classified as a member of the genus *Chloriridovirus.* The impact of this virus in the natural environment is unknown. Although, it is not known whether or not this iridovirus can infect any other mosquito species, many members of the *Iridoviridae*, such as IIV-6 and ranaviruses, are known to have large host ranges.

Interest in invertebrate iridoviruses has been limited by the perception that they have little potential as biological control agents against insect pests. However, there is now growing awareness of the potential impact of sublethal IIV disease on the dynamics of insect populations, including insect vectors of medical importance worldwide. Studies with insects indicate that sublethal iridovirus infections can also seriously impact host fitness through reduction in reproductive capacity, body size, and longevity [7]. The mosquito iridovirus *Ae. flavescens* can be promising biological control agent against insect pests, as it causes covert disease and reduces mosquito stocks.

Therefore, future experiments are required to establish whether AfIV is a new species within the genus *Iridovirus* or just an isolate of a previously known virus species. In addition, it would be worthwhile to investigate the susceptibility of AfIV to other species of *Aedes* and to explore the prevalence of these viruses in wild stocks.

Conclusion. Electron microscopy and molecular approaches yielded results consistent with the suggestion that mosquito iridovirus *Ae. flavescens* was a member of the family *Iridoviridae*. Moreover RFLP analysis of viral DNA, number of virus proteins and molecular weight of MCP distinguished iridovirus *Ae. flavescens* from another mosquito iridovirus *Ae. taeniorhynchus*, the type species of the genus *Chloriridovirus*. Presently, a large numberof mosquito iridoviruses, that have been reported in literature, are insufficiently studied and consequently they are not classified. Our observation may help to understand the relatedness of mosquito iridovirus *Ae. flavescens* within family *Iridoviridae.*

1. Family Iridoviridae. In Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses / Chinchar V.G., Essbauer S., He J.G. [et al.]. – San Diego: Elsevier/Academic Press, 2005. – P. 150–162. 2. Williams T., Barbosa-Solomieu V., Chinchar V.G. // Advanced Virus Research. – 2005. – 65. – P. 173–248. 3. Molecular cloning: a laboratory manual, 3rd ed / Sambrook J., Russell D.W. – New York: Cold Spring Harbour, 2001. 4. Laemmli U.K. // Nature. – 1970. – 227. – P. 680-685. 5. Oshima S., Hata J., Segawa C. [et al.] // Analytical Biochemistry – 1996. – 242. – P. 15–19. 6. Delhon G., Tulman., E.R, Afonso C.L. [et al.] // Journal of Virology. – 2006. – 80. – P. 8439-8449. 7. Chinchar, V.G., Hyatt A., Miyazaki, T. [et al.]. // Current Topics in Microbiology and Immunology. – 2009. – 328. – P. 123-170. 8. Ahne W., Bearzotti M., Bremont M. [et al.] // Journal of Veterinary Medicine B. – 1998. – 45. – P. 373–383. 9. Knell R. Mopane worm diseases. – London, 2006. – 119 p. 10. Tang K.F.J., Redman R.M., Pantoja C.R. [et al.] // Journal of Invertebrate Pathology. – 2007. – 96. – P. 255-260.

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DETECTION AND IDENTIFICATION OF VIRUSES AFFECTING IRISES IN LITHUANIA AND UKRAINE

Робота виконана згідно зі спільною Литовсько-Українською програмою співробітниками кафедри вірусології Київського національного університету імені Т. Шевченка та лабораторії вірусів рослин інституту ботаніки в рамках проекту "Моніторинг складу вірусних патогенів декоративно-квіткових культур в Україні та Литві". Мета даної роботи – дослідження вірусних захворювань півників (Iris L.) ботанічних садів Литви та України; ідентифікацію збудників проводили за допомогою класичних та новітніх, молекулярно-біологічних, методів. Вірус помірної мозаїки півників (IMMV) був виявлений в усіх досліджених зразках як Литви так і України. Методами рослин індикаторів, електронної мікроскопії, імуноферментного аналізу, в модифікації DAS, та зворотно-транскрипційної полімеразної ланцюгової реакції (RT-PLR) з деяких литовських зразків були ізольовані та ідентифіковані віруси тютюнової мозаїки (TMV), кільцевої плямистості тютюну (TRSV) та кільцевої плямистості томатів (ToRSV).

