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SEVERAL VIRAL DISEASES OF *LYCOPERSICON ESCULENTUM* CIRCULATING IN UKRAINE

This paper describes detection of some typical plant viruses infecting Lycopersicon esculentum Mill. plants in Ukraine. Diagnostics using enzyme-linked immunosorbent assay (ELISA) confirmed presence of antigens of viruses belonging to Tobamovirus (PMMoV, ToMV), Cucumovirus (CMV) and Tobravirus (TRV) genera in sap of tomato plants. When studying viral diseases of tomatoes, monoinfection was shown to be prevalent. Tomato mosaic virus (ToMV) was most common.

Keywords: viral diseases, Tobamovirus, Cucumovirus, Tobravirus, tomato.

Introduction. Plant diseases are one of the factors suppressing the efficiency of agriculture. In this context, viruses infecting vegetable cultures are considered as pathogens having most prominent influence. Viral diseases can barely be controlled, lead to decreased yield and serious commercial losses in agriculture [1]. *L. esculentum* plants may be infected by a wide range of viruses from different families including *Bromoviridae* (*Cucumber mosaic virus* (CMV), *Tomato aspermy virus* (TAV)), *Virgaviridae* (*Tomato mosaic virus* (ToMV), *Potyviridae* (*Potato virus Y* (PVY)), *Secoviridae* (*Tomato ringspot virus* (ToRSV), *Tobacco ringspot virus* (TRSV), *Tomato black ring virus* (TBRV)), *Bunyaviridae* (*Tomato spotted wilt virus* (TSWV)), *Alphaflexiviridae* (*Potato virus X* (PVX), *Pepino mosaic virus* (PepMV)) and *Geminiviridae* (*Tomato yellow leaf curl virus* (TYLCV)) [2, 3].

Today, novel viruses appear and spread rapidly on tomato culture. These include *Tomato yellow leaf curl virus* (TYLCV), *Tomato Torrado virus* (ToTV) and *Pepino mosaic virus* (PepMV) [4].

Earlier, CMV, ToMV, Tobacco mosaic virus (TMV), TSWV [5], PVM and PVY have been detected on tomato plants in Ukraine [6].

This paper aims at studying diversity of viruses circulating in Ukraine and infecting *L. esculentum* plants.

Materials and methods. *L. esculentum* plants collected from different regions of Ukraine with virus-like symptoms were the objects of this study. Plant sample collection basing on the visual symptoms is considered to be the simplest and most common method. It is based on the ability of many viruses of inducing characteristic symptoms of infection developing in the form of bands/streaks of leaf blades, their deformations, shortening of stems and shoots (rosette-like appearance), leaf discoloration, formation of necrotic spots on leaves, etc. For this study, we collected samples of tomato with typical viral symptoms under open ground conditions in Kyiv, Poltava, Zhytomyr, Vinnytsya regions of Ukraine and in AR Crimea during 2012-2013 years.

For detection of virus antigens, we conducted DAS-ELISA with commercial test systems of Loewe (Germany) according to the manufacturer's recommendations in 96-well polystyrene plates (Labsystem, Finland). For ELISA, plant samples (vegetative organs and fruits) were homogenized in 0,1 M PBS + 0,001 M EDTA (1:2, v/v) with following sedimentation at 4000 rpm for 20 min at 4^0C using PC-6 centrifuge [7]. Such homogenate was used for ELISA. Collected samples were analyzed for presence of following viruses: *Cucumber mosaic virus*, *Tomato mosaic virus*, *Tobacco mosaic virus*, *Tobacco rattle virus*, *Tomato ringspot virus*, *Pepper mild mottle virus* and *Tomato yellow leaf curl virus*. Optical density values were registered using ELISA reader Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at the wavelength of 405/630 nm [7]. The percentage of infected plants was determined following generally accepted method of calculation described in [8]. Morphology of virions was analyzed using transmission electron microscope Jeol (JEM 1400) after staining with 2% uranil acetate [9].

Results and discussion. The disease symptoms were observed on *Solanaceae* plants (tomatoes, peppers and eggplants) in 1-1,5 month after sawing, during budding. First signs of infection were prominent on young leaves in the form of yellow spots and tissue clearing along the major veins. Further, diseased plants developed yellow or green mosaics, chloroses and necrotic spotting. Tomatoes were collected in Kyiv, Poltava, Zhytomyr, Vinnytsya regions of Ukraine and in AR Crimea. During the sampling, different symptoms were noted both on leaves and fruits.

On the leaf blades, varying sorts of mosaic symptoms were observed including bright green and yellow green mosaics, both interveinal and developed along the veins. Also, chlorosis and deformations were evident. Bronze coloring of leaves (typical for TSWV) has not been observed. Some fruits showed yellowish irregularly shaped spots or ring spots (Fig.1).

