japan | Monocot | 98.6 | 98.5 | 98.6 | 98.5 | [7] |
| 3 | AM884180 | Lisianthus | *Eustoma russellianum* | Taiwan | Monocot | 98.4 | 98.5 | 98.4 | 98.5 | [9] |
| 4 | MG002647 | Saffron2 | *Crocus sativus* | China | Monocot | 98.2 | 100 | 98.2 | 100 | [15] |
| 5 | AF185961 | Masdevallia | *Masdevallia sp.* | Germany | Monocot | 98.2 | 99.5 | 98.2 | 99.5 | [8] |
| 6 | AB029438 | E-24N | gladiolus | Japan | Monocot | 98.2 | 99.5 | 98.2 | 99.5 | [8] |
| 7 | AB029435 | S-22N | gladiolus | Japan | Monocot | 97.9 | 99.5 | 97.9 | 99.5 | GenBank |
| 8 | MK516282 | BYMV-CB1 | *Senna bicapsularis* | China | Monocot | 97.9 | 100 | 97.9 | 100 | [16] |
| 9 | OL555723 | Bean-MP-2019 | *Phaseolus vulgaris* | Ukraine | Monocot | 95.9 | 98.4 | 95.9 | 98.4 | GenBank |
| 10 | AY845012 | VM-23 | *Vanilla fragrans* | India | Monocot | 97.5 | 96.9 | 97.5 | 96.9 | GenBank |
| 11 | AB079886 | M11 | *G. hybrida* | Japan | Monocot | 97.2 | 98.6 | 97.2 | 98.9 | GenBank |
| 12 | AB079887 | IbG | *G. hybrida* | Japan | Monocot | 96.6 | 97.9 | 96.6 | 97.9 | [7] |
| 13 | HG970866 | BYMV-LP | *Lupinus pilosus* | Australia | Lupin | 92.1 | 99.5 | 92.1 | 99.54 | [3] |
| 14 | EU082124 | R-Lut-1 | *Lupinus luteus* | Russia | Lupin | 92.7 | 99.5 | 92.7 | 99.5 | [3] |
| 15 | HG970868 | LPexFB | *Vicia faba* | Australia | Lupin | 92.0 | 98.9 | 92.0 | 98.9 | [3] |
| 16 | JQ026005 | BYMV-Iraq12 | *Vicia faba* | Iraq | Broad bean | 85.7 | 98.9 | 85.7 | 98.9 | [9] |
| 17 | AB041970 | V 124 | *Vicia faba* | Japan | Broad bean | 85.8 | 98.4 | 85.8 | 98.4 | GenBank |
| 18 | EU082116 | FBI-1 | *Vicia faba* | Australia | Broad bean | 85.5 | 97.9 | 85.5 | 97.9 | [17] |
| 19 | EU082114 | FBI-2 | *Vicia faba* | Australia | Broad bean | 85.5 | 98.4 | 85.5 | 98.4 | [17] |
| 20 | AY192568 | GDD | *Gladiolus sp.* | USA | General | 83.6 | 95.8 | 83.6 | 95.8 | [18] |
| 21 | AB439729 | Gla | *G. hybrida* | Japan | General | 83.6 | 95.8 | 83.6 | 95.8 | [19] |
| 22 | AB439730 | G1 | *G. hybrida* | Japan | General | 83.6 | 96.3 | 83.6 | 96.3 | [19] |
| 23 | AB029436 | S-22C | *Gladiolus sp.* | Japan | General | 83.4 | 94.8 | 83.4 | 94.8 | [9] |
| 24 | AB029439 | E-92C | *Gladiolus sp.* | Japan | General | 83.4 | 95.8 | 83.4 | 95.8 | [9] |
| 25 | KF155409 | CK-GL1 | *Gladiolus* | India | General | 83.0 | 95.3 | 83.0 | 95.3 | [6] |
| 26 | JQ686721 | Glad 2 | *Gladiolus* | India | General | 83.6 | 95.3 | 83.6 | 95.3 | [6] |
| 27 | AM398198 | Palampur | Gladiolus | India | General | 83.4 | 95.3 | 83.4 | 95.3 | GenBank |
| 28 | AB041972 | BH-8 | *Phaseolus vulgaris* | Japan | General | 83.2 | 94.8 | 83.2 | 94.8 | GenBank |
| 29 | EU082123 | PvB-1 | *Phaseolus vulgaris* | Australia | General | 82.7 | 95.8 | 82.7 | 95.8 | [20] |
| 30 | AF192781 | LutKP-1 | *Lupinus luteus* | Australia | General | 82.5 | 94.3 | 82.5 | 94.3 | [3] |
| 31 | D83749 | MB4 | - | Japan | General | 83.6 | 95.8 | 83.6 | 95.8 | [21] |
| 32 | MN509831 | BYMV-ALP | *Alpinia galanga* | India | General | 83.4 | 96.3 | 83.4 | 96.3 | [17] |
| 33 | AJ844916 | E441 | Gladiolus | India | General | 82.3 | 93.7 | 82.3 | 93.7 | [22] |
| 34 | X53684 | Danish | *Gladiolus hybrida* | Denmark | General | 82.3 | 94.3 | 82.3 | 94.3 | [20] |
| 35 | EF592169 | Csz | *Canna sp.* | China | Canna | 83.6 | 94.3 | 83.6 | 94.3 | GenBank |
| 36 | EF592168 | Cgz | *Canna sp.* | China | Canna | 83.0 | 94.8 | 83.0 | 94.8 | GenBank |
| 37 | DQ641248 | W | *L. albus* | USA | W | 85.5 | 95.3 | 85.5 | 95.3 | [23] |
| 38 | AB373203 | CS | *Pisum sativum* | Japan | Pea | 81.2 | 92.7 | 81.2 | 92.7 | GenBank |
| 39 | S71232 | I | *Pisum sativum* | Australia | Pea | 80.2 | 92.2 | 80.2 | 92.2 | [24] |
| 40 | AB041971 | P242 | *Pisum sativum* | Japan | Pea | 80.1 | 92.7 | 80.1 | 92.7 | [25] |

