

DETECTION OF *TOMATO ASPERMY VIRUS* IN WILD-GROWING ORCHIDS IN KYIV REGION

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*The orchids *Dactylorhiza incarnata* and *Epipactis palustris* of natural Ukrainian flora collected in Kyiv region were studied. Two types of pathogens were detected in samples of orchids. One of these pathogens was identified as *Tomato aspermy virus*.*

Key words: orchids, viruses, natural flora

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 [10, 12]. 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. The most dangerous are viruses transferred to natural biocenosis from agriecosystems because they are often highly pathogenic for the new hosts.

The antigens of *Arabis 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. Plant material was collected in Kyiv region. For further investigation we used plants of *Dactylorhiza incarnata* and *Epipactis palustris* with typical viral symptoms.

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 following test plants: *Amaranthus caudatus*, *Celosia argentea*, *Gomphrena globosa*, *Zinnia elegans*, *Brassica oleracea*, *Chenopodium amaranticolor*, *Cucumis sativus*, *Phaseolus vulgaris*; *Datura stramonium*, *Nicotiana alata*, *N. benthamiana*, *N. rustica*, *N. tabacum*, *Petunia hybrida*, *Tetragonia expansa* [5,8]. 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. The survey for visual symptoms on orchids was carried out during 2011-2012 yy. In the majority of cases plants of

D. incarnata showed necrotic spots and occasionally mild mosaic. The plants of *E. palustris* were mostly symptomless, only a few plants had distinct necrotic spots on their leaves.

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]. The ELISA testing revealed antigens of TAV in the samples of *D. incarnata* and *E. palustris* (Fig. 1).

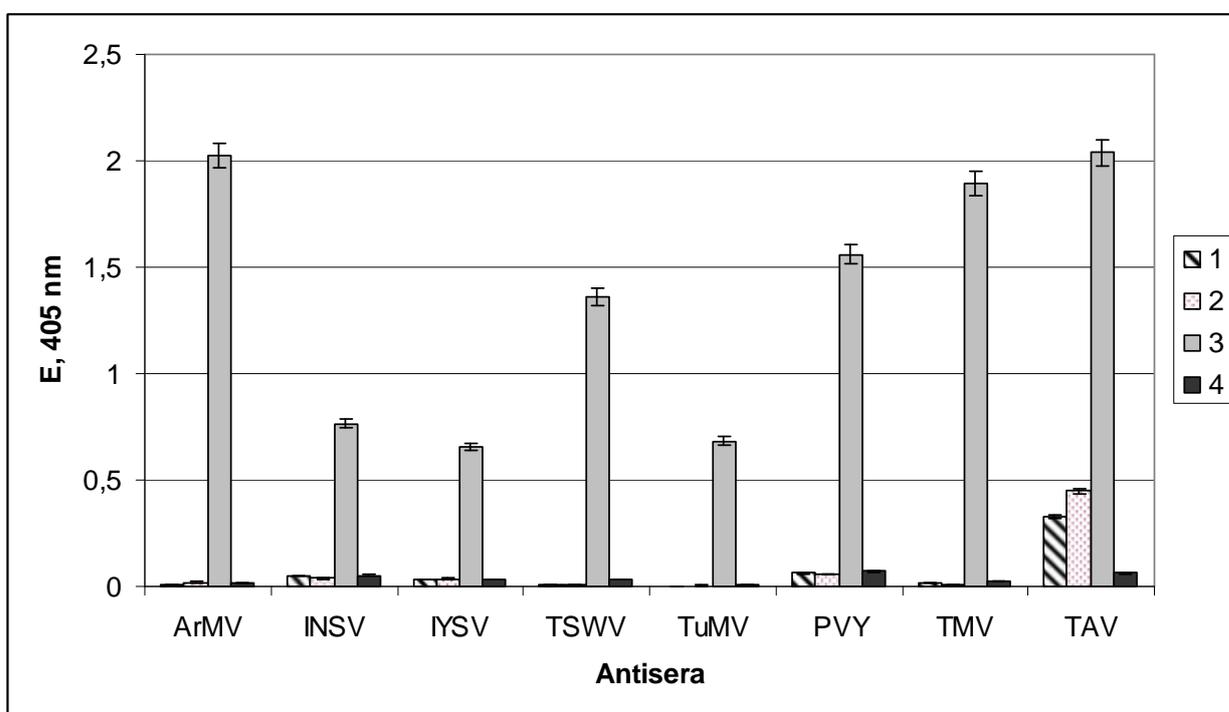


Fig. 1. The results of DAS-ELISA: 1 – *D. incarnata*, 2 – *E. palustris*, 3 – positive control, 4 – negative control.

To confirm the infectivity of the plant sap, used biological testing on the range of test plants, which are typical indicators for many viruses of ornamental plants [5, 8]. The test plants inoculated with the sap of *D. incarnata* and *E. palustris* showed only systemic reaction (s). The visual symptoms of viral infection were observed on the plants of *D. stramonium* (s), *N. benthamiana* (s), *P. vulgaris* (s), *T. expansa* (s). It should be noted that the test-plants inoculated

with the sap of *D. incarnata* and *E. palustris* reacted similarly, the difference was only in the level of the symptoms manifestation (Table).