According to the joint Lithuanian–Ukrainian program the research workers of Department of Virology T. Shevchenko Kyiv National University and Plant Virus Laboratory of Nature Research Center Institute of Botany are fulfilling research project "Monitoring of diversity of virus pathogens infecting ornamental plants in Ukraine and Lithuania". The aim of this work was to investigate viral diseases affecting irises (Iris L.) grown in Botanical gardens in Lithuania and Ukraine; to identify their agents applying the classical and modern, molecular biology methods. Iris mild mosaic virus (IMMV) was detected in all investigated Lithuanian and Ukrainian samples; Tobacco mosaic (TMV), Tobacco ringspot (TRSV), and Tomato ringspot (ToRSV) viruses have been isolated and identified in some investigated Lithuanian samples by the methods of test–plants, electron microscopy, double–antibody sandwich enzyme–linked immunosorbent assay (DAS–ELISA), and reverse transcription polymerase chain reaction (RT–PCR).

Introduction. Irises have been used for centuries both as ornamentals and as a source of perfumes and medicine. This wide-ranging genus of more than 200 species is native to the temperate regions of the Northern hemisphere. There are many hybrids. Irises are divided into main two groups: rhizomatous and bulbous.

Spread of viruses in irises remains a serious problem. Until now 15 viruses affecting irises have been described in literature: *Iris mild mosaic virus*, *Narcissus latent virus*, *Iris severe mosaic virus*, *Bean yellow mosaic virus*, *Tomato spotted wilt, Impatiens necrotic spot virus*, *Turnip yellow mosaic virus* [1], *Tobacco ringspot virus, Tobacco mosaic virus, Tobacco rattle virus*, *Iris fulva mosaic virus*, *Cucumber mosaic virus, Broad bean wilt virus*, *Lilac chlorotic leafspot virus*, *Iris germanica virus*.

According to the joint Lithuanian–Ukrainian program the research workers of Department of Virology T. Shevchenko Kyiv National University and Plant Virus Laboratory of Nature Research Center Institute of Botany are fulfilling joint research project "Monitoring of diversity of virus pathogens infecting ornamental plants in Ukraine and Lithuania". The aim of this work was to investigate viral diseases affecting *Iris L.* grown in Botanical gardens in Lithuania and Ukraine; to identify their agents applying the classical and modern, molecular biology methods.

Materials and methods. Plant material was collected in Botanical Garden of Vilnius University, Experimental Station of Field Floriculture and collection of iris breader O. Griniuviene in Lithuania; Botanical garden of Kiyv T. Shevchenko university, and N. N. Griško Kyiv National Botanical Garden in Ukraine.

Viruses have been identified by the methods of test–plants [2], electron microscopy (EM), double–antibody sandwich enzyme–linked immunosorbent assay (DAS–ELISA) and reverse transcription polymerase chain reaction (RT–PCR) [3, 4, 5, 6].

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The test–plants were inoculated in early stages of growth by mechanical sap transmission, applying carborundum as an abrasive. The inocula were prepared by homogenizing infected plant tissue in 0.1 M phosphate buffer pH 7.0, containing 1 % nicotine acide as virus–stabilizing additive.

Virus particles were examined in leaf dip EM preparations negatively stained with 3 % uranyl acetate using a JEOL–100S electron microscope, at magnification 25000.

