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МОЛЕКУЛЯРНО-ГЕНЕТИЧНІ ОСОБЛИВОСТІ ПОВЕРХНЕВИХ ТА НЕСТРУКТУРНИХ БІЛКІВ ПАНДЕМІЧНИХ ВІРУСІВ ГРИПУ A(H1N1)PDM09 В СЕЗОНІ 2015-2016 РОКІВ

Метою дослідження було виявити молекулярно-генетичні зміни в генах гемаглютиніну (HA), нейрамінідази (NA) та неструктурного білку (NS1) пандемічних вірусів грипу, що циркулювали в Україні в 2015-2016 роках. Зразки були проаналізовані методом полімеразної ланцюгової реакції (ПЛР) в реальному часі. Філогенетичні дерева будували в програмі MEGA 7. 3D структури будували в програмі Chimera 1.11.2rc. Віруси, виділені в Україні в сезоні 2015-2016 років, належать до генетичної групи 6B, в якій в цьому сезоні виникли дві нові підгрупи 6B.1 та 6B.2, за генами HA та NA. Ці підгрупи визначаються специфічними для них амінокислотними заміщеннями. В білку NS1 були виявлені нові амінокислотні заміщення D2E, N48S та E125D в сезоні 2015-2016 років. В антигенних сайтах HA були виявлені специфічні заміни, проте віруси зберегли подібність до вакцинного штаму. Білок NS1 набув заміщення, пов'язане з підвищенням вірулентності вірусу грипу.

Ключові слова: віруси грипу A(H1N1)pdm09, амінокислотне заміщення, антигенний сайт, не структурний білок.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ ПОВЕРХНОСТНЫХ И НЕСТРУКТУРНЫХ БЕЛКОВ ПАНДЕМИЧЕСКИХ ВИРУСОВ ГРИППА A(H1N1)PDM09 В СЕЗОНЕ 2015-2016 ГОДОВ

Целью исследования было определение молекулярно-генетических изменений в генах гемаглютинина (HA), нейраминидазы (NA) и неструктурного белка (NS1) пандемических вирусов гриппа, которые циркулировали в Украине в 2015-2016 годах. Образцы были проанализированы методом полимеразной цепной реакции (ПЦР) в реальном времени. Филогенетические деревья построили в программе MEGA 7. 3D структуры построили в программе Chimera 1.11.2rc. Вирусы выделенные в Украине в сезоне 2015-2016 годов, принадлежат к генетической группе 6B, в которой в этом сезоне возникли две новые подгруппы 6B.1 и 6B.2, по генам HA и NA. Эти подгруппы определяются специфическими для них аминокислотными замещениями. В белке NS1 были обнаружены новые аминокислотные замещения D2E, N48S и E125D в сезоне 2015-2016 годов. В антигенных сайтах HA были обнаружены специфические замещения, но вирусы сохранили подобие к вакцинному штаму. Белок NS1 приобрел замещение, связанное с повышением вирулентности вируса гриппа.

Ключевые слова: вирусы гриппа A(H1N1)pdm09, аминокислотное замещение, антигенный сайт, неструктрурный белок.

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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. Obtained 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.

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, cucurbitaceous 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, Odesa, Mykolaiv and Cherkasy regions of Ukraine during 2013-2015 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 [3]. Such homogenate was used for ELISA. 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 [4]. 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 and ZYMV (expected product size – 800 bp, 600 bp respectively) [5]. This genome region is variable among different subgroups, and used for determination of group attribution of ZYMV and WMV-2 [2, 6,7].

Then obtained amplicons were purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big

Dye terminators, version 3.1 (Applied Biosystems, USA). Phylogenetic analysis was conducted using Neighbor-Joining method in MEGA 6.

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 mosaic, vein banding and dark mottle on leafs, deformation of fruits and stunting (Fig.2).

A B

Figure 1. The symptoms of Zucchini yellow mosaic virus:
A – Filamentary and dark green mosaic on squash, B – Knobs on pumpkin fruit

A B

Figure 2. The symptoms of Watermelon mosaic virus-2:
A – Dark green mottling, leaf distortion, abnormal leaf shapes on pumpkins yellowing,
B-Deformation of squash fruit

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 WMV2 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).

