UDK 578.85/.86

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DETECTION OF *ARABIS MOSAIC* AND *TOMATO ASPERMY VIRUSES* INFECTING ORCHIDS OF NATURAL UKRAINIAN FLORA

The orchids collected from natural biocenosis and from collection of M. M. Gryshko' National Botanical Garden were studied. Two types of viral pathogens were identified in the samples of orchids: Arabis mosaic virus and Tomato aspermy virus.

Key words: orchids, Arabis mosaic virus, Tomato aspermy virus.

Introduction. *Orchidaceae* Juss. is one of the biggest families of flowering plants including about 35 000 species. They can be found all over the world excluding deserts and Polar Region [5]. Viral diseases of orchids are known from the middle of XX century [7]. As for today, more over 30 viruses of orchids have been described [9, 11]. The majority of these viruses were detected in tropical orchids cultivated *in situ*. On the other hand the viruses of terrestrial orchids of temperate zone are studied not enough and require immediate attention as they endanger rare plant species.

All orchids of Ukrainian natural flora are listed in the Red Book of Ukraine [2]. These plants are characterized by complicated developmental biology and require specific symbiotic fungi and distinct entomophily. Therefore, orchids have low degree of recovery, high sensitivity to environmental changes and anthropogenic factors. Viruses pose a biotic stress factor and can directly affect the general condition of the gene pool in plants' populations. Viruses transferred to natural biocenosis from agriecosystems are the most dangerous because they are often highly pathogenic for the new hosts.

The antigens of A*rabis mosaic virus* (ArMV)*, Bean yellow mosaic virus* (BYMV)*, Tomato aspermy virus* (TAV), *Tobacco rattle virus* (TRV) and *Turnip mosaic virus* (TuMV) were detected in orchids of natural Ukrainian flora (from Carpathians and Crimea regions) in previous investigations [2]. Also, the infection of *Cypripedium* sp., *Orchis sp.*, *Ophrys* sp. by TRV and TuMV has been described [7].

Materials and methods. The samples of 20 different species of orchids were surveyed during 2011-2013 yy. The plants were collected in Cherkasy (Kaniv Natural Reserve), Crimean, Kherson, Kyiv, Lviv, Zakarpattia regions and in collection of M. M. Gryshko' National Botanical Garden.

Virus identification was carried out using standard DAS-ELISA [1]. The samples were prepared by homogenizing plant tissue with 0.1 M phosphate buffer (pH 7.4) in the ratio 1:3 (m/v), and followed by centrifugation at 5000 rpm for 20 min. For the diagnostics were used polyclonal antiserums to TMV (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), TuMV (antiserum kindly provided by Lesemann D.E., Julius Kühn Institute, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Germany), TAV, ArMV, *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Iris yellow spot virus* (IYSV), (Loewe, Germany) and *Potato virus Y* (PVY) (Prime Diagnostics, The Netherlands).

Biological properties of viruses were studied using the range of test plants. 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. EM was carried out using a JEOL-1400 electron microscope at the magnification 40 000 and 60 000.

Results and discussion. For detection and identification of viruses affecting terrestrial orchids were used 47 samples of plants collected in Ukraine.

To confirm the infectivity of the plant sap, we used biological testing on the range of test plants: *Amaranthus caudatus, Brassica oleracea, Capsicum annuum, Celosia argentea, Chenopodium amaranticolor, Cucumis sativus, Datura stramonium, Gomphrena globosa, Lycopersicon esculentum, Nicotiana alata, N. benthamiana, N. rustica, N. tabacum, Petunia hybrida, Phaseolus vulgaris, Tetragonia expansa, Zinnia elegans.* Such range of test plants is typical for many viruses of ornamental plants [5, 8].

According to biotesting the most typical symptoms were necrotic lesions, yellowing and deformation of leaves on *Datura stramonium*; chlorotic spots and deformation of leaves on *Petunia hybrida*; necrotic lesions and mosaic on leaves of *N. benthamiana*; necrotic lesions and vein chlorosis on leaves of *Chenopodium amaranticolor*; necrotic lesions, deformation of leaves and wilting on *Tetragonia expansa* (fig. 1).

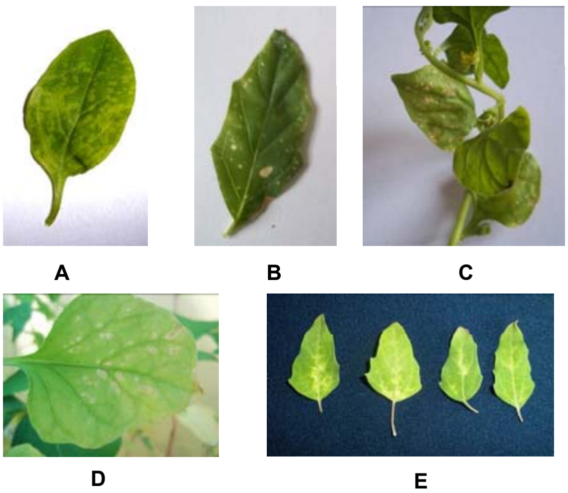

Figure 1. Symptoms on test plants inoculated with the sap of orchids: A – chlorotic spots on the leaves of *Petunia hybrida*; B – necrotic spots on the leaves of *Datura stramonium*; C – necrotic lesions and deformation of the leaves of *Tetragonia expansa*; D – necrotic spots on the leaves of *Nicotiana benthamiana*; E – vein chlorosis symptoms on the leaves of *Chenopodium amaranticolor*