DAS–ELISA was carried out using commercial kits (DSMZ Plant Virus Collection, Germany), according to standard procedure. Immunoglobulins (IgGs) and IgG alkaline phosphatase conjugates were used at a dilution of 1/1000. 50 mg of sample leaf tissue was extracted in 1 ml of sample buffer. 0.1 % p–nitrophenylphosphate was used as substrate. The reactions were measured after 90 min incubation with substrate photometrically at 405 nm (Labsystems Multiskan RC).

RT–PCR was accomplished using the TMV specific primers: F (5'-GAC CTG ACA AAA ATG GAG AAG ATC T-3') and R (5'-GAA AGC GGA CAG AAA CCC GCT G -3') [4].

RT–PCR for TRSV identification has been carried out using forward primer 5'– CTT GCG GCC CAA ATC TAT AA–3' and reverse primer 5'–ACT TGT GCC CAG GAG AGC TA–3' which anneal to the conserved region in coat protein gene. The reaction produced an amplification product of 348 bp [5].

Primers for ToRSV identification by RT–PCR included U1, 5'– GACGAAGTTATCAATGGCAGC–3' (nt 1,078 to 1,098) and D1, 5'–TCCGTCCAATCACGCGTAATA–3' (nt 1,506 to 1,527) of putative viral polymerase gene, resulting in a 449 bp amplification product [6].

Total RNA was extracted from symptomatic plant material stored frozen at –20^{o}C using Plant RNA Purification TRIzol reagent (Carlsbad, CA. USA). Extraction procedure was carried out according to the manufacturer's instructions.

All PCR procedures were carried out in Biometra Tgradient Thermocycler. RNA denaturation mixture (for each sample) of 9 µl RNA and 1 µl of 20 pM reverse primer was incubated 5 min at 70 ^{0}C and 5 min at 4 ^{0}C.

Reverse transcription (RT, cDNA synthesis) reaction mixture (for one sample): 4 µl 5x PCR buffer; 1 µl RNasin; 2 µl 10 mM dNTPs; 1 µl 200 U/µl MulRev Transcriptase and 11 µl of denatured RNA mix. Reaction was performed incubating at 42 ^{0}C for 60 min, at 70 ^{0}C for 10 min and at 4 ^{0}C for 5 min.

TMV PCR mixture contained (for one sample): 10 µl cDNA; 34.75 µl PCR water, 4 µl 2 mM dNTPs, 1 µl 20 pM primer F, 1 µl 20 pM primer R, 5 µl 10xPCR buffer, 3 µl $MgCl_2$, 0.25 µl 5 U/µl *Taq* DNA polymerase. Reaction mixtures were incubated at 94 ^{0}C for 2 min (for first step), 35 cycles of 94 ^{0}C for 30 sec, 62 ^{0}C for 45 min, 72 ^{0}C for 1 min, and at 72 ^{0}C for 5 min (final step).

TRSV PCR reaction mixtures contained (for one sample): 10 µl cDNA; 34.75 µl PCR water; 1 µl 20 pM forward primer; 1 µl 20pM reverse primer; 4 µl 2mM dNTPs; 5 µl 10xPCR buffer; 3 µl $MgCl_2$; 0.25 µl 5 U/ µl *Taq* DNA polymerase. Reaction was performed incubating at 94 ^{0}C for 4 min (for first step), 40 cycles of 94 ^{0}C for 1 min, 52 ^{0}C for 2 min, 72 ^{0}C for 2 min, and at 72 ^{0}C for 10 min (final step).

ToRSV PCR reaction mixture contained (for one sample): 10 µl cDNA; 34.75 µl PCR water; 1 µl 20 pM forward primer; 1 µl 20pM reverse primer; 4 µl 2mM dNTPs; 5 µl 10xPCR buffer; 3 µl $MgCl_2$; 0.25 µl 5 U/ µl *Taq* DNA polymerase. Reaction was performed incubating at 94 ^{0}C for 4 min (for first step), 40 cycles of 94 ^{0}C for 1 min, 53 ^{0}C for 2 min, 72 ^{0}C for 2 min, and at 72 ^{0}C for 10 min (final step).

