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G. Korotyeyeva, PhD
Taras Shevchenko National University of Kyiv, Kyiv

IDENTIFICATION OF VIRUSES AFFECTING GLADIOLUS IN KYIV REGION

The plants of different cultivars of Gladiolus from Kyiv region were analyzed for viruses. The viruses were identified by the methods of bioassay, ELISA and electron microscopy. Tomato aspermy virus was identified in the samples of Gladiolus plants.

Key words: ELISA, viruses, gladiolus.

Introduction. The *Gladiolus* genus belongs to the *Iridaceae Juss* family. It includes more then 150 species. Most of them are native to Africa and few originated from the Mediterranean area, Asia and South Europe. Cultivars of gladiolus exhibit a great diversity of colour, size, shape, flowering time, and bulbing and dormancy behaviour [2]. Gladiolus is an important ornamental plant grown for commercial purposes, bouquets, floral baskets and cut flowers in Ukraine. Viruses represent a major problem for gladiolus and other bulb crops because the plants are propagated each year by bulbs that may harbor a virus. These viruses can cause extensive losses in the quality and quantity of flowers and corms. They cause significant economic losses to floriculture. The most widespread symptoms of virus infections in gladiolus plantings are overall stunting, color-breaking, flower distortion, reduced flower and cormel production, severe mosaic in leaves, stunted plants, and color -breaking in flowers, notched leaf blade margin symptoms.

Gladiolus plants may be infected by a number of different viruses or by strains of a particular virus [1]. The main viruses reported from gladioli in different countries are: *Tomato ringspot virus* (ToRSV), *Tomato black ring virus* (ToBRV), *Tomato spotted wilt virus* (TSWV), *Tobacco mosaic virus* (TMV), *Tobacco ringspot virus* (TRSV), *Arabis mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV), *Tobacco streak virus* (TSV) [3, 4].

None of the viruses found on gladioli was specific to the crop species or to the *Iridaceae* family. All of these viruses have a wide host range as well as geographic distribution and some of them can be transmitted by vectors. Unfortunately the early diagnosis of virus diseases is not essential for establishing management measures. In addition to the aboveground parts of plants the corms could be a source of virus infection. To improve the quality of planting material and minimizing viral affection of gladiolus the timely diagnosis is of essential importance. Thus, identification of the source of infection, including the presence of the pathogen in planting material, is effective in controlling spread of viruses of gladioli and termination of epiphytoties.

The aim of the work was to investigate and identify the viruses affecting gladiolus.

Marerials and methods. For detection and identification of viruses affecting gladiolus we used 22 samples of 3 different cultivars ('Nizhnist', 'Nichnyj bluz', 'Asol') from private gardens of flower growers collected in Baryshivka town in Kyiv region. The samples were collected from gladioli expressing viral symptoms on leaves and flovers.

Virus identification was carried out using standard DAS-ELISA and indirect ELISA [9]. The samples were prepared by homogenizing plant tissue with 0.1 M phosphate buffer (pH 7.4) in the ratio 1:3 (m/v), followed by centrifugation at 5000 rpm for 20 min. For the diagnostics we used polyclonal antiserums to TMV (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), *Turnip mosaic virus* (TuMV) (antiserum kindly provided by Lesemann D.E., Julius Kühn Institute, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Germany), *Tomato aspermy virus* (TAV), ToRSV, TRSV, CMV, *Arabis mosaic virus* (ArMV), *Pepper mild mottle virus* (PMMoV), *Tomato spotted wilt virus* (TSWV), *Tobaco rattle virus* (TRV) (Loewe, Germany).

Futher, viruses were identified by bioassay and study of virus particle morphology. Biological properties of viruses were studied using the range of test plants: *Nicotiana tabacum, Cucumis sativus, Lycopersicon esculentum, Phaseolus vulgaris, Capsicum annum, Tetragonia expansa, Zinnia elegans*. The test plants were inoculated on early growth stages by mechanical sap transmission, applying carborundum as an abrasive.

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate. Electron microscopy (EM) was carried out using a JEOL-1400 electron microscope at the magnification of 40 000 and 60 000.

