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).

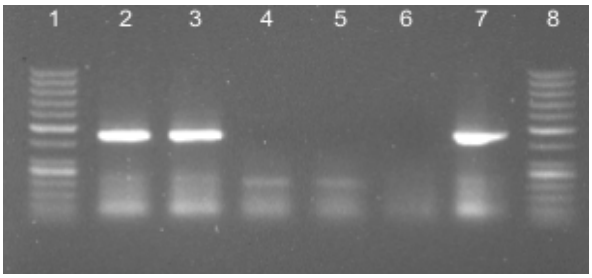

Fig. 7. RT–PCR products of amplified DNA fragments from ToRSV (EF 1 % agarose gel).
1, 8 – DNA size standard SMO371;
2 – iris (host-plant); 3 – *C. quinoa* (S); 6 – K–; 7 – K+.
Size of ToRSV specific product 449 bp

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]

in a circulative, non-propagative manner [17] therefore the occurrence of diseased plants in the field depends on the presence of the vector.

WDV belongs to the genus *Mastrevirus* (family *Geminiviridae*) infecting monocotyledonous plants. Mastreviruses have a monopartite single-stranded genome of circular DNA and the genome encodes four different proteins: movement protein (MP) and coat protein (CP) on the viral-sense strand, while two replication-associated proteins (Rep and RepA) on the complementary-sense strand [4]. The presence of an intron in the Rep gene makes it possible for WDV to produce two different forms of the replication protein. The non-coding long intergenic region (LIR) and short intergenic region (SIR) contain sequence elements necessary for viral replication and transcription. The LIR comprises the origin of rolling circle replication of the virus [6]. The SIR contains polyadenylation signals, and a region to which a short complementary primer for the second strand synthesis binds [10].

Two different forms of WDV exist: a wheat-adapted form (WDV wheat strain) and a barley-adapted form (WDV barley strain) [2, 14, 17]. Both strains infect plants in the family Poaceae [17]. There are, however, contradictory reports as to whether the wheat strain can infect barley, and barley strain can infect wheat [13, 17, 22, 27]. According to Kundu et al. (2009) [13], barley strain is restricted to the barley host, while the wheat strain is present in both wheat and barley plants.

The genomes of the barley and wheat strains of WDV share an average of 85% identity, whereas the isolates within wheat strain show high degree of homology (>98%), when the isolates of barley strain are more variable (>94 %). As the demarcation criterion for mastrevirus species has been set to 75% nucleotide sequence identity by the International Committee for Taxonomy of Viruses, both strains are currently considered to belong to the same species. Recently Schubert et al. (2007) [22] surveyed cereal samples and based on DNA sequence differences they proposed the new mastrevirus species *Barley dwarf virus* (BDV) and *Oat dwarf virus* (ODV*)*. ODV was accepted as a new tentative mastrevirus species sharing 70% genome-wide nucleotide sequence identity with the wheat and barley strains of WDV.

The aim of this study was to make molecular characterisation of WDV isolates from Hungary and Ukraine and compare them with the available sequences of WDV.

Materials and Methods. *Virus isolates.* The symptoms of viral infection were found during spring observations carried out in wheat crops in Martonvasar (Middle Hungary), Pula (Southern Hungary) and Mironivka (Middle Ukraine). Plants manifesting yellowing of leaves or dwarfing were placed in an insect-proof greenhouse and were tested for WDV with ELISA using a WDV kit (Bio-Rad No. 31202) or rabbit polyclonal WDV-specific antisera (Loewe, Germany). The collected WDV-infected plants were replanted into clay pots and placed in an insect-proof isolation net. For use as a vector organism, thirty individuals of virus-free *Psammotettix alienus* Dahlb. were placed underneath each net. One week later the leafhoppers were transferred to young seedlings (10 cm long) of wheat. Six weeks later the plants were tested again for WDV with ELISA. Three isolates WDV-HU-2Marton:08 (collected in 2008 from Martonvasar), WDV-HU:Pula:07 (collected in 2007 from Pula) and WDV-Uk:Mironivka:09 (collected in 2009 from Mironivka and maintained in our greenhouse by subsequent transmission) were selected for further studies. Ten wheat samples from Odessa region (South Ukraine) and one from Glevakha (Central North Ukraine) were initially tested by PCR. Two samples (WDV-Uk:Odessa:09 and WDV-Ukg:08 collected from Odessa and Glevakha respectively) were selected for molecular characterization.