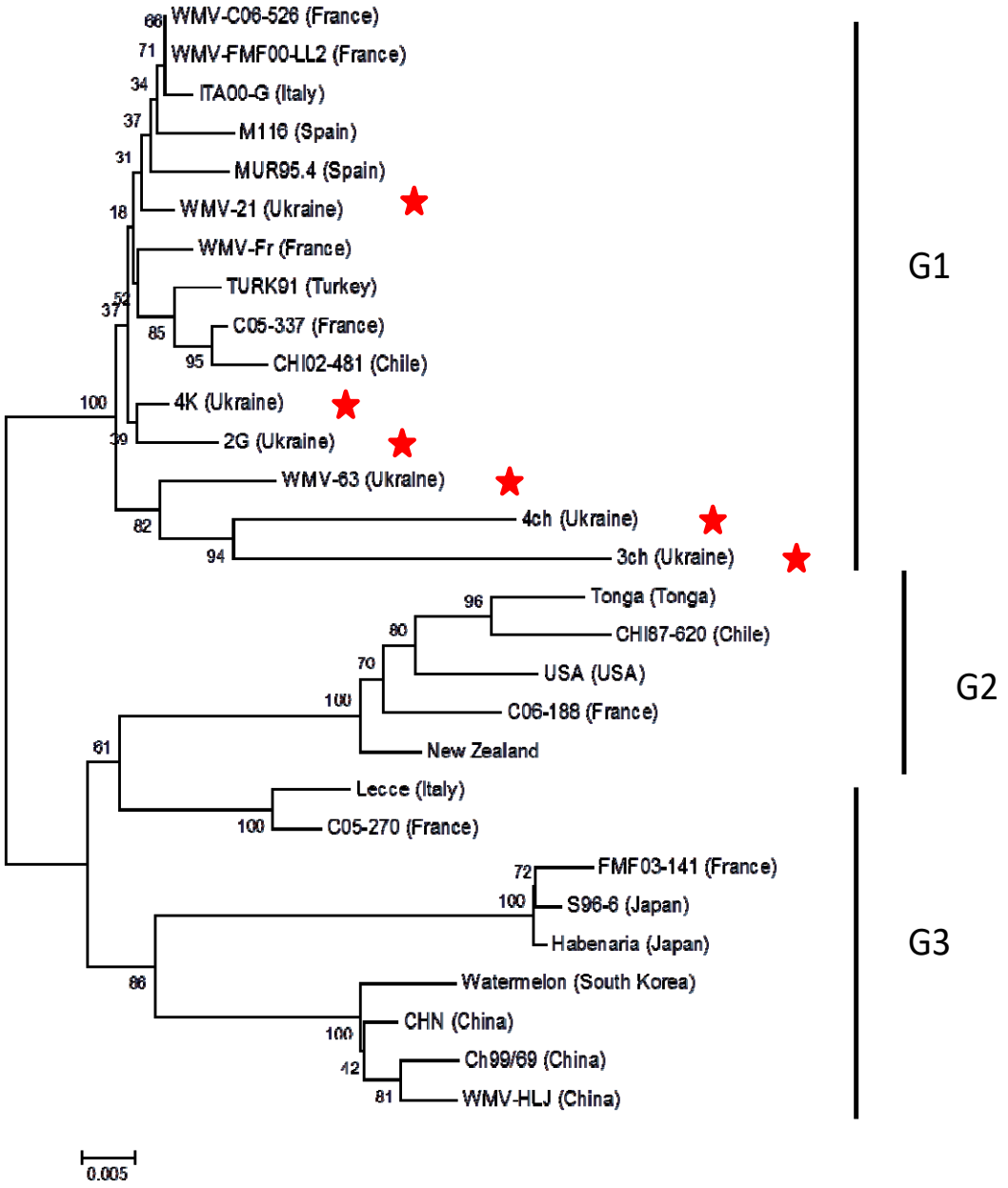

Figure 3. Phylogenetic tree of WMV-2 isolates constructed using Neighbor-Joining method

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 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),) i 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 AI (Fig. 4).

