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NOVEL TOBAMOVIRUS ISOLATED FROM VEGETABLE CROPS IN UKRAINE

Here we report on the novel tobamovirus, Pepper mild mottle virus (PMMoV), endangering vegetable crops of Solanaceae family which has been isolated in Ukraine for the first time. Virus particles' size and morphology are studied. It has been established that Ukrainian PMMoV isolate belongs to a cluster with strains from Spain and Korea.

Keywords: Tobamovirus, polymerase chain reaction, phylogeny, PMMoV.

Introduction. During the recent years plant virologists (as well as other scientists) have witnessed an increased interest to the advance of knowledge at the level of population and similar ecologically-oriented research. This trend is common for many traditional molecular biology studies. The development of a new generation of diagnostic methods for plant viruses (such as ELISA, RIA, RIPA, PCR, RT-PCR, etc.) during the last decade enabled a new level of studying plant viruses' spread in the environment.

The relevance of this question lies in both its fundamental and practical significance. The determination of virus spread, mechanisms of virus transmission, natural range of host plants, research on virus response to environmental changes makes it possible not only to more fully characterize a given representative of the Vira kingdom, but also to predict the emergence and development of viral diseases for developing sound strategies of combating the viral infections. This includes search for resistant varieties, control of virus reservoirs and carriers, obtaining virus-free planting material, etc.

At present, the representatives of *Tobamovirus* genus are widespread in Ukraine and remain the point of interest for field virologist due to the harm they cause for crops, particularly for plants of *Solanaceae* family.

This work was aimed at establishing diversity and spread of tobamoviruses infecting plants of *Solanaceae* family, phylogenetic analysis of nucleotide sequences for coat protein of newly discovered pathogen (*Pepper Mild Mottle Virus*).

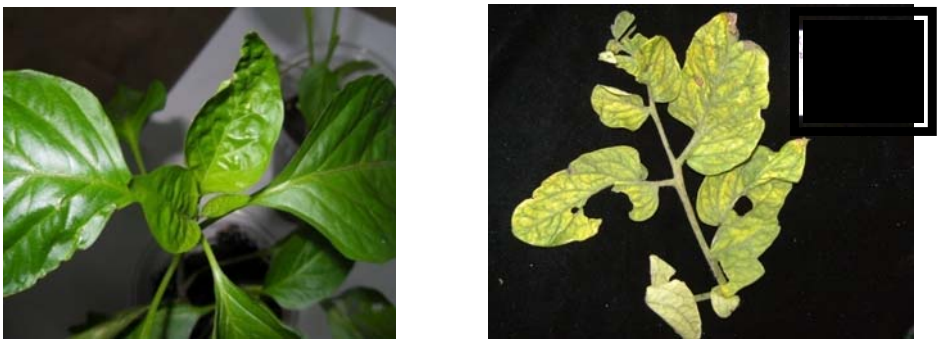
Materials and methods. We used samples of sweet pepper plants (*Capsicum annuum*) and tomato plants (*Lycopersicon esculentum*) collected in Crimea, Vinnytsia, Zhytomyr, Kyiv and Poltava regions – very differing parts of Ukraine in geographical terms. Sampled plants were characterized with virus-like symptoms.

These samples were then tested for the presence of antigens of *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), and *Pepper mild mottle virus* (PMMoV) (typical tobamoviruses infecting these cultures) by DAS-ELISA using commercial antisera from Loewe (Germany) and Prime Diagnostics (The Netherlands).

We have also used the following metods: transmission electron microscopy, total RNA extraction, NA electrophoresis, RT-PCR, nucleic acid sequencing, phylogenetic analysis. For RT-PCR, we have used primers specific to a part of the coat protein gene of PMMoV: 5-TAC TTC GGC GTT AGG CAA TC-3 (forward), 5-GGA GTT GTA GCC CAG GTG AG-3 (reverse).

Results and discussion. DAS-ELISA analysis showed that 22% of symptomatic pepper samples collected in Poltava region contained PMMoV, and 22% of tomato samples were contaminated by ToMV. In Zhytomyr region, 11% of tomato samples contained PMMoV. In Kiev region, 33% of pepper samples were positive for ToMV, as well as 11% of tomato samples. In Vinnytsia region, we have shown that 22% of pepper samples and 22% of tomato plants were infected by ToMV. In Crimea (probably the biggest region in Ukraine for growing tomato and pepper in the open field conditions), 11% of pepper samples and 33% of tomatosamples were shown positive for ToMV.

Collected samples of *Capsicum annuum* and *Lycopersicum esculentum* plants had typical virus-like symptoms (Fig. 1A, 1B).



A B

Figure 1. Virus-like symptoms:
A – deformation of leaves of *Capsicum annuum*; B – light green mosaics on leaves of *Lycopersicum esculentum*

We have used transmission electron microscopy for confirmation of DAS-ELISA results and to study the morphology of virus particles (ToMV, PMMoV).