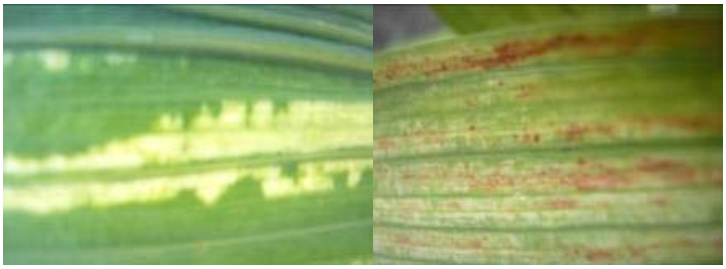
Results and discussion. Viral symptoms on gladiolus plants are not specific and common for wide range of viruses. Moreover, imbalance of mineral elements, their lack, high intensity lighting, damage by insects and mites, bacterial and fungal infections or genetic disorders can cause symptoms similar to those of viral infection. Although viral infection could be diagnosed in advance by specific symptoms such as ring spot, mosaic and the necrosis.

The samples were collected from gladioli expressing viral symptoms on leaves and flovers. Leaves of naturally infected gladiolus plants exposed chlorotic spots and streaks of irregular shape. Some samples demonstrated coarse breaking patterns, expressed as disappearance of pigment in flowers (Fig.1). Severe color breaking and deformation of flowers are the most common symptoms associated with CMV infection in gladiolus [9].

A B C

Fig.1. White and light gray blotches in flowers of *Gladiolus* 'Nichnyj bluz' (A), 'Asol' (B) and 'Nizhnist' (C)

Such symptoms as green mosaic, local necrotic lesions and striped mosaic had been detected on leaves of gladiolus 'Nizhnist' (Fig.2A). According to the literature data, the symptoms of mosaics could be caused by BYMV and TRV [5, 6]. As the mixed viral infection is common in gladiolus, the symptoms may be very variable. The different cultivars of gladiolus also contribute to variability of symptoms [7]. As the symptoms can be not only of viral origin, identification of the virus, based on symptoms alone, is difficult because the gladiolus can react differently even to the same virus.



A **B**

Fig.2. Leaf of infected gladiolus showing green mosaic (A), local necrotic lesions and striped mosaic (B)

To confirm the infectivity of the plant sap and to detect the biological properties of pathogens, we used biological testing on the range of test plants typical for gladioli viruses [7]. They produced different virus-like symptoms (Table 1).

***Table* 1. Symptoms on leaves of test plants inoculated with the sap of infected gladiolus**

Test plants	Symptoms					
	Nicotiana tabacum	*Cucumis sativus*	*Lycopersicon esculentum*	*Tetraggonia expansa*	*Capsicum annum*	*Phaseolis vilgaris*
Gladiolus 'Nichnyj bluz'	-	M	-	-	-	N
Gladiolus 'Nizhnist'	M	Cl.sp.	M, D	-	-	N
Gladiolus 'Asol'	-	M	-	-	-	-

M – mosaic; N – necrotic lesions; D – deformation of leaves; Cl.sp. – chlorotic spots; – symptomless.

Mosaic symptoms were observed on leaves of *Nicotiana tabacum* after inoculation with sap from plant *Gladiolus* 'Nizhnist'. These symptoms are typical for wide range of gladiolus viruses, such as TAV, CMV. However, symptoms of small local chlorotic spots on cotyledons of *Cucumis sativus* are typical for TAV infection. Besides, this virus causes necrotic reaction on leaves of *Phaseolus vulgaris* [8]. Symptoms registered on leaves of *Nicotiana tabacum* and *Phaseolus vulgaris* are characteristic for BYMV [6]. Other inoculated test plants showed no reaction on inoculation with gladiolus sap.

Hence, the biotesting confirmed the infectivity of the sap samples of gladiolus plants. Absence of symptoms on other testing plants after sap inoculation, in our opinion, cannot provide evidence of non-infectious nature of the disease because the pathogens may not be readily transmitted by mechanical inoculation and also may have different diagnostically susceptible host species.

For the virus identification we used DAS-ELISA and indirect ELISA with antisera to viruses, which are reported from gladioli: TMV, CMV, PMMoV, ToRSV, TRSV, TuMV, TAV, ArMV, TSWV and TRV. According to ELISA results, the antigens of TAV were detected in the samples of gladiolus 'Nizhnist', while the results of the testing of gladiolus 'Nichnyj blus" and 'Asol' with this serum were negative. Antigens of TMV, CMV, PMMoV, ToRSV, TRSV, TuMV, ArMV, TSWV and TRV have not been detected in tested samples.

The electron microscopy of the plant sap was carried out simultaneously with biotesting. One type of spherical virus-like particles was revealed in infected plant tissues. The particles of about 30 nm in diameter were detected in plants of Gladiolus 'Nizhnist' with stripe mosaic symptoms (Fig.3).

