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OCCURRENCE OF *CUCUMBER MOSAIC VIRUS* IN VEGETABLE CROPS IN UKRAINE

Symptomatic plants of Cucurbitaceae and Solanaceae families collected in Ukrainian agriecosystems used for commercial cultivation of vegetables have been analyzed. According to the ELISA results, 38 samples (of 126 samples in total, i.e., 30%) have been infected with Cucumber mosaic virus. CMV is widespread in Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. We have obtained a cDNA of 500 bp corresponding to the coat protein gene of Ukrainian CMV isolate.

Key words: Cucurbitaceae and Solanaceae families collected, ELIS.

Introduction. Viral infections cause considerable economic losses to farms engaged in vegetable growing. Usually large-scale assessment of such damages is conducted visually. However, the similarity of symptoms on plants caused by pathogenic agents of different nature, such as viruses, bacteria, fungi, in practice makes impossible to use effective means of treatment and prevention without well-defined identification of pathogens [1].

The only efficient way of controlling viral diseases is their timely diagnostics and putting in place respective preventive measures with regard to eliminate vectors and reservoirs of viral antigens, introducing resistant cultivars of plants and obtaining virus-free material [2].

Cucumber mosaic virus (CMV) belongs to genus *Cucumovirus* of family *Bromoviridae*. CMV infects about 200 species of plants belonging to 60 families, but most known for damaging cucumbers cultivated in the open field conditions [3]. CMV cause severe symptoms on plants and fruits, and greatly reduces the yield of the pumpkin crops. In recent years new data confirmed the expansion of this viral disease onto new territories together with spread of mixed and latent infections, emergence of new strains with altered pathogenicity.

The purpose of the work was to establish the distribution of *Cucumber mosaic virus* in vegetable crops in Ukraine.

Materials and methods. The samples were selected following the visual examination of virus symptoms. For virus detection, plant material was homogenized in 0.1M phosphate buffered saline (PBS), pH 7.4, 1:2 (m/v). Plant components were removed by centrifugation at 5.000 g for 20 minutes at +4˚C using centrifuge PC-6 [4]. The supernatant was taken for further using ELISA. ELISA was performed according to the recommendations of test-system manufacturer (DAS-ELISA) using 96-well plates ("Labsystem"). The results were read at the wavelength of 405/630 nm using microplate reader Termo Labsystems Opsis MR (USA) with software Dynex Revelation Quicklink [5]. Loewe test-system were used in ELISA for CMV detection. Morphology of purified virions was assessed under electron microscope JEM-1400 using 2% uranyl acetate as contrasting agent [6]. Total RNA extraction was carried out using RNeasy Plant Mini kit (Qiagen, UK). The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel. Reverse transcription reaction (RT-PCR) was performed using primers specific to the coat protein gene of CMV:

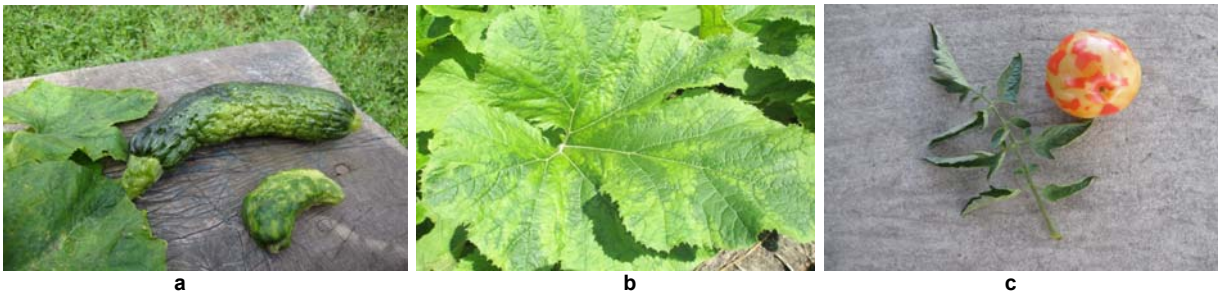
forward primer –
5' TATGATAAGAAGCTTGTTTCGCGCA-3'
reverse primer – 5'
TTTTAGCCGTAAGCTGGATGGACAACCC-3'

PCR products were analyzed by electrophoresis in 1.5% agarose gel using markers Gene Ruller 100 bp DNA Ladder plus (Fermentas, USA) [7].