Microscopy studies demonstrated the presence of rod-shaped virus particles typical of *Tobamovirus* genus, namely, for the *Pepper mild mottle virus* and *Tomato mosaic virus* whose size constituted 310±3 x 15±3 nm and 300±3 x 19±3 nm, respectively (Fig. 2, 3).

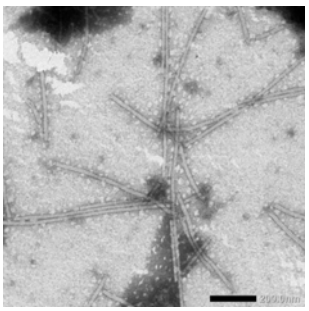


Figure 2. Electron micrography of PMMoV

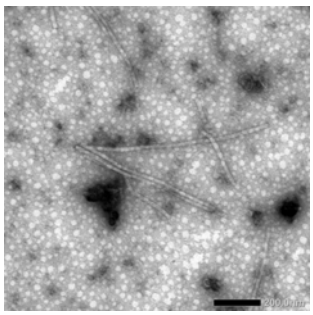


Figure 3. Electron micrography of ToMV

Further, we have extracted a total RNA preparation from PMMoV-infected tomato plants to use the resulting product in RT-PCR.

The total RNA extraction was carried out RNeasy Plant Mini kit (Qiagen, UK) following the manufacturer's recommendations. The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel using TBE buffer.

The next step was the RT-PCR using primers specific to the coat protein gene of PMMoV (Fig. 4).

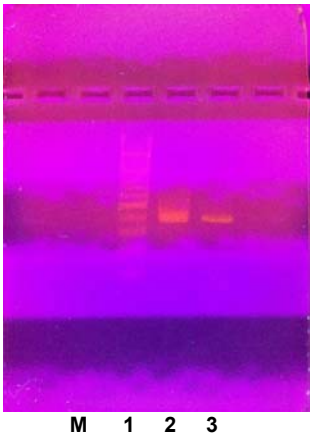


Figure 4. Results of RT-PCR for PMMoV:
M – marker (100bp, Fermentas);
1 – cDNA of capsid protein gene of PMMoV; 2 – positive control; 3 – negative control

We have obtained the cDNA of expected size of 387 bp which was further used for sequencing and construction of a phylogenetic tree (Fig. 5). Phylogenetic tree was constructed using the Neighbor-Joining method.

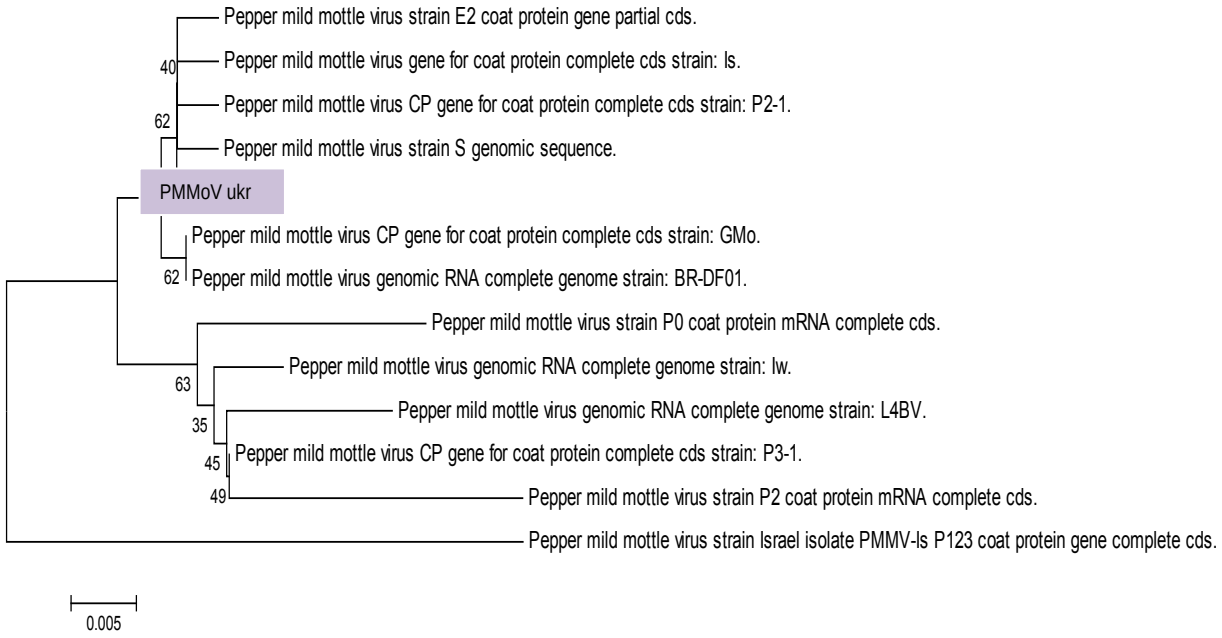


Figure 5. Phylogenetic tree (NJ) showing evolutionary relations of Ukrainian isolate of PMMoV with published virus sequences (based on the coat protein gene)

Thus, the Ukrainian PMMoV isolate belongs to a cluster containing virus strains found in Spain and Korea.