So, both Ukrainian BYMV isolates cluster with isolates from the Monocot group and have the highest level of the amino acid and nucleotide sequences identity with isolates from *Gentiana plants* (AB097090) and *Crocus sativus* plants (MG002647). This fact is consisted withe the hypothesis that *Gentiana* plants could be infected from *Gladiolus* plants growing nearby [3, 8, 9]. Possibly, the 'monocot group might have coevolved with the domestication of the host species *Iris* or *Crocus*, the ancestors of which are indigenous to Eurasia and are now widely grown as ornamental plants worldwide' [3].

Ukrainian BYMV isolate AI38 from *Phaseolus vulgaris* (KT923791) and isolate AN from soybeans (KT923790) exhibit the highest nucleotide sequence identity with Russian and Australian isolates from lupine plants, and Argentinean isolate from soybean [26]. Hence both belong to the lupine group. These facts confirm previously shown high host-specificity of the BYMV isolates and suggest that GIMP-17 and GIMP-18 isolates were introduced into the Ukraine from other geographical regions.

**Conclusions.** Identity of CP gene sequences of GIMP-18 and Bean-MP-2019 (isolate from bean plants, Poltava region) is only 95.9 % nt and 98.4 % aa and these isolates are located distantly on the phylogenetic tree that indicates about their possible different origin. GIMP-18 isolates in 2018 from gladiolus Pamyat' in Poltava region shares 100 % nt and aa *CP* identity with BYMV-GI-SV-20 from var. Galyna Zelenobirska, Kyiv region 2020. Identity of two gladioli BYMV isolates GIMP-18 i BYMV-GI-SV-20 from geographically remote regions of the country and in different years of selection indicates a possible common origin of the isolates and probable their distribution of planting material.

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## ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ ІЗОЛЯТІВ ВІРУСУ ЖОВТОЇ МОЗАЇКИ КВАСОЛІ ІЗ ГЛАДІОЛУСІВ В УКРАЇНІ

***Вірус жовтої мозаїки квасолі (ВЖМК) є одним із найбільш поширених та шкодочинних вірусів, що уражують рослини гладіолусів у світі. Метою роботи було провести філогенетичний аналіз двох українських ізолятів ВЖМК, виділених із гладіолусів різних областей України, і визначити їхні філогенетичні зв'язки з іншими ізолятами цього вірусу. У дослідженні були використані такі методи: візуальна діагностика; імуноферментний аналіз у модифікації "сандвіч"; виділення тотальної РНК, ЗТ-ПЛР; філогенетичний аналіз; статистичні методи оброблення даних. Результати досліджень показали, що гладіолуси сортів Пам'ять (Полтавська обл., 2018) та Галина Зеленобірська (Київська обл., 2020) із симптомами хлоротичної штрихуватої мозаїки на листках і розривом кольору квітки інфіковані ВЖМК. Послідовності фрагмента гена CP українських гладіолусних ізолятів вірусу жовтої мозаїки квасолі, названі GIMP-18 (MK416160) та BYMV-GI-SV-20 (MZ286966), депоновані до NCBI GenBank. Нуклеотидні послідовності цих ізолятів, які відповідають ділянці гена CP розміром 578 нуклеотидів, локалізованій у позиціях 8727–9305 вірусного геному, а також амінокислотні послідовності порівнянні із 40 відомими ВЖМК ізолятами/штамами. Філогенетичний аналіз показав, що GIMP-18 і BYMV-GI-SV-20 мають між собою ідентичність 100 % і за нуклеотидною, і за амінокислотною послідовністю. Обидва українські ізоляти належать до групи Monocot. Ідентичність двох гладіолусних ізолятів GIMP-18 і BYMV-GI-SV-20 за ділянкою гена CP із територіально віддалених областей країни та в різні роки відбору свідчить про їхнє спільне походження і ймовірне поширення посадковим матеріалом.***

***Ключові слова: гладіолус, вірус жовтої мозаїки квасолі, філогенетичний аналіз.***