ВІСНИК
КИЇВСЬКОГО НАЦІОНАЛЬНОГО УНІВЕРСИТЕТУ ІМЕНІ ТАРАСА ШЕВЧЕНКА

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Подано експериментальні дані про особливості будови, розвитку і функціонування рослинних і тваринних організмів, флору і фауну України, одержаних науковцями НДІ фізіології імені академіка Богача та ННЦ "Інститут біології та медицини" та інших наукових установ. Викладено також нові дані про патофізіологічні закономірності й біохімічні механізми регуляції процесів на клітинному та органному рівнях після впливу різноманітних фізико-хімічних чинників.

Для викладачів, наукових співробітників, аспірантів і студентів.

Подано экспериментальные данные об особенностях строения, развития и функционирования растительных и животных организмов, полученных учеными НИИ физиологии имени академика Богача и УНЦ "Институт биологии и медицины" и других научных учреждений. Изложены также новые данные о патофизиологических закономерностях и биохимических механизмах регуляции процессов на клеточном и органном уровнях после воздействия различных физико-химических факторов.

Для преподавателей, научных сотрудников, аспирантов и студентов.

The experimental dates development and function of the plant and animal organisms of research institute and ESC "Institute of Biology and medicine". Results of newly pathophysiological aspects and biochemical mechanisms of cell and organism processes regulation under the influence of different factors are presented.

For scientists, professors, aspirants and student.

ЗМІСТ

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UDC 578.3+582.974

L. Dudar, PhD stud., V. Polischuk, DSc., Biol, Prof.,
I. Budzanivska, DSc., Biol, Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
Gyula Balka, PhD., Attila Csagola, PhD.
University of Veterinary Medicine, Budapest, Hungary

COMPLETE GENOME SEQUENCE OF PORCINE CIRCOVIRUS TYPE 2 UKRAINIAN ISOLATES

Porcine circovirus type 2 (PCV2) is associated with distinct syndromes and diseases in swine, collectively known as porcine circovirus-associated diseases (PCVAD), which include postweaning multisystemic wasting syndrome (PMWS), PCV2-associated pneumonia as a part of the porcine respiratory disease complex (PRDC), PCV2-associated enteritis, PCV2-associated reproductive failure, and porcine dermatitis and nephropathy syndrome (PDNS) (1–3). PCV2-infection is widespread and essentially all pig herds are infected with PCV2. Porcine circovirus 2 (PCV2), a member of the genus Circovirus in the family Circoviridae, is a very small single-stranded negative-sense DNA virus of approximately 1.7 kb (4). The genome of PCV2 encodes three major open reading frames (ORFs) encoding the replicase proteins (ORF1), the viral capsid protein (ORF2), and a protein with suggested apoptotic activity (ORF3) (5). Previous reports showed that there were five PCV2 genotypes, including PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e (6–9). Here, we report the complete genome sequence of Ukrainian PCV2 isolates from different regions of Ukraine.

Key words: Porcine circovirus type 2, porcine circovirus-associated diseases, multisystemic wasting.

Introduction. Porcine circovirus type 2 (PCV2) was first recognized as a causative agent of postweaning multisystemic wasting syndrome (PMWS), a multi-factorial disease in swine in Canada in 1991 (Harding and Clark, 1997). Subsequently, it has been reported in almost all intensive pig production countries worldwide (Allan and Ellis, 2000; Chae, 2005). PCV2 causes several clinical and pathological conditions in pigs including porcine respiratory disease complex (PRDC), reproductive failures, porcine dermatitis and nephropathy syndrome (PDNS), proliferative and necrotizing pneumonia and congenital tremor (Darwich et al., 2004; Chae, 2005). Currently, these associated diseases and conditions linked to PCV2 are called porcine circovirus associated diseases (PCVAD).

PCV2 belonging to the family Circoviridae, is a smallest mammalian, non-enveloped, single-stranded DNA virus encoding a circular genome about 1.76 kb (Mankertz et al., 1997). The genome of PCV2 contains 3 major open reading frames (ORFs): ORF1, ORF2 and ORF3. The Cap protein is the main structural and major immunogenic protein of PCV2, which is encoded by ORF2. As a result, ORF2 is commonly used for reconstruction of the phylogenetic tree similarity to the whole PCV2 genome study (Olvera et al., 2007) Several studies suggested that PCV2 could be divided into 2 major genotypes (Carman et al., 2006; Cheung et al., 2007; Ma et al., 2007; Takahagi et al., 2008; Kim et al., 2009). Recently, both genotypes were proposed and referred to PCV2a (PCV2- genotype 2) and PCV2b (PCV2-genotype 1). However, PCV2c genotype has been described, but only found in Denmark (Segales et al., 2008). Interestingly, the virulence of PCV2a and PCV2b isolates was similar in the conventional SPF pig model, but the virulence of the isolates within the same cluster differed (Opriessnig et al., 2008). Alternatively, PCV2 can be classified into 8 subgroups 1A to 1C and 2A to 2E (Olvera et al., 2007), but those were not associated with the disease conditions or geographic areas. Recently, a new type of PCV referred to PCV1/2a was reported and later found to be a chimeric virus containing ORF1 of PCV1 and ORF2 of PCV2a in Canada in 2009 (Gagnon et al., 2010).

In Ukraine, PMWS caused by PCV2 has been reported from 2000. However, genetic information about PCV2, spreaded in swine herds of Ukraine has been still unavailable. Therefore, the objective of this study was to determine the genetic characterizations of complete genome of current PCV2 isolates from Ukraine pigs from different regions of Ukraine (included Cherkasy, Kharkiv, Chernigiv, Zaporiggia regions).

Materials and Methods.

Field samples: Clinical samples (serum samples and lymph nodes) from different farms in high pig density provinces of Ukraine submitted to Molecular Diagnostic Laboratory at CVD (Center of Veterinary Diagnostics) during 2014-2015 years were included in this study. These samples were kept at -80°C until performing DNA extraction and PCR. Viral DNA was extracted from lymphoid tissue homogenates and serum samples using NucleoSpin Extract Viral DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

PCR amplification: A full-length ORF2 gene of PCV2 was amplified by PCR with forward primer, PCV2-f1 (5'-CCA TGC CCT GAA TTT CCA TA-3') and reverse primer PCV2-r1 (5'-ACA GCG CAC TTC TTT CGT TT-3') published by Takahagi et al. (2008), in a 50 µl reaction mixture. The amplification reaction was performed with an initial step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min and a final extension step at 72°C for 7 min. The PCV2 positive samples of 702 nt were used for DNA sequencing.

Viral sequences and phylogenetic analysis: The PCR products were separated by 1.5% agarose gel electrophoresis and purifiedwith NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) for the sequences. DNA sequencing was carried out with primers used in the previous PCR reaction. A total of 4 sequences from Ukraine pigs were obtained and translated intoamino acid sequences for analysis. The 4 Ukraine PCV2 sequences were analyzed together with representative complete genome sequences reported in GenBank. A phylogenetic trees was constructed by MEGA 6 software (Tamura et al., 2007) using the neighbor-joining (NJ) method with 1000 bootstrapping replicates (Saitou and Nei, 1987).