Resulting PCR products were analyzed by electrophoresis through 1 % agarose gel, stained with ethidium bromide. DNA bands visualized and documented using Bio-Rad Gel Doc XR.

Results. Samples for investigations have been collected from irises bearing symptoms of chlorotic and necrotic streaks; various shape spots, including ringspots on leaves and flower bud sheaths; flower breaking. For investigations 47 samples of symptomatic iris have been collected in Lithuania and 10 in Ukraine.

EM investigation of negatively stained dip preparations from naturally infected plants revealed the presence of flexuous filamentous particles, 750 nm long and isometric particles 30 nm in diameter. Filamentous particles have been detected in all investigated Lithuanian and Ukrainian samples.

Symptomatic host plants and inoculated test–plants were tested in DAS–ELISA using the IgGs and IgG alkaline phosphatase conjugates specific for ArMV, CMV, TMV, TRSV, ToRSV. Reaction was considered positive when absorbance at 405 nm was twice and higher the mean of healthy plants (negative controls). All collected Ukrainian samples gave negative reaction to tested viruses. TMV, CMV, ToRSV infection was reliably confirmed for one, ArMV, TRSV for two tested Lithuanian iris samples.

***Iris mild mosaic virus* (IMMV)** was detected in all investigated Lithuanian and Ukrainian iris plants. **IMMV c**auses mosaic in the leaves consisting of pale-green and yellowish green stripes. Flowers show a breaking pattern as stripes or spots (Fig. 1).

Fig. 1. Flower breaking symptoms induced by IMMV on iris flower

The test–plants were inoculated by mechanical sap inoculation. Virus infected only *Chenopodium quinoa* causing chlorotic local lesions. EM investigation of negatively stained dip preparations from naturally infected plants and inoculated *C. quinoa* plants revealed the presence of flexuous filamentous particles, 750 nm long (Fig. 2).

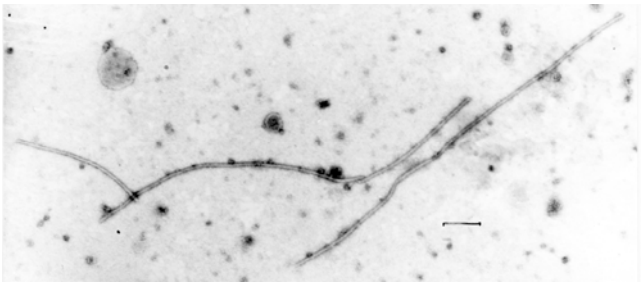

Fig. 2. IMMV particles. Bar represents 100 nm

IMMV is a member of *Potyvirus* genus. Virus is distributed worldwide, most important cultivars are totally infected [1]. Virions filamentous; usually flexuous with modal length of 750 nm. Transmissible in non persistent manner by *Aphis gossipi, Macrosiphum euphorbiae*, and *Myzus persicae* [2].

***Tobacco mosaic virus* (TMV) w**as isolated from irises bearing symptoms of chlorotic turning to necrotic mottling and streaks on leaves (Fig. 3).

Fig. 3. Symptoms of TMV on iris leaves

The test–plants were inoculated by mechanical sap inoculation. The most specific reaction to this virus was induced on *Nicotiana glutinosa* expressed by local dark necrotic ringspots, which appeared in 2–3 days after inoculation. Local (L) lesions were induced by virus infection on following test-plants: *Amaranthus caudatus, A. paniculatus, Chenopodium amaranticolor, C. foetidum, C. quinoa, C. urbicum*; local necrotic spotting on *Datura metel, Datura stramonium, Gomphrena globosa, Nicandra physalodes; Petunia hybrida, Physalis floridana, Tetragonia expansa* reacted expressing grey necrotic spots and leaf necrosis. *Nicotiana debneyi* and *N. tabacum* 'Samsun' showed local necrotic spotting, followed by systemic (S) mosaic and leaf distortion. *N. rustica* developed local necrotic spots; systemic reaction was expressed by vein and plant top necrosis. EM investigation of negatively stained dip preparations from naturally infected plants and inoculated test-plants revealed the presence of rod-shaped particles, 300 nm long. Symptomatic host plant and inoculated test–plants were tested in DAS–ELISA. All tested plants gave clearly expressed positive reaction confirming TMV infection.