Results and discussion. Approximately 126 plant samples belonging to the *Cucurbitaceae* and *Solanaceae* families were selected and tested for CMV. Plant samples were collected from following regions of Ukraine: Autonomic Republic of Crimea, Vinnytsia, Zaporizhzhia, Kyiv, Kirovohrad, Odessa, Poltava, Cherkasy and Chernihiv regions. Plants of *Cucurbitaceae* family (cucumber, squash, pumpkin, and zucchini) showed puckering, distortion, vein banding, yellowing, filamentary, yellow mosaic on leaf blade; dark green spots of different size, knobs and malformations on fruits (Fig.1). Plants of *Solanaceae* family (tomato, pepper, eggplant) showed disease symptoms in month after seedtime during flowering. The first symptoms were yellow spots and vein clearing on young leafs followed by systemic yellow and green mosaics, chloroses and local necroses (Fig.1).

Visual observation of external symptoms is an unreliable method for detection and identification of viral infection, because the appearance of viral infection mainly depends on interaction between a virus and a host. Besides, the strains of the same virus can often cause a variety of symptoms changing from hypersensitive to asymptomatic reaction on plants of the same species. The growing conditions and the presence of mixed infection can also effect the development of symptoms. For example, sometimes only mixed infection of pepper may lead to the appearance of mosaics and mottling. That's why the diagnosis of viral infection should be confirmed by specific methods of examination and identification of viruses, particularly by serological tests.

Thus, for detection of viral antigens in plant samples DAS-ELISA was carried out with using commercial test-system of Loewe (Germany). 126 samples of vegetable crop were checked and 38 of them were found to be infected by CMV. Virus-infected plants were detected in agriecosystems of Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. On plants of *Cucurbitaceae* family, CMV was found in Vinnytsia, Kyiv, Odessa, Poltava and Cherkasy regions. Virus was found in Vinnytsia, Kyiv, Odessa, Poltava and Cherkasy regions on plants of *Solanaceae* family. *Cucumber mosaic virus* was mostly detected as the single agent of infection with several cases of mixed infection. For example, CMV was found with *Watermelon mosaic virus 2* and *Zucchini yellow mosaic virus*. In accordance with ELISA results, we may conclude on the significant spread of the viral infection caused by contamination of healthy seed tissues and coats. In addition, CMV can be transmitted from the weeds (reservoir of infection) and through the soil as plant residues may contain virus.



a b c

Figure 1. Viral symptoms on plants of *Cucurbitaceae* and *Solanaceae* families induced by *Cucumber mosaic virus*:
a – dark green blistering on fruit coat of squash and cucumber caused by CMV;
b – dark green vein banding of leaf blade on squash caused by CMV;
c – leaf blade deformation (rolling) and yellow spots (discoloration) on tomato fruits

Altogether, obtained results point on rather serious situation with CMV spread in Ukrainian agriecosystems.

For direct detection of CMV, indication of its morphology, size of viral particles and confirmation the results of ELISA electron microscopy was used. As a result of these studies of highly purified and concentrated preparation spherical particles 29 nm in diameter were detected. According to the literature data they are typical for *Cucumovirus* genus, in particular for *Cucumber mosaic virus* (Fig.2).

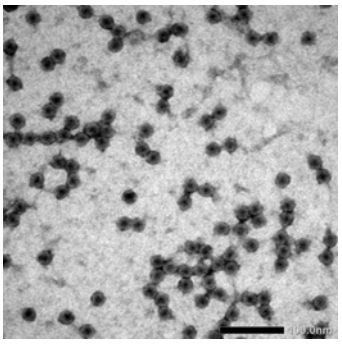


Figure 2. Electron micrograph of *Cucumber mosaic virus*

Thus, the presence of CMV in plant samples was confirmed by electron microscopy.

Further, we have extracted a total RNA preparation from CMV-infected squash plants to use the resulting product in RT-PCR (Fig. 3).

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 CMV.

Figure 3. Results of RT-PCR for CMV:
M – marker (100bp, Fermentas);
1 – cDNA of capsid protein gene of CMV №8;
1 – cDNA of capsid protein gene of CMV №13

We have obtained the cDNA of 500 bp which was further used for sequencing and construction of phylogenetic tree.

Conclusions. Symptomatic plants of *Cucurbitaceae* and *Solanaceae* families collected in Ukrainian agriecosystems used for commercial cultivation of vegetables have been analyzed. According to the ELISA results, 38 samples (of 126 samples in total, i.e., 30%) have been infected with *Cucumber mosaic virus*. CMV is widespread in Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. We have obtained a cDNA of 500 bp corresponding to the coat protein gene of Ukrainian CMV isolate. Purified CMV preparation has been obtained and included in the collection

of plant virus isolates at the Department of Virology, and have been used for comparison with other CMV isolates.