Results and discussion.

Genetic characterization: All 4 Ukraine of PCV2 sequences in this study had a genome length of 1768 nt and revealed nucleotide identities ranged between 100-95% (Table 1), indicating no significant differences between PCV2 genotype.

***Table* 1. Comparison of Ukrainian isolates (AK and NT sequences of complete genome) with PCV2 reference strains in the same group by nucleotide and deduced amino-acid sequences**

Isolate PCV2	subgroup	AK, %	NT, %
swine_Chernigiv	1A/B	100	100
swine_Zaporigga	1A/B	100	100
swine_Cherkasy	2	87,1	95,2
swine_Kharkiv	2	87,1	94,9
PCV2_Uy_2_Cap	1A/B	91,9	96
PT-15152-03_cap	1A/B	92,7	97,1
NL_Control_1_capsid_protein	1A/B	91,1	96
FJFQ0511_cap	1A/B	91,1	95,7
YZ_cap_protein	1A/B	91,1	95,7
HN1-5_cap	1A/B	91,1	95,7
IL_capsid_protein	1A/B	91,9	96,3
09JX_Cap	1A/B	90,3	95,5
CHST_cap	1A/B	91,1	95,7
09GD_Cap	1A/B	91,9	96,3
C7201-1_capsid_protein	1A/B	90,3	95,2
CH3_capsid_protein	1A/B	100	99,5
PT-34765-06_cap	1A/B	100	99,5
NIVS-5_putative_capsid_protein	1A/B	100	99,5
WB-H-1_capsid_protein	1A/B	100	99,5
ZHZ1_cap_protein	1A/B	100	99,7
DK442case_capsid_protein	1A/B	99,2	99,5
Fd1_capsid	1A/B	100	99,5
AUT5_capsid_protein	1A/B	100	99,7
NL_Control_3_capsid_protein	1A/B	99,2	99,2
Henan_cap_protein	1A/B	100	99,5
SXTY14_ORF2	1A/B	100	99,7
ZHZ1_pig_gi1033208412	1A/B	100	99,7
AUT5_gi37791490	1A/B	100	99,7
SXTY_PCV_gi1031916872	1A/B	100	99,7
1397/2011_Vicenza_36_07/07/2011_	1A/B	100	99,7
SNUVR000463_gi573463974	1A/B	100	99,7
DK558control_gi156193221	1A/B	100	99,7
1C-China	1C	91,1	96,3
PCV2_Gen2-_Hungary	2	89,5	96

Genome consists of at least three ORFs, encoding 2 major proteins, the Rep and Cap proteins. Multiple sequence alignment was completed by means of MEGA6 with other available strains from the GenBank nucleotide database. Ukraine isolates from Chernigiv and Zaporigga shares a 100 % identity to each other and high identity (95% to 99.7%) with the strains from 1A/B subgroup. Ukraine isolates from Cherkasy, Kharkiv shares near 100 % identity to each other and high identity (94% to 96%) with the strains from subgroup 2.

Phylogenetic analyses: The phylogenetic analysis in this study reconstructed from the 4 Ukraine complete sequences from Cherkasy, Kharkiv, Chernigiv, Zaporiggia regions of Ukraine and sequences published in GenBank database representing all PCV2 genotypes shown in Fig. 1.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.11324677 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method [3] and are in the units of the number of base substitutions per site. The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 387 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

Two Ukraine isolate PCV2 sequence belonged to genotype 1 (PCV2b) and two Ukraine isolate PCV2 sequence belonged to genotype 2 (PCV2a) according to the classification proposed by Grau-Roma et al. (2008). Based on the subgroup terminology described previously (Olvera et al., 2007), nucleotides 262-267 and amino acids 88-89 of ORF2 were compared and classified. The nucleotide sequences "CCCCGC", "CCCCTC" and "AAAATC" are the signatures motif for PCV2b subgroup 1A/B, 1C and PCV2a, respectively. The amino acid "PR" was enclosed with subgroup1A/B, while the PL and KI were related with subgroup 1C and PCV2a (Cheung et al., 2007). Ukraine isolate PCV2 from Chernigiv and Zaporiggia genotype 1 were divided into 1A/ B subgroups. Ukraine isolate PCV2 from Cherkasy, Kharkiv were divided into genotype 2.

The sequence and phylogenetic analyses performing in this study did not show any evidence of recombination as reported in PCV type 2 isolated in Hong Kong, Korea and USA (Ma et al., 2007; Choi and Chae, 2008; Hesse et al., 2008).