Total RNA for performing RT–PCR was extracted from frozen leaf tissue of naturally infected iris plant and inoculated *C. quinoa* (L), *N. glutinosa* (S) and *N. rustica* (S) test-plants. Leaf tissue from healthy *N. glutinosa* was used as negative control (K–). TMV isolate from *Petunia hybrida* was used as positive control (K+). Specific for TMV PCR products were obtained with all investigated samples and positive control, but not with negative control. Specific bands in agarose gel of analyzed products after electrophoresis at a position corresponding to the expected size of amplification product of 422 bp were obtained, confirming TMV identity (Fig. 4).

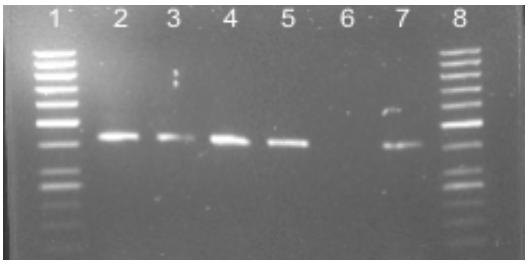

Fig. 4. RT–PCR products of amplified DNA fragments from TMV (EF 1 % agarose gel).
1, 8 – DNA size standard SMO371,
fragment sizes: 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50 bp.;
2 – iris (host-plant); 3 – *C. quinoa* (L);
4 – *N. glutinosa* (S); 5 – *N. rustica* (S); 6 – K–; 7 – K+.
Size of TMV specific product 422 bp

On the basis of particle morphology data, symptom expression on inoculated test–plants, positive reaction in DAS–ELISA test, RT–PCR results, and in accordance with published descriptions of TMV [2], the virus with rod-shaped particles isolated from irises was identified as TMV.

Tobacco ringspot virus **(TRSV)** was isolated from irises expressing symptoms of chlorotic streaks and ringspots (Fig. 5).

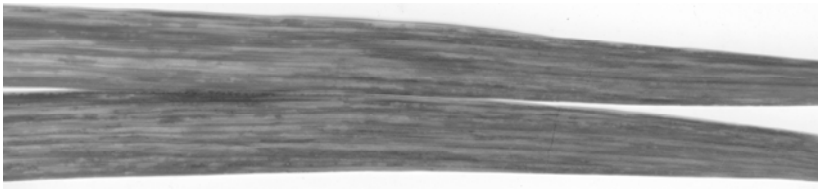

Fig. 5. Symptoms of TRSV on iris leaves

Virus has been identified basing on positive reaction in DAS–ELISA, EM data and test–plant inoculation results. Specific for TRSV local symptoms have been observed on *Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Petunia hybrida, Tetragonia expansa*, systemic symptoms on *Cucumis sativus, Datura stramonium, Nicandra physalodes, Nicotiana tabacum, Phaseolus vulgaris.* The inoculated test–plants have been tested in DAS–ELISA. Both tested plants gave clearly expressed positive reaction confirming TRSV infection. EM investigation revealed isometric particles 30 nm in diameter in symptomatic test–plants.

RT–PCR was carried out with tissues from two naturally infected plants. TRSV isolate from *Echinacea purpurea* was used as positive control (K+), leaf tissue from healthy *N. glutinosa* as negative control (K–). Specific for TRSV bands in agarose gel of analyzed products after electrophoresis in 1 % agarose gel at a position corresponding to the expected size of the amplification product of 348 bp have been obtained with both iris samples and positive control, but not with negative control (Fig. 6).