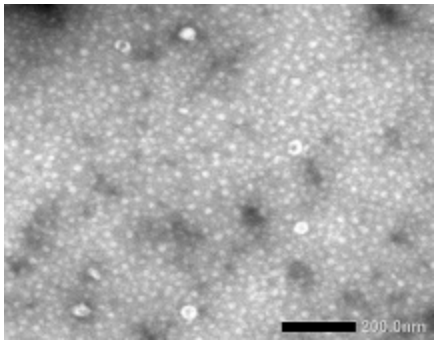


Fig.3. Electron micrograph of virus particles in the sap of gladiolus plants cultivar 'Nizhnist', bar 200 nm

On the basis or test plants' reaction data, ELISA, particle morphology and literature data [4, 8] the virus was identified as TAV.

Conclusion. The plants of different cultivars of gladiolus from Kyiv region were analyzed for presence of viruses. The pathogen detected in gladiolus plant was identified as *Tomato aspermy virus*. Methods of controlling viral diseases assume growing and propagating healthy planting material tested as virus-free, inspection of plants during vegetation for occurrence of symptoms and elimination of affected plants.

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Г. Коротєєва, канд. біол. наук
КНУ імені Тараса Шевченка, Київ

ІДЕНТИФІКАЦІЯ ВІРУСІВ, ЯКІ УРАЖУЮТЬ ГЛАДІОЛУС У КИЇВСЬКІЙ ОБЛАСТІ

Проведено обстеження рослин різних сортів роду Gladiolus, відібраних на території Київської області, на наявність вірусних патогенів. Віруси детектували за допомогою методів біотестування, імоунофрментого аналізу та електронної мікроскопії. У зразках рослин було ідентифіковано вірус аспермії томатів.

Ключові слова: імуноферментний аналіз, віруси, гладіолус

А. Коротеева, канд. біол. наук
КНУ имени Тараса Шевченко, Киев

ИДЕНТИФИКАЦИЯ ВИРУСОВ, КОТОРЫЕ ПОРАЖАЮТ ГЛАДИОЛУС В КИЕВСКОЙ ОБЛАСТИ

Проведено обследование растений разных сортов рода Gladiolus, отобранных на территории Киевской области, на наличие вирусных патогенов. Вирусы детектировали с помощью методов биотестирования, имоунофрментого анализа и электронной микроскопии. В образцах растений был идентифицирован вирус аспермии томатов.

Ключевые слова: иммуноферментный анализ, вирусы, гладиолус

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L. Leibenko, PhD, S. Babii, PhD stud., L. Radchenko, PhD stud., O. Smutko, stud.
Taras Shevchenko National University of Kyiv, Kyiv

PHYLOGENETIC ANALYSIS OF B/VICTORIA-LIKE INFLUENZA VIRUSES ISOLATED IN 2008-2012 IN UKRAINE

The article presents results of phylogenetic analysis of B/Victoria-like influenza viruses isolated in 2008-2012 epidemic seasons in Ukraine. Key mutations in amino acid sequences in hemagglutinin and neuraminidase proteins of Ukrainian isolates were analyzed. All Ukrainian B/Victoria-like influenza viruses type B located in Brisbane/60 cluster as the most isolates from all over the world according our data.

Key words: B/Victoria-like, phylogenetic analysis, Ukraine, Brisbane/60.

Introductoin. Family *Ortomyxoviridae* contains five different genes: *Influenza virus* A, *Influenza virus* B, *Influenza virus* C, *Isavirus* and *Togotovirus* [1]. Influenza B viruses belonged to the two genetic lineages: B/Victoria/2/87 and B/Yamagata/16/88 since the end of the XX century. Influenza viruses type B are less discovered than A type. Type B haven't animal host reservoir and pandemic potential. However, influenza B viruses could cause severe diseases and become the main infectious agent of influenza epidemic every three years [2,3].

The phylogenetic analysis applied to new influenza isolates allows monitor the rate and intensity of virus variations practically in real time. Moreover, the comparative analysis of their protein sequences allows reveal point amino acid replacements providing the mechanism of virus adaptation to human immune system [4]. Sequences of surface antigens – hemagglutinin (HA) and neuraminidase (NA) – are usually used for genetic analyses [5].

The **aim** of our work was to analyze the genetic diversity of B/Victoria-like influenza viruses isolated in Ukraine in 2008-2012.

Materials and methods. Nasal-throat swabs taken from patients with influenza-like illnesses (ILI) or severe acute respiratory infection (SARI) from different regions of Ukraine during 2008-2012 epidemic seasons in the study. They were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses type B were isolated in MDCK cell culture from samples positive in PCR. Hemagglutinin (HA) and neuraminidase (NA) gene sequences of Ukrainian isolates were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 5 software [6].