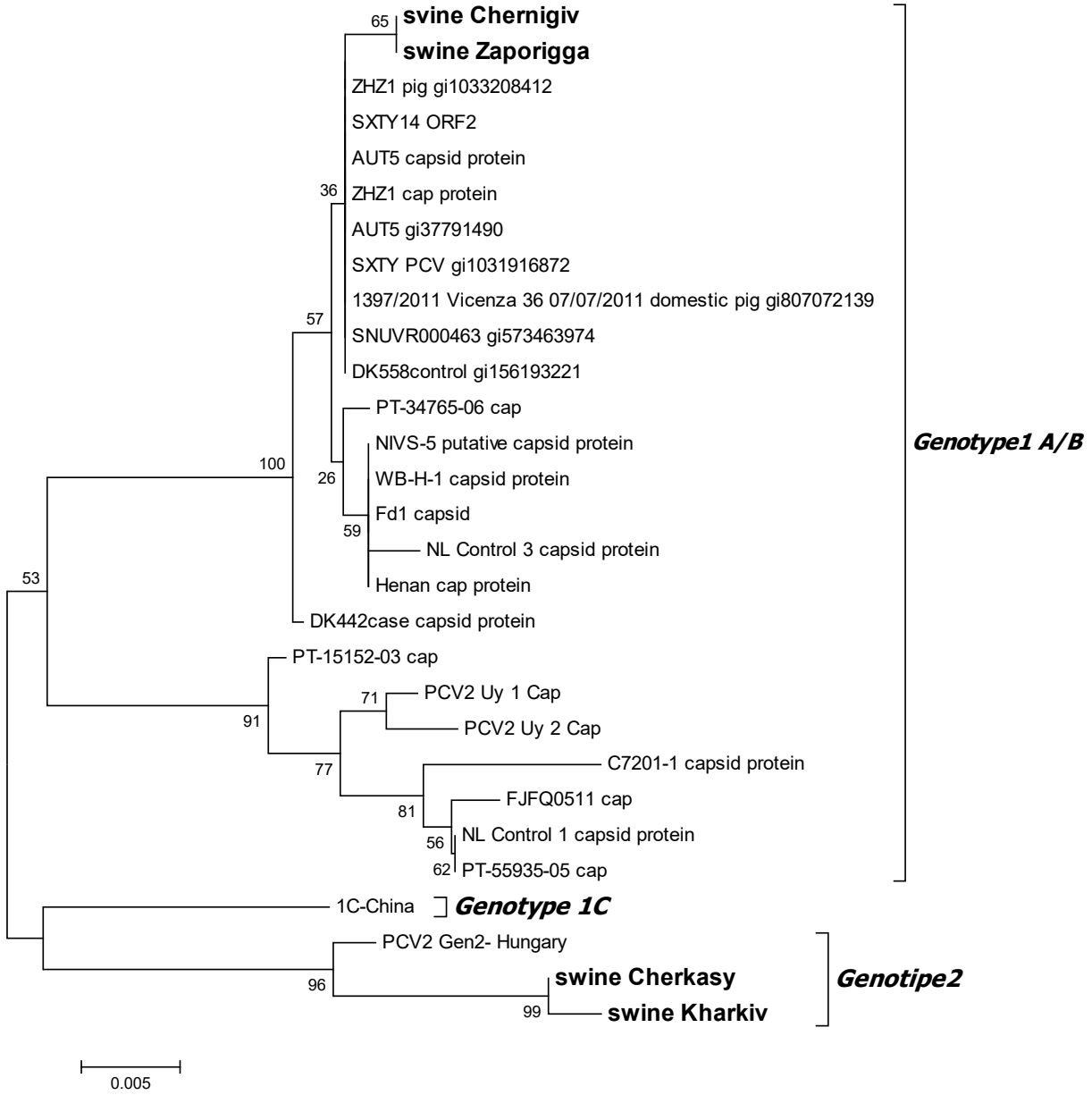

Figure 1. Evolutionary relationships of PCV2

However, a few amino acid replacements among Ukraine sequences in this study were observed. Due to the high nucleotide substitution rate of PCV2 compared to other single-stranded DNA viruses, it was estimated approximately $1.2x10^3$ substitutions/site/year (Firth et al., 2009). Therefore, the emerging of any new PCV2 genotype is possible in the future. Since the samples in this study were collected from the highest pig density provinces, the results yielded in this study can demonstrate at least 2 introductions of PCV2 into Ukraine. Imported swine breeders and semen appear to be the major route or transmission. Another evidence of introducing new virus strain into the swine herds is using improper killed chimeric vaccine in Canada (Gagnon et al., 2010).

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Л. Дудар, асп., В. Поліщук, д-р біол. наук, проф., І. Будзанівська, д-р біол. наук, проф.
Київський національний університет імені Тараса Шевченка, Київ, Україна,
Гула Балка, канд. біол. наук, Аттила Цагола, канд. біол. наук
Університет ветеринарної медицини, Будапешт, Угорщина

ПОВНОГЕНОМНИЙ СИКВЕНС УКРАЇНСЬКОГО ІЗОЛЯТУ ЦИРКОВІРУСУ СВИНЕЙ 2-ГО ТИПУ

Свинячий цирковірус 2 (PCV2) асоціюється з різними синдромами і хворобами свиней, які відомі під загальною назвою свинячих цирковірус-асоційованих захворювань (PCVAD), які включають синдром мультисистемного розладу (PMWS) PCV2-асоційовані пневмонії (PRDC), PCV2, асоційований з ентеритом, PCV2, асоційований з репродуктивною функцією, а також свинячі дерматит і синдром нефропатії (PDNS) (1-3). PCV2-інфекція широко поширена і по суті всі свині стада заражені PCV2. Свинячий цирковірус 2 (PCV2), член роду* Circovirus *родини Circoviridae. Має ол- ДНК вірусу приблизно 1,7 кб (4). Геном PCV2 кодує три основних відкритих рамок зчитування (ORF,), які кодують репліказу (ORF1), вірусний білок капсида (ORF2), і білок, із запропонованою апоптичною активністю (ORF3) (5). Попередні дані показали, що існує п'ять генотипів PCV2, в тому числі PCV2a, PCV2b, PCV2c, PCV2d і PCV2e (6-9). В нашій роботі ми провели секвенування повного геному генома українськогих ізолятів PCV2з різних регіонів України.

Ключові слова: свинячий цирковірус 2, свинячий цирковірус-асоційованих захворювань, мультисистемний розлад.

Л. Дудар, асп., В. Полищук, д-р биол. наук, проф., И. Будзанивская, д-р биол. наук, проф.
Киевский национальный университет имени Тараса Шевченко, Киев, Украина,
Гула Балка, канд. биол. наук, Аттила Цагола, канд. биол. наук
Университет ветеринарной медицины, Будапешт, Венгрия

ПОЛНОГЕНОМНЫЙ СИКВЕНС УКРАИНСКОГО ИЗОЛЯТА ЦИРКОВИРУСА СВИНЕЙ 2-ГО ТИПА

Свиной цирковирус 2 (PCV2) ассоциируется с различными синдромами и болезнями свиней которые известны под общим названием свиных цирковирус-ассоциированных заболеваний (PCVAD), которые включают синдром мультисистемных нарушений (PMWS) PCV2-ассоциированной пневмонии, PCV2, ассоциированный с энтеритом, PCV2, ассоциированных с репродуктивной функцией, а также свиные дерматит и синдром нефропатии (PDNS) (1-3). PCV2-инфекция широко распространена и по существу все свиньи стада заражены PCV2. Свиной цирковирус 2 (PCV2), член рода* Circovirus *семейства Circoviridae Геном – очень маленькая оц- ДНК вируса примерно 1,7 кб (4). Геном PCV2 кодирует три основных открытых рамок считывания (ORF,), кодирующие репликазуи (ORF1), вирусный белок капсида (ORF2), и белок, с предложенной апоптотической активностью (ORF 3) (5). Предыдущие данные показали, что существует пять генотипов PCV2, в том числе PCV2a, PCV2b, PCV2c, PCV2d и PCV2e (6-9). Здесь мы сообщаем полную последовательность генома украинских изолятов PCV2из разных регионов Украины.

Ключевые слова: свиной цирковирус 2, свиной цирковирус-ассоциированных заболеваний, синдром мультисистемных нарушений.

UDC 616-084+714:615.015.8+612.6

N. Babii, PhD
SI "L.Gromashevsky Institute of epidemiology and infectious diseases of NAMSU", Kyiv, Ukraine

PREVALENCE OF DRUG RESISTANT HIV STRAINS IN HIV-INFECTED PATIENTS OF REPRODUCTIVE AGE

The prevalence of drug resistant HIV strains among HIV-positive reproductive aged persons with ineffective antiretroviral therapy (ART) was assessed. The prevalence of drug resistant strains of HIV was 73.8% in the group of women and 89.29% in the group of men (totally in 80.0% of patients). In the spectrum of drug resistance mutations (DRMs) the most prevalent mutation associated with high-level resistance to nucleoside reverse transcriptase inhibitors was substitution M184V (80.36%); in addition, the high prevalence of K65R (26.79%) was indicated. The most common mutations causing a high-level resistance to non-nucleoside reverse transcriptase inhibitors were G190S/A (57.14%), Y181C (37.50%), K101E (33.93%). The DRMs to protease inhibitors were indicated with significantly less frequent (5.36%).