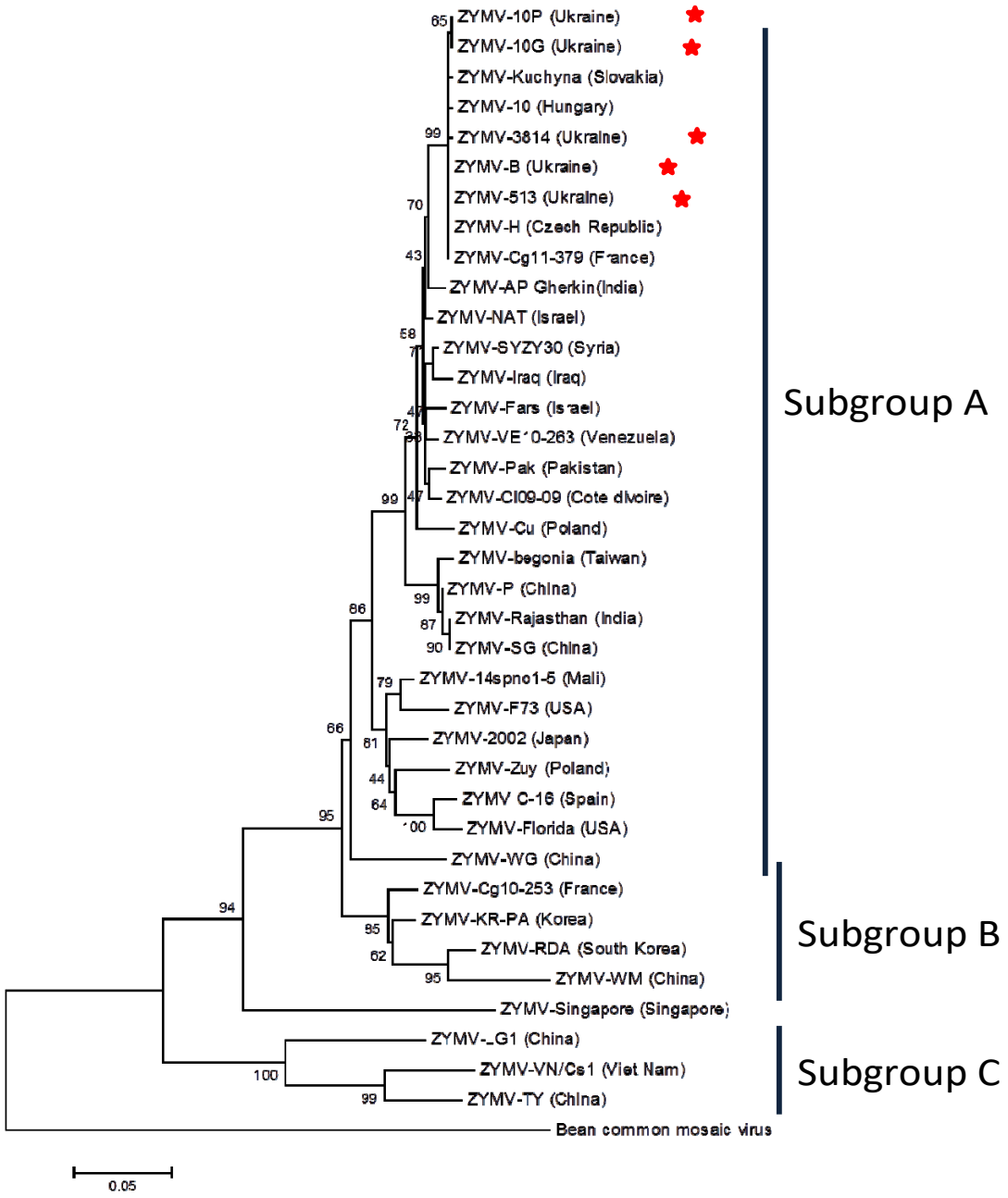

Figure 4. Phylogenetic tree of ZYMV isolates constructed using Neighbor-Joining method

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.

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ПОТІВІРУСНА ІНФЕКЦІЯ ОВОЧЕВИХ КУЛЬТУР В УКРАЇНІ

Робота присвячена детекції вірусів овочевих культур на території України. Відібрані зразки рослин були тестовані на наявність вірусу жовтої мозаїки цукіні та вірусу мозаїки кавуна-2. Отримані ізоляти вірусу жовтої мозаїки цукіні утворювали один кластер із підгрупою AI разом із ізолятами з Словенії, Угорщини, Чешської республіки, Австрії та Франції. За топологією філогенетичного дерева, побудованого на основі сиквенсів NIb-CP ділянки геному вірусу мозаїки кавуна-2, досліджувані ізоляти належать до групи G1. Таким чином, в Україні, віруси, що інфікують рослини родини Гарбузових, філогенетично належать до груп представлених широкорозповсюдженими в Європі ізолятами.

Ключові слова: вірусні хвороби, Potyvirus, овочеві культури.

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ПОТИВИРУСНАЯ ИНФЕКЦИЯ ОВОЩНЫХ КУЛЬТУР В УКРАИНЕ

Работа посвящена детекции вирусов овощных культур на территории Украины. Отобранные образцы растений были тестированы на наличие вируса желтой мозаики цуккини и вируса мозаики арбуза-2. Полученные изоляты вируса желтой мозаики цуккини образовывали один кластер с подгруппой AI вместе с изолятами из Словении, Венгрии, Чешской республики, Австрии и Франции. Согласно топологии филогенетического дерева, построенного на основе последовательности NIb-CP генома вируса мозаики арбуза-2, исследуемые изоляты относятся к группе G1. Таким образом в Украине вирусы, заражающие растения семейства тыквенных, филогенетически относятся к группам представленных широкорозповсюдженимы в Европе изолятами.

Ключевые слова: вирусные болезни, Potyvirus, овощные культуры.

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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. Studied indicators can be used for the differentiation of ABE.

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

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 respons 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 experiments on animals", approved National Congress of bioethics . The animals experimentally simulated acid burns the esophagus (ABE) solution CCl_3COOH 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