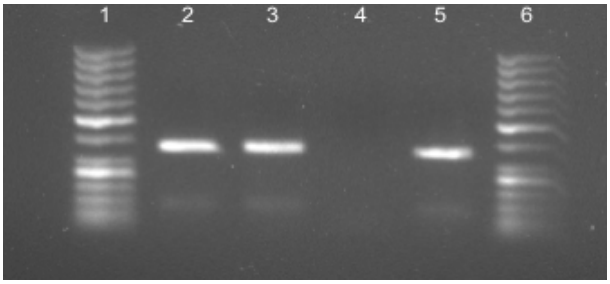

Fig. 6. RT–PCR products of amplified DNA fragments from TRSV (EF 1 % agarose gel).
1, 6 – DNA size standard SMO371;
2, 3 – irises (host-plants); 4 – K–; 5 – K+.
Size of TRSV specific product 348 bp

On the basis of test–plant reaction, morphology of virus particles, DAS–ELISA, RT–PCR results and according to literature data [2] it was established, that virus with isometric particles affecting iris plants was TRSV.

TRSV is a type member of *Nepovirus* genus. It is transmitted by nematodes *Xiphinema americanum* and other closely related *Xiphinema* spp.*, Thrips tabaci*, some aphid and mite species are reported as vectors of this virus. Reports of natural spread are largely confined to North America but the virus has been disseminated to many countries in infected planting material. It causes serious disease problems in those regions where the nematode vectors also occur. Particles are 30 nm in diameter sedimenting as three components and with a bipartite RNA genome. The virus is readily transmitted by sap inoculation and has a wide host range, including woody and herbaceous plants, annuals and perennials [2]. Ornamental hosts including *Anemone* L.*, Begonia* L.*, Crocus* L.*, Gladiolus* L.*, Hyacinthus* L.*, Hydrangea* L.*, Impatiens* L.*, Iris* L.*, Lilium* L.*, Narcissus* L.*, Rosa* L.*, Tulipa* L. have been reported as host plants of TRSV. TRSV has been isolated and identified from *Dicentra* Bernh.*, Gladiolus* L.*, Echinaceae* Moench. and *Tulipa* L. in Lithuania [7].

Tomato ringspot virus **(ToRSV)** was isolated from irises bearing viral symptoms, similar to symptoms induced by TRSV, chlorotic streaks and ringspots on leaves.

EM investigation of negatively stained dip preparations from naturally infected plants revealed the presence of isometric particles, 30 nm in diameter. The test–plants were inoculated by mechanical sap inoculation. Virus induced local and systemic reaction in inoculated test–plants except *Nicotiana glutinosa*, *N. rustica* and *Physalis floridana*, which reaction was only local. *Celosia argentea, Gomphrena globosa* and *Chenopodium quinoa* developed the most conspicuous and specific for ToRSV reactions. Inoculated leaves of *C. argentea* showed dark brown ringspots, which later extended along veins and became necrotic. Systemic reaction was expressed by malformation of leaves, vein chlorosis, small brown spots and lines located mainly on the base of leaf lamina. *G. globosa* developed local necrotic spots. Systemic reaction was expressed by mild leaf distortion and light green spots on leaves. Inoculated *C. quinoa* leaves showed local chlorotic spots, necrosis, distortion, systemic reaction was expressed by distortion of young leaves, chlorotic and necrotic spots on the base of leaf lamina. Tip of plant was distorted with necrosis and turned downwards.

EM investigation of negatively stained dip preparations from infected test–plants revealed the presence of isometric particles, 30 nm in diameter.

Symptomatic host plants and inoculated test–plants were tested in DAS–ELISA. All tested plants gave clearly expressed positive reaction confirming ToRSV infection.