Key words: HIV, drug resistant mutations, antiretroviral therapy.

Introduction. Antibiotic and antiretroviral resistance has become a major clinical and public health problem nowadays. The selection of resistant forms of pathogens is based on natural processes of microbes adaptation to conditions of environment. Uncontrolled, inappropriate and overabundant using of antimicrobial, antiviral and antifungal drugs, absence of strong clinical protocols of there using, extensive applying of them in agriculture and animal husbandry, the

strong pressure of civilization as a whole lead to the activation of pathogens genetic variability, the emergence of new pathogens and fast evolution of existing ones.

The development of resistance HIV to antiretroviral drugs (ARVs) is the main cause of antiretroviral therapy (ART) virological inefficiency. Biological properties of HIV – fast reproduction of new viral particles, high level of genetic variability connected to features of the reverse transcription, archiving of all variants of HIV genoms as proviral DNAs, high probability of genetic recombination – are the factors, which determine fast evolution of viral population and replacement of wild viruses on resistant strains under selective pressure of suboptimal concentrations of ARVs.

Consequences of HIV resistance development are the growing of risk of drug resistant HIV strains spreading, the appearing of the necessity of switch to more expensive second- and third-line regimens, the loss of ART and prophylaxis effectiveness. During few last years the main HIV spreading way in Ukraine is sexual way, connected to growing risk of HIV vertical transmission. Considering that, the spreading of drug resistant HIV strains among reproductive aged people can lead to the growing of the HIV-infection incidence.

The aim of the work was the definition of level of prevalence drug resistant HIV strains among HIV-positive reproductive aged people with virological ineffective ART.

Materials and methods. A total of 70 HIV-infected individuals at reproductive age (a median age 36.4years) with virological failure of ART were enrolled: among them 42 were female and 28 were male. All men and 24 women (received ART scheme 2 nucleoside reverse transcriptase inhibitors (NRTIs)+non-nucleoside reverse transcriptase inhibitor (NNRTI), 18 (28.57±6.9%) women received scheme 2NRTIs+1 protease inhibitor (PI).

In 42 (60.0±5.86%) patients there were no substitutions of ART regimens in anamnesis, 10 (14.29±4.18%) patients had one replacement of therapy scheme, in 18 (25.71±5.22%) patients the scheme of therapy has been changed twice or more.

In the group of women indexes of HIV viral loud (VL) were from $2.4x10^3$ to $3.18\ x10^6$ HIV RNA copies/ml of plasma; in the group of men – from $4.7x10^3$ to $2.55x10^6$ HIV RNA copies/ml of plasma.

For the HIV genome sequencing was performed on the genetic anlizer ABI PRISM 3130 using the test-system ViroSeq®HIV-1 Genotyping System v.2.0 (Celera Corporation, USA). Data analysis and interpretation were performed using Stanford University Database (http://hivdb.stanford.edu). Data were analyzed by program R (version 2.13.1; 2015-06-18).

Results and discussion.

HIV resistance is developing as a result of forming of specific mutations in the HIV genome, which lead to changes of amino acids structure of viral proteins – targets of ARVs. The selection of drug resistant variants of HIV is carried under the selective pressure of sub-optimal doses of ARVs, usually as a result of low adherence of patients to therapy. The test-systems, what were used in that work, allow determining the DRMs to three classes of ARVs, which are used in Ukraine: to NRTIs, NNRTIs, and PIs.

Among 70 tested blood samples obtained from HIV-positive patients with ineffective ART, 56 (80.0±3.7%) were positive for the presence of drug resistant HIV strains. DMRs to NRTIs (in 71.43±5.4% of samples) and to NNRTIs (in 75.71±5.13% of samples) were most frequent, and in 68.57±5.55% of samples the DMRs to both these classes of ARVs have been detected. The DRMs to PIs were much rarely – in 4.29±2.42% of samples.

Among 42 blood samples of women 31(73.80±6.78%) were positive for the presence of HIV strains with resistance to ARVs (Table 1). DMRs to NRTIs and NNRTIs have been detected with approximately equal frequency – in 27 (64.28±7.39%) and 28 (66.67±7.27%) samples respectively. In 25 (59.52±7.57%) samples DRMs to both classes of drugs were detected. Drug resistance strains of HIV have been detected in samples women's blood, who received the therapy scheme with 2NRTIs+1NNRTI, and in 38.89±11.45% of women on regimen with PI (2NRTIs+1PI). Due to results of the analysis of DRMs combinations 28 women (66.67±7.27%) needed for therapy regimen correction; in all cases the presence of DRMs to NNRTIs determined necessity to change the therapy regimen. And in all cases the presence of DRMs to NNRTIs required for the choice of ART regimen with NNRTI to scheme with PI.

***Table* 1. The incidence of drug-resistant strains of HIV in persons of reproductive age**

Marker	The frequency of detection					
	Female (n=42)		Male (n=28)		Total (n=70)	
	Abs.	Rel., M±m, %	Abs.	Rel., M±m, %	Abs.	Abs, M±m, %
Any DRMs to ARVs	31	73.80±6.78	25	89.29±5.76	56	80.0±3.7
Necessity in correction of the therapy regimen	28	66.67± 7.27	24	85.71±6.57	52	74.29±5.22
DRMs to NRTIs	27	64.28±7.39	23	82.14±7.16	50	71.43±5.4
DRMs to NNRTIs	28	66.67± 7.27	25	89.29±5.76	53	75.71±5.13
DRMs to NRTIs+NNRTIs	25	59.52±7.57	23	82.14±7.16	48	68.57±5.55
DRMs to PIs	3	7.14±3.97	0	0	3	4.29±2.42

Among men the percentage of persons with resistant strains of HIV was higher: DRMs have been detected in 25 samples of men plasma (89.29±5.76%). The necessity for ART regimen correction was indicated in 24 cases (85.71±6.57%) (tab.1) DRMs associated with resistance to NRTIs, were detected in 23 samples (82.14±7.16%), to NNRTIs – in 25 samples (89.29±5.76%), to both classes of ARVs – in 23 samples (82.14±9.56%). In all cases the combination of DRMs led to ineffectiveness of all ARVs, concluded into ART regimen. In contrast to results, obtained in women's group, there were no DRMs to PIs in the men's group.

DRMs to NRTIs. The mechanism of action of NRTIs is termination of HIV proviral DNA synthesis by incorporating into the growing DNA chain [6]. The development of HIV resistance to this class of ARVs can be carried out in two ways:

1. The accumulation of mutations, which make it impossible to conclude NTRIs in DNA chain: substitutions M184V, non-thymidine analogue mutations K65R, K70E/G, L74V, Y115F and mutations of Q151M-complex.