Isolation of virus DNA, cloning and sequence analysis of the WDV isolates. DNA extraction and amplification were done according to Shepherd et al. (2008) [23]. A small leaf sample (approximately 40 mg) from WDV-positive plants was placed into an Eppendorf tube covered with 50 µl extraction solution (Extract-n-Amp^{TM} Plant PCR kit, Sigma) and heated at 95^{o}C for 10 min. Lastly 50 µl of dilution solution (also supplied with the kit) was added and the sample was then stored at -20^{o}C or used directly as a template for rolling circle amplification (RCA) of the WDV genome [5]. One microliter of the final Extract-n-Amp DNA solution was mixed with 4 µl of Templi Phi^{TM} sample buffer (TempliPhi^{TM}, Amersham Biosciences), heated for 2 min at 94 ^{o}C, and then brought to room temperature. Five µl of reaction buffer and 0.2 µl of enzyme mix were added to the cooled mixture and the Templi Phi^{TM} extension reaction was run at 30 ^{o}C for 18 – 20 h. WDV genome concatemers generated during Phi29 DNA polymerase amplification were digested with *Hind*III to release unit-length genomes. After digestion genomic DNA was separated in 1 % agarose gels and extracted by a DNA purification kit (Fermentas DNA Extraction Kit). The WDV genome was inserted into a *Hind*III digested pBSK+ plasmid (Stratagene). The recombinant plasmids were transformed into *Escherichia coli* DH5α [21].

Clones containing inserts with the expected size of 2.7 kb were sequenced with the DyeDeoxyTerminator Kit (Applied Biosystems) using reverse, universal (-20) and internal primers. Sequence analysis was performed using University of Wisconsin Genetics Computer Groups (GCG) sequence analysis software package version 9.1.

In order to determine the phylogenetic relationships between different isolates complete genomes were analysed. Sequence alignment, tree formation, and bootstrap analysis were done with the help of the software Clustal X 1.83.

Results. *WDV spread in Ukraine*. Large scale survey of agriecosystems where grain cereals are regularly cultivated in Ukraine has been conducted for WDV diagnostics. In particular, fields in Kyiv (North), Kharkiv (East), Cherkassy (Center), Khmelnytsk (West), Kherson and Odessa (South) regions have been screened giving rather large cover of the country. Winter wheat and winter barley plants were sampled in each region. In addition, in Kyiv, Kherson and Odessa regions wild cereals have also been identified with typical WDV symptoms or sampled symptomless. These plants have further been put into *Deschampsia sp.* genus. In total, more than 500 samples have been collected and subsequently analyzed serologically, and also some of them via TEM. It should be said that apart from symptomatic plants sampled in Kharkiv and Kyiv region we have also detected vectors for this virus – leafhoppers *Psammotettix alienus*. Their occurrence is an indirect proof for significant spread of WDV in Ukrainian agriecosystems and also points on changes in climatic conditions towards an increase in average annual temperature.

Further, WDV diagnostics have been carried out for taken samples by DAS ELISA (LOEWE kits, Germany). Most of the samples were shown WDV-positive. Electronic microscopy of some of WDV-positive samples confirmed presence (however, in very low quantity) of typical geminivirus particles of about 22-24 nm in diameter.

Level of WDV spread of Ukraine has been established and tentative list of cultivars/lines of wheat and barley (cultivated in surveyed regions) which are susceptible or insusceptible/tolerant/resistant has been proposed. Statistical data shows that average level of WDV infection spread in cultivated cereals was 6-9% in 2009, comparing to maximum 5% in 2006. Coupled with other outcomes, it highlights the tendency towards positive dynamics of WDV spread in Ukrainian ecosystems. WDV has probably become the second biggest virus threat to cereals in Ukraine, with BYDV being the first.

Virus isolates. The collected wheat plants were WDV infected as confirmed by ELISA tests and PCR. The virus was transmitted by *P. alienus* and maintained on wheat plants. Nucleic acids were isolated from plants infected with WDV-HU-2Marton:08, WDV-HU:Pula:07, WDV-Uk-g:08 and WDV-Uk:Odessa:09, WDV-Uk-g:08 and WDV-Uk:Mironivka:09.