Figure 1. Yellow spotting on tomato fruit

Observed symptoms were similar to those known to be induced by vegetable viruses, including *Cucumber mosaic virus*, *Tobacco rattle virus*, *Tomato mosaic virus*, *Tobacco mosaic virus*, *Tobacco ringspot virus*, *Tomato ringspot virus* and many others. However, the similarity (convergence) of symptoms induced by different viruses on the same plants

cannot allow determining virus species. Thus, our further efforts were directed on establishing taxonomic positions of these viruses using additional methods. For identification of antigens of seven viruses in collected *L. Esculentum* samples, we have used double-antibody sandwich (DAS) ELISA with commercial test systems of Loewe (Germany). As such, we have confirmed the occurrence of four viruses: *Cucumber mosaic virus*, *Tomato mosaic virus*, *Tobacco rattle virus* and *Pepper mild mottle virus*.

This allowed evaluating the percentage of virus-infected samples of *L. esculentum* in the general number of samples used (Fig.2).

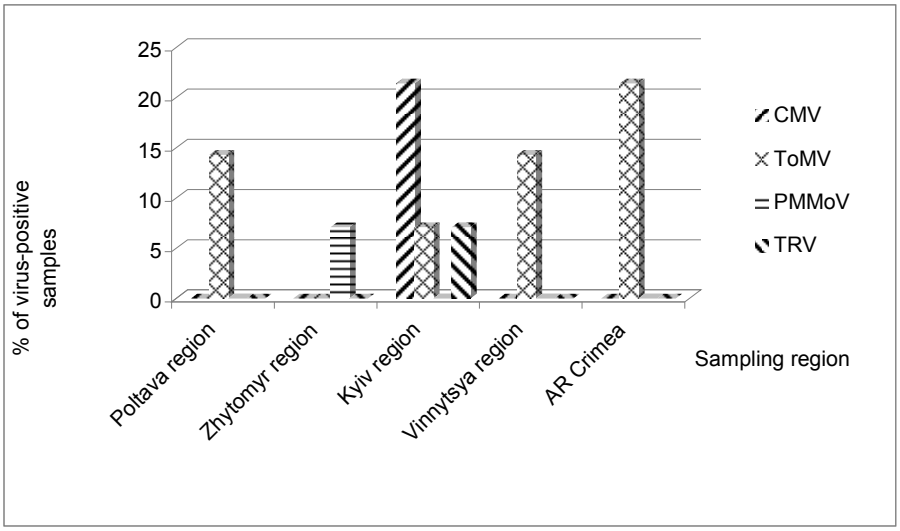

Figure 2. Percentage of samples of *L. esculentum* infected by *Tobamovirus*, *Cucumovirus*, and *Tobravirus* species in different regions of Ukraine

Therefore, 14,3% of tomato plants sampled in Poltava region contained ToMV antigens. 7,1% of tomato plants sampled in Zhytomyr region contained PMMoV antigens. In Kyiv region, 7,1% of tomato plants contained ToMV antigens, 7,1% – TRV antigens, and 21,4% – CMV antigens. In Vinnytsya region, 14,3% of tomato plants contained ToMV antigens, whereas in AR Crimea this pathogen has been found in 21,4% of samples.

Transmission electron microscopy (TEM) was used for direct indication of viruses in *Lycopersicum esculentum* plants, and to study virus morphology, particle dimensions, and to confirm ELISA results as well.

TEM results indicated the presence of rod-shaped virions of 300±3 x 19±3 nm typical for PMMoV (Fig.3).

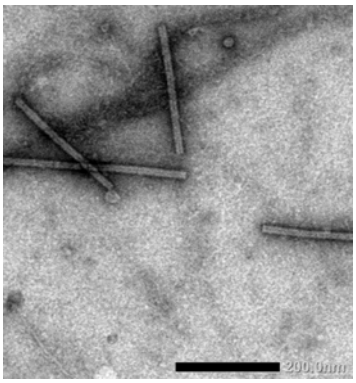

Figure 3. Electron micrograph of rod-shaped PMMoV virus particles

In addition, spherical virions have been found of 30 nm typical for CMV (Fig.4).

Therefore, screening of commercial tomato plantings using different approaches (ELISA and TEM) confirmed their infection induced by ToMV, PMMoV, CMV and TRV.

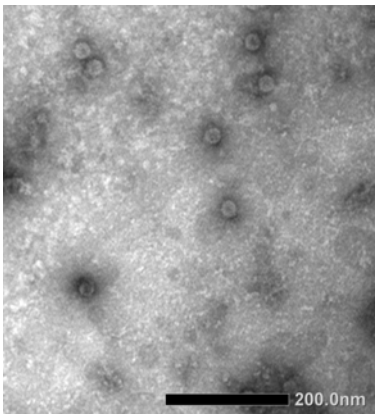

Figure 4. Electron micrograph of spherical CMV particles

Conclusions. Most typically tomato plants are affected by tomato mosaic disease induced by ToMV. Quite often plants are simultaneously infected by the complex of viruses which may include Cucumber mosaic virus (CMV), Potato virus Y (PVY), Potato virus X (PVX), Tomato mosaic virus (ToMV), Aspermy tomato virus (AsTomV), etc. In such cases the disease typically progresses more severely leading to higher yield losses making further plant cultivation unreasonable from the commercial point of view. Even genetic-based tomato resistance to certain viruses can be of no use as the resistance barrier becomes 'ruined' under the stress exerted by various pathogens at the same time [3]. The occurrence of antigens of viruses belonging to *Tobamovirus* (PMMoV, ToMV), *Cucumovirus* (CMV) and *Tobravirus* (TRV) genera has been confirmed by ELISA and, in part, by TEM. For sampled *L. esculentum* plants, viral monoinfection was predominant. *Tomato mosaic virus* has been most widespread in this study.