2. The accumulation of mutations that facilitate excision of 3'-terminal chain-terminating inhibitors from blocked DNA chain through phosphorolysis mediated by ATP or pyrophosphate. These mutations, known as thymidine analogue resistance mutations (TAMs), include M41L, D67N, K70R, L210W, T215F/Y and K219E/Q [16].

There are two different ways of TAMs forming: first – by the accumulation of substitutions M41L, L210W, T215Y

(Type 1 TAMs), second – includes mutations D67N, K70R, T215F, K219Q/E (Type 2 TAMs). Factors that lead to selection of mutations by first or second way are unknown. Perhaps this is a random process, or it can be connected to genetic features of HIV, to immunologic characteristics of patient, to the list and sequence ART regimens and other reasons [16]. TAMs of 1 Type have a greater negative impact on virological response to an ABC-, ddI-, or TDF-containing regimen than do TAMs of Type 2 [11].

The frequency of detection of different DRMs was calculated with respect to the total quantity of samples with at least one DRM. Among mutations connected to first mechanism of NRTI-resistance development the mutation M184V was dominant in samples obtained from women and men. In some samples the substitution M184I was detected – mutation that usually appeared before M184V because it results from a more common HIV-1 nucleotide substitution. M184I has the similar effect on HIV resistance. Generally, the detection rates of M184V/I were 77.42±5.34% in a group of women and 80.0±4.74% in a group of men (Tab.2). The selection of this substitution occurs under the press of therapy with 3TC and FTC, and leads to 100-fold decrease of HIV sensitivity to these drugs. Besides, M184V/I appear at treating by ABC and ddI causing low-level HIV resistance to them. In the same time, M184V increases HIV susceptibility to AZT, d4T and TDF and slows down the development of resistance to them. M184V/I are associated with reduced HIV replication *in vitro* and *in vivo*. That is why M184V/I are not contraindications to continued treatment with 3TC or FTC. The combination of TDF, AZT or d4T + 3TC/FTC inhibits HIV with M184V [9].

In addition to this mutation, in the spectrum of DRMs to NRTIs, detected in women's samples of plasma, non-TAMs mutations K65R (38.71±8.69%), Y115F (22.58±7.44%), L74V/I (19.35±7.02%) were prevalent. Among TAMs substitution K219Q/E was dominant (22.58±7.44%), it refers to Type 2 TAMs.

***Table* 2. The detection rate of major and accessory mutations in the pol gene of HIV-1 associated with resistance to NRTIs[1]**

Mutation	The frequency of detection in the spectrum of DRMs					
	Female (n=31)		Male (n=25)		Total (n=56)	
	Abs.	Rel.,M±m,%	Abs.	Rel.,M±m,%	Abs.	Rel.,M±m,%
M41L	0	0	2	8.0±5.33	2	3.57±2.22
A62V	3	9.68±3.53	3	12.0±6.42	6	10.71±4.01
K65R	12	38.71±5.82	3	12.0±6.42	15	26.79±5.29
D67N	2	6.45±2.94	5	20.0±7.94	7	12.50±3.95
T69N	2	6.45±2.94	1	4.0±3.78	3	5.36±2.84
K70E	3	9.68±3.53	5	20.0±7.94	8	14.29±4.18
K70R	1	3.23±2.11	2	8.0±5.33	3	5.36±2.84
L74V/I	6	19.35±4.72	6	24.0±8.48	12	21.43±4.9
V75I	0	0	4	16.0±7.26	4	7.14±3.29
Y115F	7	22.58±4.5	3	12.0±6.42	10	17.86±4.58
Q151M	0	0	0	0	0	0
M184V/I	24	77.42±5.34	20	80.0±7.94	45	80.36±4.74
L210W	0	0	2	8.0±5.33	2	3.57±2.27
T215F/Y	1	3.23±2.11	3	12.0±6.42	4	7.14±3.29
K219Q/E	7	22.58±4.5	2	8.0±5.33	9	16.07±4.80

It is known that mutation K65R leads to 2-fold decreasing of HIV susceptibility to ABC, TDF, d4T, ddI, and 5-10-fold – to 3TC and FTC, but increase HIV sensitivity to AZT (accept of cases of combination with Q151M substitution) [9]. It should be taken into account for ART regimen changing.

The L74V is selected by therapy of ABC and ddI. In combination with M184V it is the most common substitution for patients receiving ART with ABC/3TC; together these mutations lead to 5-fold decreasing of HIV susceptibility to ABC and 2-fold – to ddI. Mutation L74I is selected by therapy with the same ARVs and TDF; its effect on the resistance is less pronounced [9].

Y115F is selected by ABC and TDF. Alone, Y115F reduces ABC susceptibility about 3-fold but has a little phenotypic effect on TDF susceptibility. In combination with K65R or Q151M, Y115F synergistically reduces ABC and TDF susceptibility [14]. Mutation K219Q/E in combination with other TAMs reduces susceptibility about 3-fold to AZT and d4T.

In the samples of men, except of dominant M184V/I, the substitutions L74V/I, K70E and two TAMs (Type 2) – D67N and K70R were detected with higher frequency (Tab.2). D67N and K70R reduce of HIV susceptibility to AZT and d4T. In combination with other TAMs it leads to decreasing of HIV susceptibility to ABC, ddI and TDF. The frequency of K65R detection in the samples, obtained from men, was lower compared to group of women – 12.0±6.42%. Mutation K70E reduces HIV susceptibility to ARVs only in combination with other NRTI-resistance mutations.

Except of major DRMs to NRTIs, some accessory mutations have been detected (A62V, T69N, V75I) with low frequency. Without major mutations they don't influence on HIV resistance largely. For example, among mutations of Q151M complex, only additional substitutions were detected – A62V and V75I – with the frequency 10.71±4.01% and 7.14±3.29% respectively. Without main mutation of this complex – Q151M – additional substitutions do not affect the HIV resistance. Mutation A62V is a common polymorphic substitution for HIV strain circulating in Russian Federation which is entrenched in that area due to the "funder effect" [2]. The prevalence of A62V in HIV strains detecting in Russian Federation is about 13%.

Interestingly, that analysis of DRMs prevalence has indicated some gender differences: TAMs (Type1 and Type2) were detected in men's samples of plasma more frequent. The connection between the gender and the prevalence of some DRMS was detected in other investigations [3, 15]. However, based on our data it is difficult to draw conclusions on the causes of this phenomenon, the additional study is necessary.

DRMs to NNRTIs. NNRTIs can block HIV reverse transcriptase by binding to special hydrophobic region of the enzyme (so-colled "pocket"). That binding leads to the change of the spatial configuration of reverse transcriptase

[1] Bold – high-level resistance mutations, plane – reduced HIV susceptibility in combination with other mutations, italics – accessory mutations

active center. The development of resistance to NNRTIs is caused by forming of mutations in the "pocket" region.

