Introduction. Watermelon mosaic virus 2 (WMV-2) and Zucchini yellow mosaic virus (ZYMV) belongs to Potyvirus genus, Potyviridae family [1]. In experimental conditions, Watermelon mosaic virus 2 infects more than 170 plant species from 26 families. However, cucurbiteaceous plants (Cucurbitaceae family) are the major natural hosts for viruses, which were found in both field and greenhouse conditions. ZYMV infects 15 plant species from 7 different families. An occurrence of ZYMV was reported from more than 50 countries. It causes yield losses ranging from 25 to 50% depending on the pathogenicity of the virus strain [2]. Vegetable crops are widely cultivated in Ukrainian fields. Through characterization of viral population possible migration patterns of ZYMV and WMV-2 dissemination from other countries to Ukraine as well as from Ukraine to other countries may be determined.

Therefore, current study was aimed at detection and characterization of viruses infecting vegetable crops in Ukraine.

Materials and methods. Vegetable plants collected from different regions of Ukraine with virus-like symptoms were the objects of this study. Plant sample collection based on the visual symptoms is considered to be the simplest and most common method. For this study, we collected samples with typical viral symptoms under open ground conditions in Kyiv, Poltava, Zhytomyr, Vinnytsya, and Poltava regions, as well as samples from vegetable crops cultivated in Kyiv region. Samples were screened by three methods:

1. For detection of virus antigens, we conducted DAS-ELISA with commercial test systems of Loewe (Germany) and Dynex Technologies (UK) based on the ELISA of commercial test systems. All tests were performed in accordance with the manufacturer's recommendations in 96-well polystyrene plates (Labsystem, Finland).

2. Total RNA was extracted from plant samples using RNeasy Plant Mini kit (Qiagen, UK). RT-PCR was accomplished using specific primers to NIb-CP region of WMV-2 virus [3]. Such homogenate was used for ELISA testing of viral antigens. A portion of the amplified product was purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye Terminator Cycle Sequencing Ready Reaction kit protocol.

3. Phylogenetic analysis was performed using the neighbor-joining method based on sequences of NIb-CP genome region obtained WMV-2 isolates showed that belong to subgroup AI. For this study, we used a database of the Neighbor-Joining tree based on sequences of NIb-CP genome region obtained WMV-2 isolates showed that belong to subgroup AI. According to the phylogenetic analysis, the presence of Zucchini yellow mosaic virus and Watermelon mosaic virus-2. Obtained isolates of Zucchini yellow mosaic virus were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup AI.

POTYVIRUSES INFECTING VEGETABLE CROPS IN UKRAINE

This paper describes detection of some potyvirus infecting vegetable crops in Ukraine. Collected samples were screened for the presence of Zucchini yellow mosaic virus and Watermelon mosaic virus-2. Isolated isolates of Zucchini yellow mosaic virus were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup AI. According to the topology of Neighbor-Joining tree based on sequences of NIb-CP genome region obtained WMV-2 isolates showed that belong to group G1. Viruses infecting cucurbits in Ukraine presented by phylogenetic groups widespread in Europe.

Keywords: viral diseases, Potyvirus, vegetable crops.

Results and discussion. Symptomatic plant samples were collected in different regions of Ukraine. Collected samples were screened for the presence of Zucchini yellow mosaic virus (ZYMV) and Watermelon mosaic virus-2 (WMV-2). Detection of viral antigens was carried out by DAS-ELISA using commercial test systems.

ZYMV caused yellow mosaics, leaf blade deformation, knobs and malformations of fruits (Fig. 1). The symptoms of WMV-2 included dark green mosaics, vein banding and dark mottle on leaves, deformation of fruits and stunting (Fig. 2).

For deeper understanding epidemiology of viruses under study further we conducted the phylogenetic analysis of obtained isolates and previously reported strains from NCBI.

We have sequenced partial Nuclear Inclusion protein (NIb)-CP sequences of ZYMV (825bp), WMV-2 (605bp) isolates found in Ukraine. This genome region is variable among different subgroups, and used for determination of group attribution of ZYMV and WMV-2.

In 2000, new strains of WMV-2 referred as ‘emerging’ (EM) strains were detected in South-eastern France. EM strains are generally more severe and phylogenetically distinct from those previously reported in this country and referred as ‘classic’ (CL) strains [6].

WMV-2 isolates were also obtained from various plants in different regions: WMV-2G, WMV-21 (extracted from Cucurbita pepo L. in Poltava region), WMV-4K (extracted from Cucumis sativus L. in AR of Crimea, WMV-3ch and WMV-4ch (extracted from Cucurbita pepo L. in Cherkasy region), WMV-63 (extracted from Cucurbita pepo L. in Kyiv region). The homology ranged from 94 to 99%.

The topology of Neighbor-Joining tree based on sequences of NIb-CP genome region showed that Ukrainian isolates of WMV-2 belong to group G1 (Fig. 3).
Group G1 consists of non-recombinant isolates reported from different countries [6,7].

According to the topology of phylogenetic tree built using the Nib/CP genome region, the ZYMV isolates form three distinct groups: subgroup A is the most numerous group, which consists of members of different geographic origin subgroup B includes five isolates from Reunion and neighboring islands, subgroup C consists of several Chinese, Polish and Australian isolates [2].