Virus DNA isolation, cloning and sequence analysis of the WDV strains. The Extraction-n-Amp DNA extraction method was very rapid and simple in order to isolate the full length WDV genomes. Combined with the RCA method, a vast number of virus genome concameters were produced, which were digested with *Hind*III and released as unit-length genomes (Fig. 1) with sticky ends. The size of the full length genome prepared from WDV-HU-2Marton:08 and WDV-HU:Pula:07 were 2750 nucleotides, WDV-Uk-g:08 and WDV-Uk:Mironivka:09 were 2749 nt, and WDV-Uk:Odessa:09 was exactly 2734 nucleotides. The very special properties of this isolate that it has originated from wheat plant. In the literature there is no data that barley strain of WDV could be isolated from naturally infected wheat plant. The genome contained all four expressed mastrevirus ORFs (MP, CP, Rep, RepA), and the intergenic regions LIR and SIR.

The nucleotide sequences were deposited into GenBank as FN806783 (WDV-Uk-g), FN806784 (WDVUk-Miron), FN806785 (WDV-HU-2Marton), FN806786 (WDV-HU-Pula) and FN806787 (WDV-Uk-Odessa), and were further compared to previously characterized WDV isolates [26, 27, 28].

The sequence analysis of the full genome revealed high levels of identity among wheat strains and high level of diversity among barley strains. The sequences' identities between isolates of the wheat strain from different geographical origin are very similar (>98.7 %). Inside the movement protein (MP) and coat protein (CP) we observed high sequence identity (>98.8 %) at amino acid level, in some cases MP were even identical (between Hungarian and Swedish isolates), or CP (between Hungarian isolates originating from different part of the country). In the short intergenic region (SIR) and large intergenic region (LIR) we observed higher variability (97 % and 96.6 % respectively) (data not shown). Regarding the diversity of WDV isolates of barley strain we observed higher variability (96.3 % – 99.4%). Interestingly, MP and CP at amino acid level revealed high level of identity among barley strain isolates originating from different geographical region (98.5 % – 100 %). Barley strain isolates showed greater variability also in the LIR and SIR. Molecular characterization of Ukrainian and Hungarian WDV isolates was followed by phylogenetic analysis in order to compare the relationships with previously characterized wheat and barley isolates from the GenBank (Fig. 1). The phylogenetic analysis of WDV isolates showed that they are clearly distinguishable, both barley and wheat strains form two clades. Clade 1 could be subdivided into two groups formed by wheat isolates from Hungary and Sweden in one and wheat isolates from China in the other group. In Clade 2 WDV-Uk-Miron and WDV-Swe-Enk2 clusterized in one subgroup and the other Ukrainian isolate WDV-UK-g grouped with isolates from Czeh Republic and Germany. Similar situation can be observed with barley isolates forming two clades, which could be subdivided into more subgroups. Both clades represent a pool a divergent isolates. Clade 3 could be devided into 3 or 4 subgroups formed by German and Czech isolates.

Clade 4 could be grouped into three subgroups, the first is formed by Hungarian barley isolates, Ukrainian and Bulgarian isolates belong to subgroup two and Barley isolate from Turkey form the third subgroup. Within this clade subgroups correspond to the geographical origin of isolates of WDV barley strain.

The phylogenetic analysis showed some isolates are out clades like WDV-Ge-SxA22, WDV-Ge-ScBB21, WDV-Cz19 and WDV-Swe-SE and ODV-Ge-SxA25. The last one is understandable since *Oat dwarf virus* has only about 70 % genome-wide nucleotide sequence identity with barley and wheat strain isolates of WDV.

Discussion. The research confirms rather wide spread of Wheat dwarf virus in Ukraine with positive tendency and infavorable forecast. There no WDV-insusceptible/resistant varieties of cereals cultivated in Ukraine. Unique virus vector has been repeatedly detected in Ukrainian ecosystems proving need for careful virus/vector monitoring and selection/growing of tolerant cultivars.

Five isolates (two Hungarian and three Ukrainian) originated from wheat plants were sequenced and compared with previously characterized barley and wheat strains of WDV. Four isolates belonged to wheat strain of WDV, which present a high degree of identity throughout the complete genome regardless that they are originated from different geographical origin. Ukrainian isolates were 2749 nucleotides long, while Hungarian isolates consists of 2750 nt.