Among the most frequent major DRMs to NNRTIs in HIV isolates obtained from women's samples of plasma were G190S/A (54.84±8.88%), K101E (36.26±8.34%), Y181C/I (41.94±8.81%) (Tab.3). Mutation G190S is selected during the therapy with NVP and EFV. It 50-fold reduces HIV susceptibility to specified ARVs. Mutation G190A is selected by the selective pressure of the same ARVs and leads to 50-fold and more decreasing of HIV susceptibility to them. Substitution K101E is forming during the therapy with NNRTIs and leads to 3-10-fold decreasing of HIV sensitivity to NVP, 1-5- fold – to EFV, 2-fold – to ETR and RPV. Y181C/I are selected by the therapy with any NNRTIs and more-less decreases the susceptibility to every drug of that class [9].

Among HIV isolates obtained from men, in addition to aforecited mutations, accessory substitutions V90I (20.0±7.94%) and V106I (24.0±8.48%) have been indicated. Both of them increase HIV resistance to NNRTIs, but in a less degree.

DRMs to PIs. PIs can block viral protease by binding to the active center of enzyme [10]. Therefore the resistance to PIs develops as a result of forming amino acids substitutions changing spatial configuration of the active center. PIs have a high genetic barrier to resistance. It means that a significant level of resistance to PIs is forming after accumulation of 3-10 mutations in the HIV genome – major and minor. Major mutations have an effect on HIV resistance, but at the same time they decrease the HIV viability and replicative activity, because lead to structural changes in molecule of HIV enzyme. Minor mutations don't influence on HIV resistance, but they can restore the viral fitness.

***Table* 3. The detection rate of mutations in the pol gene of HIV-1 associated with resistance to NNRTIs**

Mutation	Frequency of detection					
	Female (n=31)		Male (n=25)		Total (n=56)	
	Abs.	Rel.,M±m, %	Abs.	Rel.,M±m, %	Abs.	Rel.,M±m, %
V90I	3	9.68±5.22	5	20.0±7.94	8	14.29±4.68
A98G	1	3.23±3.01	1	4.0±3.79	2	3.57±2.48
L100I	0	0	0	0	0	0
K101E	10	32.26±8.34	9	36.0±9.55	19	33.93±6.33
K103N	4	12.90±5.94	4	16.0±7.26	8	14.29±4.68
V106I	1	3.23±3.01	6	24.0±8.48	7	12.50±4.42
V108I	2	6.45±4.30	1	4.0±3.79	3	5.36±3.0
V179F	1	3.23±3.01	0	0	1	1.79±1.77
Y181C/I	13	41.94±8.81	8	32.0±9.27	21	37.50±6.47
G190S/A	17	54.84±8.88	15	60.0±9.75	32	57.14±6.61
H221Y	0	0	3	12.0±6.42	3	5.36±3.0
P225H	2	6.45±4.30	2	8.0±5.33	4	7.14±3.44

The major DRMs to PIs were found in three plasma samples (5.36±3.0%) obtained from women: M46I/L, V82F/A, I47A (Tab. 4). In all these samples combination of substitutions M46I/L and V82F/A was found (Tab.5). And in all cases the major DRMs to PIs was accompanied by various minor substitutions – L10F, L10I, L33F, Q58E, A71V.

***Table* 4.The detection rate of major mutations in the pol gene of HIV-1 associated with resistance to PI**

Mutation	Frequency of detection (n=56)	
	Abs.	Rel., M±m, %
M46I/L	3	5,36±3,0
V82F/A	3	5,36±3,0
I47A	1	1,79±1,77

M46I/L is selected by therapy with IDV, NFV, FPV, ATV, LPV; it is often associated with V82A; in combination with other PI-resistance mutations M46I/L decrease HIV susceptibility to ATV, FPV, IDV, LPV and NFV. Substitution V82F/A appears during the treating with IDV and LPV; it causes the HIV resistance to ATV, FPV, IDV, LPV and NFV, accept of that in combination with other DRMs to PIs – to SQV and FPV. I47A is selected by the therapy with LPV or DRV and decrease HIV susceptibility to all PIs accept of ATV and SQV [9]. HIV isolate obtained from the sample 320304TroAP has two major and two minor mutations; as a result the virus has high level resistance to NFV and IDV/r and reduced susceptibility to three other drugs from that class (Tab. 5). In the sample DidKY the HIV strain was detected what has three major and three minor mutations, and, as a result, was resistant to four PIs and has a reduced susceptibility to other drugs from that class. Interestingly, that in all cases HIV strains, accept of DRMs to PIs, have the DRMs to NRTIs included in to the therapy regimen. It confirms the fact, that the development of resistance to PIs occurs after the forming of resistance to NRTIs and subsequent selection of other mutations in the HIV genome connected to decreasing susceptibility to PIs.

It should be emphasized, that in sample DidKY the HIV strain with DRMs to NNRTIs (K101E, V106I, Y181C) was detected, although the therapy regimen of that patient doesn't include that drugs. During analysis of anamnesis data it was found, that patient DikKY previously received NNRTI – until 2011 the therapy scheme was TDF/3TC+EFV (that is 2NRTI+1NNRTI). In 2011 the scheme has been changed due to virologic ineffectiveness. Thus, during four years of therapy without NNRTIs the mutations of resistance to drugs of that class were saved in the HIV genome. According to results of different investigations, some mutations disappear quickly after therapy regimen replacement – when the selective pressure of appropriate drug terminate. For example, mutation M184V disappears during few weeks after termination of therapy with 3TC or FTC, the less-fitness HIV strain comprising the mutation is replaced by the high-fitness wild strain [8]. In the same time, mutations that don't affect the HIV fitness can be saved in the viral genome during a period from few months to few years after therapy regimen change. In the plasma samples of men the DRMs to PIs were not detected because men included into investigation have not received the ART with PIs.

***Table* 5. Mutations of resistance to PIs**

№	Patient	Current treatment regimen	DRMSs to PIs		DRMs to NRTIs	DRMs to NNRTIs	Drug resistance interpretation
			Major	Minor			
1	826567 PopOV	ABC+3TC+Lpv/r (2НІЗТ+1ІП)	M46IM	L10I	M184V	V90IV	High-level resistance to 3TC, low level resistance to ABC, potential low-level resistance LPV/r
2	320304 TroAP	TDF/FTC/Lpv/r 2НІЗТ+1ІП	M46I, V82A	L10F, A71AV	D67DG, L74IL, M184V	none	**NRTI:** high-level resistance 3TC, ABC, ddI, FTC (but hypersusceptibility to TDF coused by M184V). **PI:** high-level resistance to NFV, IDV/r, middle-level resistance to FPV/r, LPV/r, ATV/r
3	DidKY	ABC/3TC/Lpv/r (2НІЗТ+1ІП)	M46LM V82A I47A	L10I, L33F, Q58EQ	K70E, M184V	K101E, V106I, Y181C	**NRTI:** low-level resistance to ddI, d4T, TDF, middle-level resistance to ABC, high-level resistance to 3TC and FTC (but hypersusceptibility to ZDV caused by M184V) **NNRTI:** high-level resistance to NVP, RPV, middle-level resistance to EFV, ETR. **PI:** high-level resistance to LPV/r, NFV, IDV/r, FPV/r, middle level resistance to ATV/r, low level resistance to SQV/r, DRV/r

In women (12 persons, 35.71±7.33%) and men (3 persons, 10.71±5.76%), who have not resistant HIV isolates, the therapy ineffectiveness, obviously, has developed as a result of low adherence to ART.