Conclusions. According to the DAS-ELISA, tobamoviruses PMMoV and ToMV are prevalent among vegetable crops in the studied regions of Ukraine. Electron microscopic studies confirmed the presence of rod-shaped virus particles typical of *Tobamovirus* genus, namely, for the *Pepper mild mottle virus* and *Tomato mosaic virus* whose size is approximately 310±3 x 15±3 nm and 300±3 x 19±3 nm, respectively. We have generated a cDNA of 387 bp corresponding to the coat protein gene of Ukrainian PMMoV isolate. Phylogenetic analysis of the coat protein gene of PMMoV showed that Ukrainian PMMoV isolate groups into a cluster with strains detected in Spain and Korea.

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НОВИЙ ПРЕДСТАВНИК РОДУ *TOBAMOVIRUS*, ВИДІЛЕНИЙ З ОВОЧЕВИХ КУЛЬТУР В УКРАЇНІ

Знайдено новий для України вірус овочевих культур, зокрема рослин родини Solanaceae, визначено розмір та морфологію його вірусних часток. Встановлено приналежність українського ізоляту PMMoV до кластерів штамів вірусів Іспанії та Кореї.

Ключові слова: Tobamovirus, полімеразна ланцюгова реакція, філогенія, PMMoV.

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НОВЫЙ ПРЕДСТАВИТЕЛЬ РОДА *TOBAMOVIRUS*, ВЫДЕЛЕНЫЙ ИЗ ОВОЩНЫХ КУЛЬТУР

Найден новый для Украины вирус овощных культур, в частности растений семейства Solanaceae, определен размер и морфология вирусных частиц. Установлено принадлежность украинского изолята PMMoV к кластерам штаммов вирусов Испании и Кореи.

Ключевые слова: Tobamovirus, полимеразная цепная реакция, филогения, PMMoV.

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PHYLOGENETIC ANALYSIS OF HOSTA VIRUS X ISOLATED IN UKRAINE

Hosta virus X (Potexvirus, HVX) was identified by ELISA in different cultivars of hosta selected from M.M. Hryshko National botanical garden of Ukraine (NAS of Ukraine). Coat protein gene sequences of Ukrainaian HVX isolate was determined and compared with all known strains and isolates from Gene Bank. Phylogenetic trees were constructed using neighbour-joining and maximum likelihood methods. The CP of Ukrainian isolate shared 99–100% nucleotide and amino acid sequence identity with American isolates.

Key words: Hosta virus X, ELISA, Gene Bank.

Introduction. Hosta spp. are popular herbaceous perennial plants with more than 7000 varieties, and widely cultivated due to their diversity in leaf shape and color patterns, shade tolerance and pest resistance. Hosta virus X *(Potexvirus*, HVX) is a very serious problem for hosta growers. This virus was first identified and described in Minnesota, USA in 1996. Since then, HVX has been reported from other US states, Canada, Europe as well as from other continents. HVX is generally considered to be the most economically important virus infecting hostas [1]. As HVX is an emerging disease that is causing problems for growers, garden centers, and gardeners, the EPPO Secretariat felt that HVX could usefully be added to the EPPO Alert List. As HVX is sap-transmissible, it is easily transmitted during vegetative plant propagation. Hostas can also be propagated by seeds, but the possible seed transmission of HVX needs to be clarified. As is the case for other potexviruses, HVX is also spread by mechanical contact. Therefore, it is easily transmitted from plant to plant on hands and tools (e.g. pruning tools when removing old leaves or flowers). Over long distances, trade of infected plants has probably been the most significant source of the disease. In addition, it is suggested that some cultivars which have been selected and commercialized because of their 'interesting foliage' were in fact infected by HVX, which has contributed to further spreading the virus. There is no evidence that HVX might be transmitted by insects or other vectors [2, 3].

As is the case for other viruses, the control of the disease is difficult and essentially based on the use of resistant cultivars and of prophylactic measures to minimize the possibility of mechanical transmission of HVX. The production of virus-free planting material through the implementation of certification schemes could also contribute to limiting the spread of HVX [4,5].

We have previously shown that HVX infected some varieties of Hosta plants from the M. M. Gryshko National Botanic Gardens. The aim of this study was to carry out a phylogenetic analysis of Ukrainian isolate of HVX.

Materials and methods:

The samples of various hosta cultivars were collected from M. M. Gryshko National Botanical Garden (NAS of Ukraine). The investigated hosta cultivars included: Hosta Sum and substance, Hosta Striptease, Hosta Lady Guinevere, Hosta spp, Hosta Venticosa, Hosta Udulata, Hosta. Halcyon, Hosta. Crispula Maek, Hosta Gold Standart, Hosta Great Expectation, Hosta Ultraviolet light, Hosta Mediovariegata, Hosta spp, Hosta, Hosta August Moon, Hosta Twilight, Hosta Paul Glory, Hosta Siboldiana, Hosta Whirlwind, Hosta Abigua, Hosta Wide Brim. The samples were selected from plants with the following symptoms: systemic chlorosis, veinal chlorosis interveinal chlo-