WDV-Odessa isolate was a barley strain of WDV having 2734 nt long genome. This is the first data that barley isolate of WDV could infect wheat in natural conditions.

Wheat strain isolates present a low level of variability, while barley strain isolates showed higher level of diversity.

Phylogenetic analysis showed clear differences of barley and wheat strains of WDV isolates. Both strains could be divided into two clades and barley strain subdivided into more subgroups. Isolates grouped into the subgroups of barley clade 3 are in a good correlation with the geographic origin.

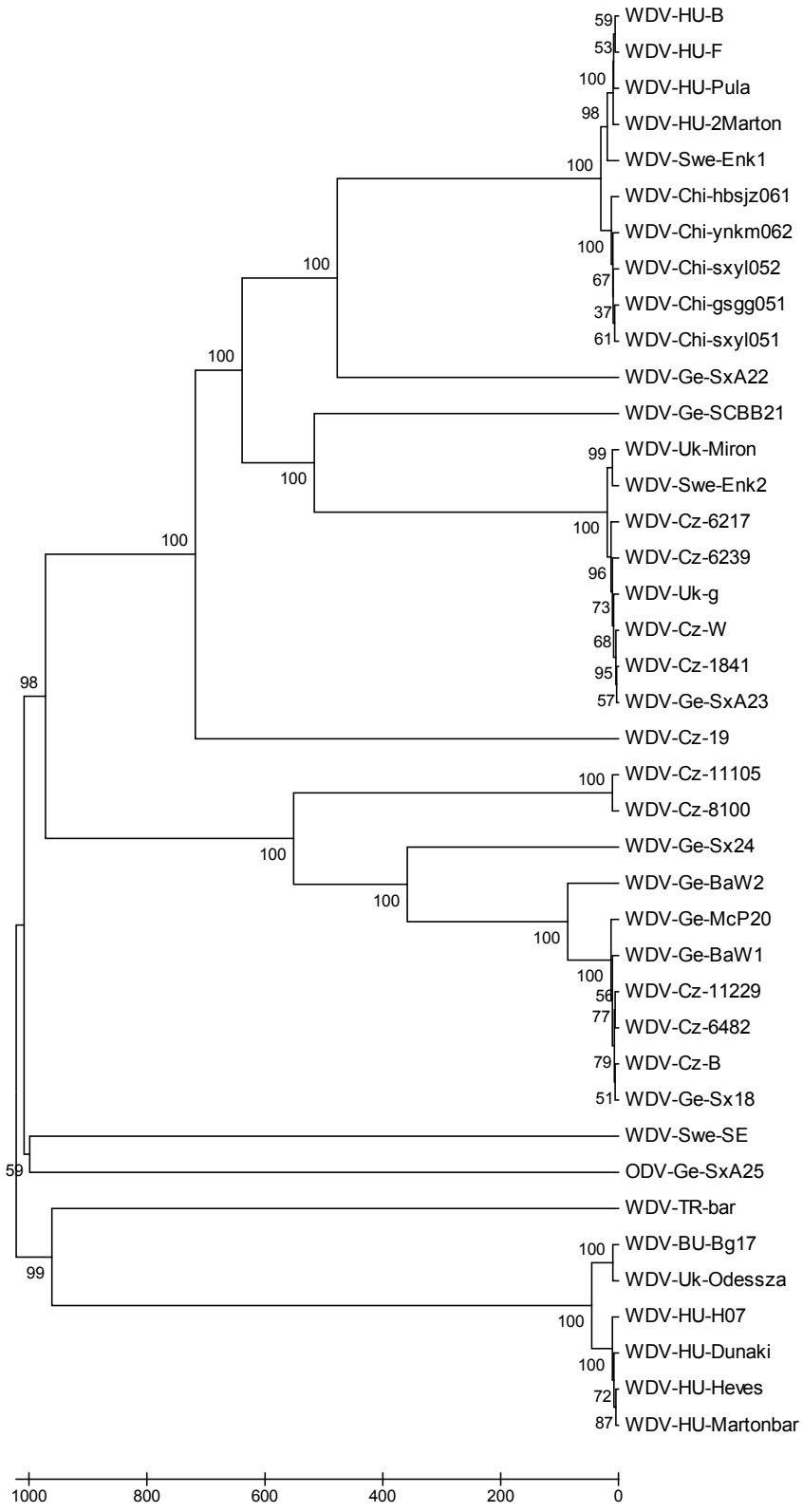

Figure 1. Phylogenetic tree constructed by UPGMA method for complete genome sequences of *Wheat dwarf virus* isolates (Bootsrap values are indicated)