The HIV viral loud levels in patients with ineffective therapy. Due to results of Wilcoxon rank sum test, there is a statistically significant difference ($p<0.05$) between indexes of HIV viral loud (VL) in women, who have HIV drug resistant strains in the blood (mean VL 75,25x103 RNA copies/ml of plasma), and women who don't have drug resistant strains (mean VL 494,25x103 RNA copies/ml of plasma). It could be explained by the effect of DRMs on the level of HIV reproduction. DRMs usually lead to conformational changes of the viral enzymes molecules, that is why drug resistant strains of HIV are less viable and have less level of reproduction than wild strains.

In the group of men high level of HIV VL was associated with the absence of drug resistant strains. Indexes of HIV VL in the samples without DRMs were from 1.45 to 2.55 $x10^6$ RNA copies/ml of plasma, mean level 2.18 $x10^6$ RNA copies. In samples were DRMs were indicated – from 5.8 $x10^3$ to 1.38 $x10^6$ RNA copies/ml of plasma, mean level – 185.0 x 10^3 RNA copies. It should be noted the high levels of HIV VL in the presence of drug resistant strains could be explained to prolonged using of ineffective therapy, that leads to accumulation of the accessory resistance mutations in the HIV genome, which recover HIV replication capacity.

We have compared the spectrum of dominant mutations to different classes of ARVs with the data of other investigations. Due to the results of A.Rakhmanova with colleagues, the most often DRMs in the HIV isolates, obtained from the HIV-positive people in Russia, were M184V, L74V, D67N (associated with resistance to NRTI), G190S/A and K103N(associated with resistance to NNRTI) [5]. During the survey of cohort of Indian HIV-positive people, it was indicated that the most often DRVs to NRTI were M184V, T215Y, D67N, K70R, to the NNRTI – Y181C, G190A, V108I [13].

In the countries of Central America among the patients with virologic ineffective ART the high prevalence of HIV strains with mutations M184V, T215Y, M41L, K103N, V108I were the most dominant. Substitution G190S was detected less frequently [6].

In aforecited investigations the frequency of mutation K65R indication was low, whereas it was present in 26.79% of HIV strains, examined in this work, (in women's samples – 38.71%, in men's samples –12.0%). It could be explained by the fact that the most of the patients included in this investigation (73.81% of women, 67.86% of men) either were obtaining ATR regimens with TDF, or have obtained of TDF-comprising regimen in anamnesis. Interestingly, mutation K65R is the antagonist of TAMs: they never appear together in one HIV genome [7]. Really, the results of the work confirm this fact. Thus, the spectrum of DRMs depends on primarily from the ARVs included in the ART regimens of examined cohort of patients.

The high prevalence of HIV strains with DRMs associated with resistance to two classes of ARVs simultaneously – to NRTIs and NNRTIs, is connected to the pharmacokinetics of reverse transcriptase inhibitors, the duration of their activity period, low genetic barrier of NRTIs and NNRTIs, due to which only one-two DRMs can lead to the loss of HIV susceptibility to that drugs. The period of half-life of NRTIs is significantly shorter compared to NNRTIs. That is why, if the patient messes the doses of ARVs, from time to time in his blood only one active drug could be – it is, practically, the monotherapy with NNRTI. Consequently, the HIV replication is continued during ART leads to the forming of HIV strains resistant to NNRTIs. Further remaining NRTIs quickly become ineffective due to the fast accumulation of DRMs associated with resistance to them.

Thus, the most cases of virologic ineffectiveness of ART in reproductive aged persons were connected to development of HIV resistance to ARVs: drug-resistant HIV strains were indicated in 73.8% women and 89.29% (generally – in 80,0% patients). It should be emphasized, that the most of indicated DRMs are related to the transmissible mutations by the WHO; it means, that the HIV strains with that mutations can be transmitted to other people, leads to grow the prevalence of primer resistance [10]. That is why, the monitoring of drug-resistance HIV strains prevalence among the reproductive aged people is the one of the main areas of the fight against the spread of HIV by the sexual and vertical ways.

Conclusions.

1. It was indicated, that the prevalence of drug resistant HIV strains was 73.80% in the group of women and 89.29% in the group of men (80.0% in total group) with virologic ineffective ART. In 74.29% of incidence of DRMs the ART regimen correction was needed.

2. Among indicated mutations the DRMs to NRTIs and NNRTIs were dominant. HIV strains with DRMs to NRTIs were found in 64.28% of women's plasma samples and 82.14% men's plasma samples; HIV strains with DRMs to NNRTIs – in 66.67% and 89.29% of samples respectively. In most cases mutations of resistance to both ARVs classes were found simultaneously. The DRMs to PIs were indicated with significantly less frequent – totally in 4.29% of all tested plasma samples.

3. In the spectrum of drug resistance mutations (DRMs) the most prevalent mutation associated with high-level resistance to NRTIs was substitution M184V (80.36%); in addition, the high prevalence of K65R (26.79%) was indicated. The most common mutations causing a high-level resistance to NNRTIs were G190S/A (57.14%), Y181C (37.50%), K101E (33.93%). The percentage of most preva-

lent DRMs to PIs (M46L/I and V82F/A) was much lower and amounted 5.36% of all detected mutations.

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Н. Бабій, канд. біол. наук
ДУ "Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України", Київ, Україна

ПОШИРЕННІСТЬ ЛІКАРСЬКО СТІЙКИХ ШТАМІВ ВІЛ ІНФЕКЦІЇ У ВІЛ – ІНФІКОВАНИХ ПАЦІЄНТІВ

Визначено рівень поширеності штамів ВІЛ, резистентних до антиретровірусних препаратів, у ВІЛ-позитивних осіб репродуктивного віку з вірусологічно неефективною антиретровірусною терапію. Частота виявлення резистентних штамів ВІЛ становила 73,0% в групі жінок та 89,29% в групі чоловіків (80,0% серед всіх обстежених пацієнтів). У спектрі мутацій, асоційованих з високим рівнем резистентності ВІЛ до препаратів класу нуклеозидних інгібіторів зворотної транскриптази, найбільш поширеною була заміна M184V (80.36%); крім того, визначено високий рівень поширеності мутації K65R (26.79%). Найпоширенішими серед мутацій, що спричиняють резистентність високого рівня до ненуклеозидних інгібіторів зворотної транскриптази, були заміни G190S/A (57.14%), Y181C (37.5%), K101E (33.93%). Мутації резистентності до інгібіторів протеази виявлялися з нижчою частотою (5,36%).