The electron microscopy of the orchids sap was carried out simultaneously with biotesting. Three types of spherical virus-like particles was revealed in infected plant tissues. The particles with diameter of about 30 nm were detected in plants of *Anacamptis laxifolia, Dactylorhiza majalis, Platanthera bifolia* (Zakarpattia region), *A. palustris, Epipactis helleborine* (Kaniv Natural Reserve), *E.palustris, D. incarnata* (Kyiv region), *Gymnodenia conopsea* and *Listera ovata* (Lviv region) (fig.2, A, B).

The virus-like particles with diameter of 50 nm were found in plants of *A. picta* (Kherson region), *E.palustris, D. incarnata* (Kyiv region) and *Platanthera bifolia* (Kaniv Natural Reserve) (fig.2, C, D). According to literature data, spherical virions with diameter of about 50 nm are typical for representatives of *Caulimoviridae*: *Caulimovirus, Cavemovirus, Petuvirus, Soymovirus* [10]. The majority of viruses belonging to the listed genera can be transmitted by mechanical inoculation. The reaction of test plants eliminates such viruses as *Blueberry red ringspot virus*, *Cestrum leaf curling virus*, *Petunia vein clearing virus* and *Tobacco vein clearing virus* which are not transmitted in mechanical manner.

The particles with diameter of about 80-100 nm, typical for representatives of *Tospovirus*, were revealed in the samples of *A. picta* (Kherson region) (fig. 2, E, F).

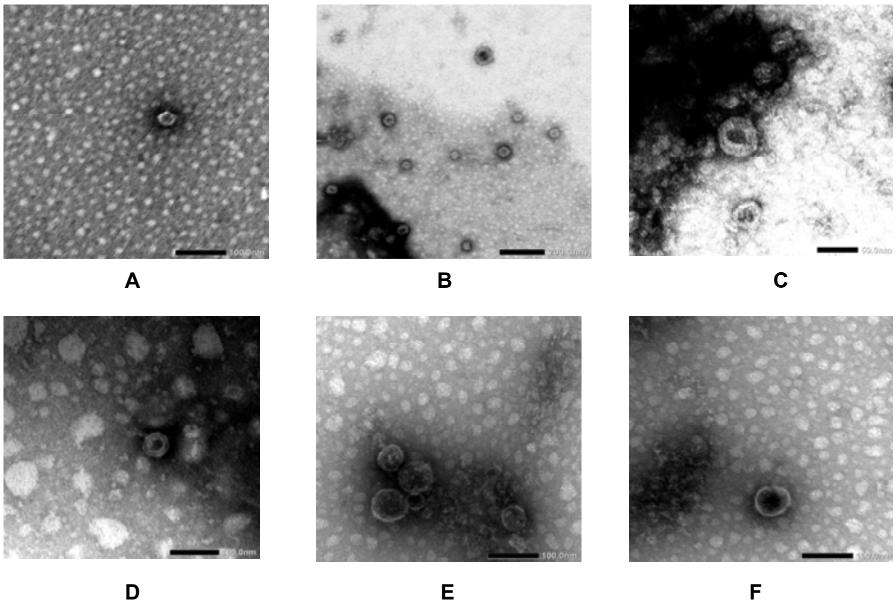

Figure 2. Electron micrograph of virus particles in the sap of infected plants. Virus-like particles with diameter of about 30 nm: A – *A. palustris* (bar 100 nm); B – *E. palustris* (bar 200 nm). Virus-like particles with diameter of about 50 nm: C – *A. picta* (bar 50 nm); D – *P. bifolia* (bar 100 nm). Virus-like particles with diameter of about 80-100 nm: E, F – *A. picta* (bar 100 nm)

For the virus identification in samples of orchids we used DAS-ELISA with antiserums to viruses, which are widespread in agriecosystems of Ukraine and to those which have been previously detected in orchids of temperate climatic zone [2, 7]. According to ELISA testing the antigens of *Tomato aspermy virus* were detected in the samples of orchids *Anacamptis laxifolia, Dactylorhiza majalis, Platanthera bifolia* (Zakarpattia region), *A. palustris, Epipactis helleborine* (Kaniv Natural Reserve), *E.palustris, D. incarnata* (Kyiv region), *Gymnodenia conopsea, Listera ovata* (Lviv region), *Cephalanthera longifolia, Cypripedium calceolus* (M. M. Gryshko National Botanical Garden); and antigens of *Arabis mosaic virus* were revealed in plants of *D. romana*, *Cephalanthera longifolia* and *P.bifolia* (M. M. Gryshko National Botanical Garden).