**Reaction of the test-plants inoculated with the sap of
D. incarnata and *E. palustris***

Test-plants	Symptoms	
	<i>Dactylorhiza incarnata</i>	<i>Epipactis palustris</i>
<i>Amarantus caudatus</i>	–	–
<i>Brassica oleraceae</i>	–	–
<i>Celosia argentea</i>	–	–
<i>Chenopodium amaranticolor</i>	–	–
<i>Cucumis sativus</i>	–	–
<i>Datura stramonium</i>	Chlorotic lesions, leaves deformation	Chlorotic lesions, wilt
<i>Gomphrena globosa</i>	–	–
<i>Nicotiana glauca</i>	–	–
<i>Nicotiana benthamiana</i>	Systemic necrosis, yellowing	Yellowing
<i>Nicotiana rustica</i>	–	–
<i>Nicotiana tabacum</i>	–	–
<i>Petunia hybrida</i>	–	–
<i>Phaseolus vulgaris</i>	Vein clearing, mottle	Mottle, yellowing
<i>Tetragonia expansa</i>	Wilt	Wilt
<i>Zinnia elegans</i>	–	–

The electron microscopy of the orchids sap was carried out simultaneously with biotesting. In the samples of *D. incarnata* and *E. palustris* two types of virus-like particles were detected with diameter of 48-52 nm and 30-33 nm (Fig. 2).

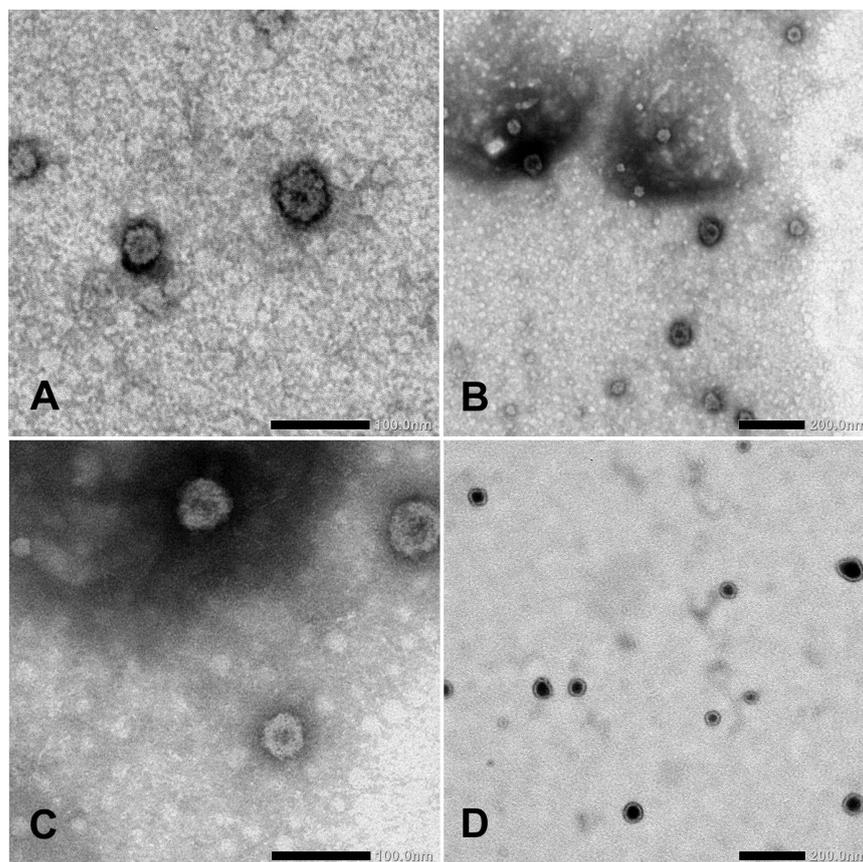


Fig. 2. Electron micrograph of virus-like particles in the sap of infected plants: A, B – *D. incarnata* (bar 100 nm and 200 nm, respectively); C, D – *E. palustris* (bar 100 nm and 200 nm, respectively)

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.

Considering the results of DAS-ELISA we can assume that the virus-like particles with diameter of about 30 nm are virions of TAV.

CONCLUSION. The biotesting of the sap samples of symptomatic plants of *D. incarnata* and *E. palustris* confirmed the infectivity of plant sap. EM studies also confirmed the presence of virus-like particles in infected plant tissues with the virions with diameter of about 30 nm and 50 nm. According to the results of

ELISA viral particles with the size about 30 nm can be attributed to *Tomato aspermy virus*.

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ВЫЯВЛЕНИЕ ВИРУСА АСПЕРМИИ ТОМАТОВ У РАСТЕНИЙ ОРХИДНЫХ ПРИРОДНОЙ ФЛОРЫ КИЕВСКОЙ ОБЛАСТИ

А.С. Перегудова, А.В. Коротеева, Т.А. Компанец, В.П. Полищук

Проведено обследование орхидных *Dactylorhiza incarnata* и *Epiractis palustris* природной флоры Украины, отобранных на территории Киевской области, на наличие вирусных инфекций. В образцах растений были детектированы два типа патогенов, один из которых идентифицировано как вирус аспермии томатов.

Ключевые слова: орхидные, вирусы, природная флора

ВІЯВЛЕННЯ ВІРУСУ АСПЕРМІЇ ТОМАТІВ У РОСЛИНАХ ОРХІДНИХ ПРИРОДНОЇ ФЛОРИ КИЇВСЬКОЇ ОБЛАСТІ

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Проведено обстеження орхідних *Dactylorhiza incarnata* та *Epiractis palustris* природної флори України, відібраних на території Київської області, на наявність вірусних інфекцій. У зразках рослин були детектовані два типи патогенів, один з яких ідентифіковано як вірус аспермії томатів.

Ключові слова: орхідні, віруси, природна флора