The identification of infected plants in 5 of 9 inspected agroecosystems suggests quite a high prevalence of the ZYMV infection in Ukraine.

For ZYMV we obtained the following Nib-CP sequences of Ukrainian isolates: ZYMV-10G, ZYMV 5/13 (extracted from Cucurbita pepo L. in Poltava region), ZYMV-10P (extracted from Cucumis melo in Vinnytsia region), ZYMV-38/14 (extracted from pumpkin (Cucurbita pepo L) in Cherkasy region), ZYMV-B (extracted from Cucumis melo in Cherkasy region). Ukrainian isolates were characterized with high homology (98-100%).

Obtained isolates were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup A1 (Fig. 4).
According to the literature data [2], subgroup AI includes the most frequently detected strains from different geographic origin.

Conclusions.

Watermelon mosaic virus and Zucchini yellow mosaic virus were detected in plant samples from different regions. Detected isolates belonged to the most frequent phylogenetic groups, which are common for other European countries: WMV-G1 and ZYMV-A. To summarize, viruses infecting cucurbits in Ukraine presented by phylogenetic groups widespread in Europe.

References


2. Desbiez C. Biological and serological variability, evolution and molecular epidemiology of Zucchini yellow mosaic virus (ZYMV, Potyvirus) with special reference to the Caribbean islands/ C. Desbiez, C. Wipf-Scheibel, H.Lecoq// Virus Res. – 2002. – Vol. 85. – P. 5-16.


References (Scopus)


Introduction. Burns of the esophagus is one of the most challenging health problems. According to statistics, 70% of patients – children, whose ages ranged from 1 to 10 years. These statistics associated with the natural curiosity of children and their most common habit to try everything that comes in their hands, to taste. Efficiency of complex intensive therapy of burn disease, occurrence of septic and toxic complications and, mostly, their results depend on the state of immunological reactivity [12].

In severe burns occurs denaturation of proteins in the underlying tissues, reduces synthesis of interferon and opsonization bacteria, inhibits proliferation and reduces cytotoxic activity and chemotaxis of lymphocytes, disrupts reticuloendothelial system develops burn disease with frequent development of secondary immunodeficiency, the severity of which is directly proportional to the depth and prevalence of burns [10].

This depletes humoral immunity and developing autoimmune reactions that lead to increased content in serum circulating immune complexes.

Despite numerous studies of humoral immunity consensus on the nature of the impact of chemical burns of the esophagus has not been made [2:3]. Need to determine the age characteristics of the immune system responses to chemical burns of the esophagus (BE) of different nature and degree.

The aim of study was to evaluate immune status, which includes determination of the parts of the humoral immune system under the experimental reproduction of acid burns of the esophagus

Materials and methods. In experiments used immature white nonlinear rats (1-month) weighing 90-110 g, are kept on a standard diet vivarium. Work carried out in accordance with the rules of the European Convention for the humane treatment of laboratory animals (European convention the protection of vertebrate animals used for experimental and other scientific purposes – Consul of Europe. Strasbourg, 1986) and the “General Principles of experience on animals”, approved National Congress of biotechnics. The animals experimentally simulated acid burns the esophagus (ABE) solution CCl3COOH 30% [11].

To obtain IgG fraction from the blood serum, 1 ml of serum was layered on a column with protein- A Sepharose (total column volume 5 ml). Nonspecifically bound proteins were washed with 0.05 M Tris-HCl buffer, pH 7.4 in a volume of tenfold of total column volume (50 ml). Elution was carried out using a glycine buffer (0.1 M glycine-HCl, pH 2.2). Samples containing protein were precipitated by pH 2.2. Samples containing protein were precipitated by pH 2.2.

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KEYWORDS: acid burns of the esophagus, IgG level, level of circulating immune complexes (CIC).

CHARACTERISTICS OF IMMUNE RESPONSE UNDER EXPERIMENTAL MODELS OF ACID BURNS OF THE ESOPHAGUS

It is well known that the immune system is actively involved in the regeneration and healing process of burn wounds. However, unanswered questions remain about the role of humoral immunity in the mechanisms of healing and complications of burn wounds. We have developed an experimental model of the acid burns of the esophagus (ABE) corresponding esophageal burns in children 1-8 years. We studied the features of humoral immunity in rats with ABE, with the observed reduction of IgG and increase levels of medium and low circulating immune complexes (CIC) on the first day after the burn of the esophagus. On 21st day after the burn, we observed an increase in the concentration of IgG and a slight accumulation of medium and low CIC.

Keywords: acid burns of the esophagus, IgG level, level of circulating immune complexes (CIC).

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Characteristics of immune parameters were studied in rats with ABE and in control rats. ABE was induced by applying to the esophagus 30% CCl3COOH solution. To assess the state of humoral immunity, we measured the level of IgG in the serum of experimental and control rats. The level of IgG was determined using a ELISA method. The level of circulating immune complexes (CIC) was determined using a CIC-ELISA method.

The results showed that the level of IgG in the serum of rats with ABE was significantly lower than in control rats. The level of medium and low CIC was also significantly higher in rats with ABE compared to control rats. The level of high CIC was not significantly different between experimental and control groups.

The results indicate that the ABE model can be used for studying the features of humoral immunity in rats with esophageal burns. Further studies are needed to determine the mechanism of the observed changes in IgG and CIC levels.