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PHYTOVIROLOGICAL STATE OF PEAR VARIETIES AND ROOTSTOCK ORCHARDS

Визначено рівні інфікованості насаджень груші вірусами ACLSV, ASGC, ASPV та AMV. Зроблено статистичну оцінку спорідненості даних вірусних агентів до окремих хазяїв на основі перевірених типів підщеп груші.

Levels of viral infection caused by ACLSV, ASGC, ASPV and AMV in pear treas are detected. On the base of tested pear rootstocks statistical analysis of the viral pathogenes affinity to different hosts was made.

Introduction. The area of industrial plantations of pear – one of the main traditional fruit crops of Ukraine, which significantly declined in 90th years, now gradually increasing. Therefore, the need for virus free planting material of this culture is growing every year. Present time Ukraine takes the last but one place in production of pear fruits and leaves behind only Poland [1]. Among factors that cause decrease of pear areal in Ukraine are negative impact of environment, presence of pests and significant spread of causative agents of fungal, bacterial and viral diseases. These factors decline productivity of pear orchards and its profitability and as a consequence make no expediency to create new gardens.

In this context immunodiagnostics of varieties and rootstocks become very important. It gives possibility to conduct not only monitoring of viruses but also to select virus free samples and to create base of virus free mother plants of economically-valuable pear varieties and rootstocks.

During the last years on the base of Department of Virology and Propagation of Fruit and Berry Cultures of Institute of Horticulture inspections of pear orchards are regularly conducted in different regions of Ukraine. These surveys enable to reveal in time trees infected with the complex of latent viruses and to use in further gardening only virus free material.

Materials and methods. For detection of viral diseases by ELISA we collected samples in productive, collection and nursery orchards of pear in 2006-2008 yeas in period of intensive growth during May-July, when concentration of virus in plant tissue is the highest [4]. Altogether 224 samples of perspective cultivars were tested, which included 47 pear varieties. Also 271 samples of pear rootstocks which included 20 traditional and new breeding forms of clone rootstock types were investigated. Surveys were conducted in gardens of Institute of Horticulture UAAS, Crimean Research Station of UAAS and Podil Research Station of UAAS. Immunodiagnostics was carried out by classic ELISA and DAS-ELISA. Certified antibodies for ACLSV, ASGV, ASPV and ApMV produced by Loewe Phytodiagnostica, Germany and Bioreba AG, Switzerland, were used for investigation purposes. Results of analysis were registered by microplate spectrophotometer STAT FAX 2100, USA.

Percent of samples infected with virus *i* in orchards of certain type *k* was calculated according to equation:

$$Fik = \frac{N_{ik}}{N_k} \cdot 100\% \qquad (1)$$

where N_k – number of tested samples, N_{ik} – number of samples infected with virus *i* in orchards of type *k*.

The general infection level of virus *i* in all types of pear orchards (Figen) was calculated according to equation:

$$Figen = \sum_{1}^{k} Nik \cdot 100\% \Big/ \sum_{1}^{k} Nk \qquad (2)$$

For the estimation of phytovirological state of the plantations of each of pear clonal rootstock the null hypothesis was checked about the random distribution of each of the four analyzed viruses in those orchards with the use of 2x2 contingency table. The data about the distribution between the samples of certain type of rootstocks infected and non infected by an individual virus in the 2x2 contingency table were contrasted to the distribution between infected and non infected samples of the rest of the rootstocks concerning the same virus. The χ^2 criteria and contingency coefficient *Phi* were calculated. Such approach made it possible to determine the total resistance potential or, on the contrary, susceptibility of certain rootstocks to each virus as compared to the rest of the pear clonal rootstock orchards. The negative *Phi* coefficient values were considered as an indication of the certain resis-