RT–PCR was carried out with leaf tissue from naturally infected host–plant and inoculated *C. quinoa* (S). ToRSV isolate from pelargonium was used as positive control (K+), leaf tissue from healthy *Nicotiana glutinosa* as negative control (K–). Specific for ToRSV bands of analyzed products after electrophoresis in 1 % agarose gel at a position corresponding to the expected size of the amplification product of 449 bp have been obtained with both samples (Fig. 7).

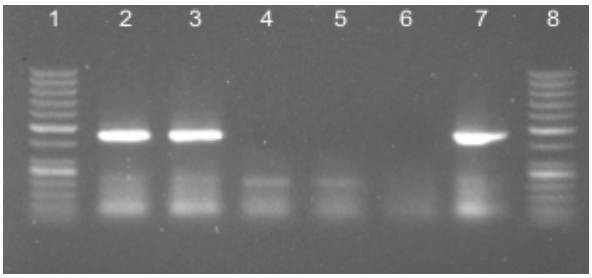

Fig. 7. RT–PCR products of amplified DNA fragments from ToRSV (EF 1 % agarose gel).
1, 8 – DNA size standard SMO371;
2 – iris (host-plant); 3 – *C. quinoa* (S); 6 – K–; 7 – K+.
Size of ToRSV specific product 449 bp

On the basis of test–plant reaction, morphology of virus particles, DAS-ELISA, RT-PCR results and according to literature data [2] it was concluded, that investigated iris plant was infected by ToRSV.

ToRSV is a member of genus *Nepovirus* and causes economically important diseases in a wide range of crops. It is readily transmissible by inoculation of sap and has a wide host range, including both woody and herbaceous plants. It is transmitted by the nematode *Xiphinema* spp. Seed transmission has been reported in several crops. The infected plants show distinctive symptoms as a shock reaction. Chronically infected plants usually exhibit no obvious symptoms but show a general decline in productivity [2]. The virus occurs in nature mostly in perennial crops. Ornamental hosts including *Anemone* L.*, Gladiolus* L.*, Hydrangea* L*, Iris* L.*, Narcissus* L.*, Pelargonium* L'Her.*, Petunia* Juss. sp. have been found naturally infected by ToRSV. The range of ToRSV host–plants in Lithuania includs 15 ornamental plant species belonging to 10 botanical families [8].

Identification of ArMV and CMV viruses by the RT-PCR have been described earlier [9, 10].

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SPREAD AND PHYLOGENETIC RELATIONSHIPS OF *WHEAT DWARF VIRUS* ISOLATED IN UKRAINE AND HUNGARY

Вірус карликовості пшениці (ВКП) є типовим патогеном злакових культур та викликає значні втрати врожаю. У даній роботі досліджувалося поширення ВКП в агроекосистемах України. Результати свідчать про високу гомологію українських ізолятів з європейськими представниками ВКП та про наявність унікального ячмінного ізоляту, здатного до інфікування рослин пшениці.

Wheat dwarf virus (WDV) is ubiquitous virus in cereals causing huge losses. This paper analyses spread of WDV in Ukrainian agriecosystems. The results show high homology of Ukrainian isolates to European representatives of WDV and presence of unique barley isolate capable of infecting wheat.

Introduction. During the last decade *Wheat dwarf virus* (WDV) became the most frequently isolated and most ubiquitous cereal-infecting virus in Hungary and now it becomes a serious problem in Ukraine [19, 25]. WDV is a frequent causal agent of dwarfing, mottling, yellowing or reddening in cereals and suppressed heading and root growth in infected plants can drastically reduce yield. WDV was first described by Vacke (1961) [29] in the former Czechoslovakia and later it has been found in Sweden [18], Bulgaria [24], Hungary [3], France [16], Germany [7], Poland [8], Finland [15], Romania [9], Spain [1], Tunisia [20], Turkey [12], Zambia [11], Ukraine (for the first time approximately in 1975, then in 2007) and China [30]. WDV is transmitted by the leafhopper *Psammotettix alienus* [29]

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