Conclusion. The biotesting confirmed the infectivity of the sap samples of orchids. EM studies also confirmed the presence of virus-like particles in infected plant tissues with the virions with diameter of about 30 nm, 50 nm and 80-100 nm. Two types of pathogens detected in orchids were identified as *Arabis mosaic virus* and *Tomato aspermy virus*.

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Received to editorial board 07.12.13

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ВИЯВЛЕННЯ ВІРУСІВ МОЗАЇКИ АРАБІСУ ТА АСПЕРМІЇ ТОМАТІВ СЕРЕД ОРХІДНИХ ПРИРОДНОЇ ФЛОРИ УКРАЇНИ

Проведено обстеження терестріальних орхідних природніх біоценозів України та колекції орхідних Національного ботанічного саду імені М.М. Гришка. Ідентифіковано два вірусних патогени серед досліджених рослин: вірус мозаїки арабісу та вірус аспермії томату.

Ключові слова: віруси орхідних, вірус мозаїки арабісу, вірус аспермії томатів

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ВЫЯВЛЕНИЕ ВИРУСОВ МОЗАИКИ АРАБИСА И АСПЕРМИИ ТОМАТА СРЕДИ ОРХИДНЫХ ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

Проведено обследование террестриальных орхидных природных биоценозов Украины и коллекции орхидных Национального ботанического сада имени Н.Н. Гришка. Идентифицированы два вирусных патогена среди исследованных растений: вирус мозаики арабиса и вирус аспермии томата.

Ключевые слова: вирусы орхидных, вирус мозаики арабиса, вирус аспермии томата

UDK 578.85/.86: [581.19:547.56]

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THE INFLUENCE OF ANTROPOGENIC LOAD AND OF THE PHYTOVIRAL INFECTION ON THE SYNTHESIS OF PHENOLIC COMPOUNDS

Have been shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds. Also, shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Key words: anthropogenic load, infestation by viruses, phenolic substances.

Introduction. One of the ecological and biochemical mechanisms of plant adaptation in the complex influence of anthropogenic factors and viral infections. are the changes in the composition and in the quantitative ratio connections of the compounds in the antioxidantal group, in particular-phenolic nature [1].

Phenolic compounds – one of the most important classes of secondary metabolites, widely represented in plants. Various functions of phenolic compounds in the plant cell, and at the same time a wide range of biological effects on humans and animals, justify study of its participation in the development of protective reactions of organisms in adverse conditions of existence [2]. The literature on the effect of stress factors on the content of phenolic compounds in plants is fragmentary and contradictory. However, it clearly indicate the variability of these parameters [3].

This fact confirms the assumption of importante role of phenolic compounds in the development of resistance of plants to changes in the intensity of natural and anthropogenic stress factors [4].

According to the all written above, the goal of the research was:

– to analyze the influence of the location of the place of grown about the factors of anthropogenic load on the fitovirusological state of plants of sugar beet (*Beta vulgaris* L) and content of phenolic compounds in them;

– to confirm the in vitro correlation between virus reproduction and synthesis of phenolic compounds.

Materials and methods. Method of random samples from agrocenosis with varying degrees of anthropogenic load on visual symptoms were selected sugar beet plants, which further were investigated by undirected enzyme-linked immunosorbent assay for the presence of viral antigens and achohol extraction method for determining the concentration of phenolic compounds [5, 6]. Leaf blades, which were selected for the ELISA, were dried and measured their content of phenolic compounds.

Analysis of results. ELISA was demonstrated the dependence of the distribution and antigen detection percent in plants from the place of sampling. The highest content of phenolic compounds in plants we recorded in the areas with a high degree of anthropogenic load and, accordingly, the greatest defeat by viral pathogens.

This interconnection has been saved in relation to other locations.

To confirm the interconnection between the accumulation of viral particles and the change in the level of concentration of phenolic compounds, we made a laboratory experiment on the model of "sugar beet plant – TMV."

The content of phenolic compounds in plants infected by a virus increased irregularly, compared with the control.

When we compared the dynamics of accumulation of viral antigens and phenolic compounds in sugar beet plants infected with TMV, this dependence can be traced as: in the period between 3 and 6 day after an infection we observed a slight increase of the concentration of viral antigens against the background of the concentration of phenolic compounds. From 6 to 10 day a number of fenolic compounds increases even faster than in the previous period. The concentration of the TMV antigens in this time wasn't changing.

Findings. This way, we have shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds.

It is shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Sugar beet – biennial plant and can't be used for laboratory model "virus – a plant" and is not suitable for laboratory researches we are doing the selection of the optimal model system for the study of interference of viruses and synthesis of phenolic compounds in the plant and the use of phenolic compounds by the plants when there are some stress factors, like abiotic and biotic nature.

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Received to editorial board 07.12.13