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ПОШИРЕННЯ ВІРУСУ ОГІРКОВОЇ МОЗАЇКИ НА ОВОЧЕВИХ КУЛЬТУРАХ В УКРАЇНІ

Аналізували відібрані в агроценозах різних регіонів України рослини родин Cucurbitaceae та Solanaceae з вірусоподібними симптомами на наявність вірусу огіркової мозаїки (ВОМ). За результатами імуноферментного аналізу встановлено, що серед 126 тестованих зразків овочевих культур – 38 зразків містили антигени ВОМ. ВОМ широко розповсюджений в агроценозах Вінницької, Запорізької, Київської, Одеської, Полтавської та Черкаської областей. Було отримано кДНК гену капсидного білку українського ізоляту ВОМ розміром 500 bp.

Ключові слова: родини Cucurbitaceae та Solanaceae, імуноферментний аналіз, вірус огіркової мозаїки.

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РАСПРОСТРАНЕНИЕ ВИРУСА ОГУРЕЧНОЙ МОЗАИКИ НА ОВОЩНЫХ КУЛЬТУРАХ В УКРАИНЕ

Анализировали отобраные в агроценозах различных регионов Украины растения семейств Cucurbitaceae и Solanaceae с вирусоподобными симптомами на наличие вируса огуречной мозаїки (ВОМ). По результатам иммуноферментного анализа установлено, что среди 126 тестируемых образцов овощных культур – 38 образцов содержали антигены ВОМ. ВОМ широко распространены в агроценозах Винницкой, Запорожской, Киевской, Одесской, Полтавской и Черкасской областей. Было получено кДНК гена капсидного белка украинского изолята ВОМ размером 500 bp.

Ключевые слова: семейства Cucurbitaceae и Solanaceae, иммуноферментный анализ, вирус огуречной мозаики.

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CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF NOVEL FLUORIC COMPOUNDS

The cytotoxicity and antiviral nucleoside activity of fluoric compounds in EBV model systems were investigated in vitro. The apoptosis-stimulating effect was found for compound SBIO-6, that makes it perspective for further research in the area of antitumor analysis. The results can be used in computer modeling of the structure-biological activity relationships of substances and will be used for development of new highly efficient antiviral agents.

Key wods: antiviral nucleoside activity, compound SBIO-6, antitumor analysis.

Introduction. Epstein-Barr virus (EBV) belonging to *Gammaherpesvirinae* subfamily, *Herpesviridae* family, is a lymphotropic DNA virus able to infect cells of lymphatic system [13]. Etiological role of EBV has been confirmed for such clinical human diseases as infectious mononucleosis (initial stage of infection), Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders [8, 9, 11]. After onset of the initial infection in human organism, herpesviruses are capable of latent infection and may be reactivated when effected by various factors, most commonly in case of immune deficiency state of a human organism. Having successfully invaded human body, the virus induces life-long persistence in its cells. Disorders of human immune system lead to virus activation with following manifestation of clinical symptoms. Prominent increase in occurrence of herpetic diseases among adults and children necessitates comprehensive research of herpetic infections and development of efficient methods of prophylaxis and treatment.

Today, chemotherapy using acyclic nucleosides is most developed. This group of preparations includes synthetic analogues of natural nucleosides forming DNA molecules of every biological species on our planet. Four preparations (acyclic analogues of nucleosides) sharing similar structure are known as main antiherpetic medicine agents: acyclovir, valacyclovir, penciclovir and famciclovir. Their efficiency has been tested and confirmed in randomized clinical trials. Every such preparation interrupts viral DNA synthesis during virus reproduction but has no effect on latent virus and extracellular virus particles, virions [1, 3, 12]. Therapy of herpetic infections remains challenging for physicians of various specializations. Novel potent preparations and therapy schemes which are being designed not only should be efficient and safe in the long term treatment, but also reasonably priced and available for wide rage of patients.

This work was aimed at cytotoxic and antiviral nucleoside activity of fluoric compounds in EBV model system *in vitro*.

Materials and methods. Epstein-Barr virus (EBV). Suspension of lymphoblastoid cells B95-8, obtained from the Institute of Virology of RAMS (Mocsow) in 1991, was used as a source of the virus. For virus accumulation, producer cells were cultivated without changing the media with the suspension density of $1x10^6$ cells/ml for 10 days. TPA (12-O-tetradecanoylphorbol-13-acetate) (Sigma, USA) was used as EBV inducer and added to B95-8 cell culture according to the manufacturer's recommendations. The virus was isolated from cells by Walls-Crawford method [4]. We have used following established cell cultures from European Collection of Animal Cell Cultures: 1) B95-8 (leucocytes of marmoset) cells which are transformed by Epstein-Barr virus (EBV) and produce it chronically were used as the source of EBV; and 2) Raji – non-differentiated lymphoblastoid human B cells isolated from Burkitt's lymphoma. Lymphoblastoid cells were