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ДЕЯКІ ВІРУСНІ ХВОРОБИ *LYCOPERSICON ESCULENTUM*, ЩО ЦИРКУЛЮЮТЬ НА ТЕРИТОРІЇ УКРАЇНИ

Робота присвячена детекції вірусів рослин, що інфікують L. esculentum на території України. За результатами ІФА визначено наявність антигенів вірусів родів Tobamovirus (PMMoV, ToMV), Cucumovirus (CMV) та Tobravirus (TRV). При дослідженні вірусних хвороб L. esculentum здебільшого зустрічалась моноінфекція. Найбільш розповсюдженим згідно наших дослідженнях виявився вірус мозаїки томату.

Ключові слова: вірусні захворювання, Tobamovirus (PMMoV, ToMV), Cucumovirus (CMV) та Tobravirus (TRV), помідори.

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НЕКОТОРЫЕ ВИРУСНЫЕ БОЛЕЗНИ *LYCOPERSICON ESCULENTUM*, ЦИРКУЛИРУЮЩИЕ НА ТЕРРИТОРИИ УКРАИНЫ

Работа посвящена детекции вирусов растений, которые инфицируют L. esculentum на территории Украины. По результатам ИФА определено наличие антигенов вирусов родов Tobamovirus (PMMoV, ToMV), Cucumovirus (CMV) та Tobravirus (TRV) При исследовании вирусных болезний L. esculentum в основном превалировала моноинфекция. Согласно нашим исследованиям более распротраненным оказался вирус мозаики томата.

Ключевые слова: вирусные заболевания, Tobamovirus (PMMoV, ToMV), Cucumovirus (CMV) та Tobravirus (TRV), томаты.

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FUSION EXPRESSION OF RECOMBINANT HUMAN BETA-DEFENSIN-3 AND ANALYSIS OF ITS BIOLOGICAL ACTIVITY

Human beta-defensins (hBDs) are small cationic antimicrobial peptides with multiple biologic activities. The aim of the study was cloning, expression in E.coli, purification and in vitro analysis of biological activity of recombinant human beta-defensin-3 (rec-hBD-3). hBD-3 cDNA was cloned into pGEX-2T vector, and recombinant plasmid was transformed into E.coli BL21(DE3) cells. Rec-hBD-3 was expressed in bacterial cells as GST-hBD-3 fusion protein, and purified by 3-step procedure via affine chromatography on glutathione-agarose, cleavage of fusion protein by thrombin, and reverse phase chromatography on Sep-Pack C18. Analysis of biological activity of rec-hBD-3 has shown that the peptide is active against Pseudomonas aeruginosa in micromolar concentrations in radial diffusion test. Rec-hBD-3 did not affect proliferation and viability of cultured human cancer cells of A431, A549, and TPC-1 lines, but was capable to potentiate cytotoxic effects of rec-hBD-2 and docetaxel in vitro.

Key Words: human beta-defensin-3, cancer cell, proliferation, viability, antimicrobial activity.

Intriduction. Human beta-defensins (hBDs) are small cationic antimicrobial peptides produced by different cell types. Human beta-defensins have been primarily recognized to possess a broad spectrum of antimicrobial activities, but as it has been shown later exhibit multiple biologic effects toward eukaryotic cells [1, 2]. In a number of studies there have been shown effects of hBDs on many important cell processes – cell proliferation, viability, differentiation, and apoptosis, and it has been shown that such effects of hBDs are concentration-dependent and could be exerted against many cell types [3-6]. Human β-defensin-3 (hBD-3; *DEFB103*) was firstly isolated in 2001 from human psoriatic lesions and cloned from keratinocytes and tracheal epithelial cells [7]. Mature hBD-3 molecule is composed from 45 aminoacid residues (molecular weight is 5.15 kDa) and possesses high cationic charge (+11). This defensin possesses potent broad spectrum activity against Gram-negative and Gram-positive bacteria but, unlike other studied members of beta-defensin family, antimicrobial activity of hBD-3 is found to be salt-insensitive. hBD-3 is an antimicrobial with an inducible expression which could be up-regulated upon stimulation with interferon-γ via STAT binding site in promoter region of *DEFB103* gene [8]. Expression of hBD-3 has been registered in many tumor types but its functional role remains unclear: hBD-3 is thought to play a role of pro-oncogenic molecule in some tumors (head and neck cancer, oral carcinoma [9, 10]) or tumor suppressor in salivary gland tumors [11]. Up to date, the role of hBD-3 in tumor cell biology is insufficiently investigated.

The present study was aimed on prokaryotic expression of hBD-3 (rec-hBD-3) and analysis of its biological activity *in vitro*.