Ключові слова: ВІЛ, мутації медикаментозної резистентності, антиретровірусна терапія.

Н. Бабий, канд. биол. наук
ГУ "Институт эпидемиологии и инфекционных болезней имени Л.В. Громашевского НАМН Украины", Киев, Украина

РАСПРОСТРАННЕНОСТЬ ЛЕКАРСТВЕННО УСТОЙЧИВЫХ ШТАММОВ ВИЧ ИНФЕКЦИИ У ВИЧ-ИНФЕЦИРОВАННЫХ ПАЦИЕНТОВ

Определен уровень распространенности штаммов ВИЧ, резистентных к антиретровирусным препаратам, у ВИЧ-положительных лиц репродуктивного возраста с вирусологически неэффективной антиретровирусной терапией. Частота выявления резистентных штаммов составила 73,0% в группе женщин и 89,29% в группе мужчин (80,0% среди всех обследованных пациентов). В спектре мутаций, ассоциированных с высоким уровнем резистентности ВИЧ к препаратам класса ненуклеозидных ингибиторов обратной транскриптазы, наиболее распространенной была замена M184V (80.36%); кроме того, был установлен высокий уровень распространенности мутации K65R (26.79%). Наиболее частыми среди мутаций, обуславливающих высокий уровень резистентности к ненуклеозидным ингибиторам обратной транскриптазы, были замены G190S/A (57.14%), Y181C (37.5%), K101E (33.93%). Мутации резистентности к ингибиторам протеазы определялись реже(5,36%).

Ключевые слова: ВИЧ, мутации лекарственной резистентности, антиретровирусная терапия.

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G. Korotyeyeva, PhD, G. Petrova, stud.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
L. Kykot, PhD.
M.M. Grishko' National Botanical Garden National Academy of Sciences of Ukraine, Kiev, Ukraine
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CARLAVIRUS IN LILY COLLECTION OF GRISHKO' NATIONAL BOTANICAL GARDEN

Screening of Lilium plants on virus diseases in the collections of M.M. Grishko' National Botanical Garden have been conducted. Basing on serological, biological and morphological properties, we suggest that filamentous virus is related to Lily symptomless virus. Another virus is not completely identified, but symptoms induced on indicator plants suggested that it was Tomato aspermy virus.

Key words: lily virus, virus of ornamental plants.

Introduction. The genus Lilium is one of the most valuable commercial market flower bulbs in the world, mainly owing to its ornamental function as a cut flower or as a potted plant. Susceptibility of lilies to infectious diseases limits their popularity as ornamental plants. Fungi and bacteria are removed during the establishment of in vitro cultures from standard sterilization of bulb scales, whereas viruses are not [1].

Virus diseases of lily are of great significance because even when present in the latent state, the viruses could be transmitted to healthy plants and cause commercial losses [2]. In spite of the fact that we could predominantly diagnose virus infection basing on specific symptoms such as ring spot, mosaic and necrotic lesions, the identification of the pathogen is not possible. Sometimes lilies do not manifest signs of virus infection [3]. Besides, some factors such as disbalance of mineral nutrition, non-compliance with the light regime, invasion by insects and mites, infections coused by bacteria, mycoplasms and fungus, or genetic disorders could lead to symptoms similar to those of virus nature. This involves necessity for serological diagnostics of the collections for preservation of their commercial value.

Materials and methods. *Lilium* plants with visual virus-like symptoms from greenhouse collections of M.M. Grishko' National Botanical Garden (Kyiv) were the objects of this research.

Infectious nature of disorders was confirmed proved using indicators plants typical for viruses normally infecting lilies such as *Cucumis sativue, Cucurbita pepo, Nicotiana tabacum* cv. Samsun, *N. Rustica, Licopersicon esculentum, Phaseolus vulgaris*. Virus identification was carried out using TAS- and indirect ELISA [4]. Same staining samples were analyzed in electron microscopy at 30,000 magnification.

Results and discussion. Virus diseases are easily spread with planting material in vegetatively-propagated crops, including various ornamentals. Several viruses have been reported to occur wildly in lily plants, often as a mixed infection, reducing their vigour and sometimes their marketability [5].

Screening of *Lilium* plants for viral diseases in the collections of M.M. Grishko' National Botanical Garden was conducted. Different virus-like symptoms were detected on lily plants: mottling, mosaic, color breaking and leaf deformation. Infectious nature of disorders was confirmed using indicator plants typical for viruses normally infecting lilies (fig.1).

A B

Fig.1. Virus-like symptoms on lily cultivars: H-Dawn' (A), 'Krema-2' (B)

To define biological properties of the pathogens, we conducted a bioassay using 6 species of indicator plants. Indicator plants were inoculated with sap obtained from lilies demonstrating virus-like symptoms. Results of the assay are presented on Table 1.

***Table* 1. Response of indicator plants inoculated with sap from symptomatic lily plants**

Plant species	Symptoms on indicator plants					
	Nicotiana tabacum cv 'Samsun'	*Nicotiana rustica*	*Cucumis sativus*	*Licopersocon esculentum*	*Cucurbita pepo*	*Phaseolus vilgaris*
Red Alert	**N**	-	-	-	-	-
Black Beauty	-	-	**M**	-	-	-
Sunburst	-	-	-	-	-	-
Krema-1	-	-	-	-	-	-
Royal Gold	-	**N**	**M**	-	**M**	**M**
H-Dawn	-	**N**	**M, D**	-	**M**	**M, N**
Krema-2	-	-	**M**	-	-	-
Mister Cas	-	-	-	-	-	-

- – negative response; M – mosaic; N – necroses, D-deformation

Mosaic symptoms on *Phaseolus vulgaris* inoculated with sap from lily cultivars H-Dawn' and Royal Gold are not typical for any known lily virus. On the contrary, mosaic symptoms observed on *Cucumis sativus* and *Cucurbita pepo* were typical for *Cucumber mosaic* (CMV) and *Tomato aspermy viruses* (TAV). Besides necrotic symptoms on *nicotiana rustica* were common for TAV and *Tobacco rattle virus* (TRV).

Thus we conducted the infectious etiology of diseases on *Lilium*. Absence of reactions on some indicator plants post inoculation with sap from diseased plants, in our opinion, couldn't be explained with non-transmittance of some viruses by mechanical inoculation or with insusceptibility of definite indicator plants to virus infection.

To determine virus nature of disease we conducted indirect and DAS-ELISA tests [4]. Same samples were analyzed in electron microscopy at 30,000 magnification.

Results of ELISA tests showed positive reactions of *Lilium* cultivar 'Royal gold', 'H-dawn' and 'Krema-2' with antiserums to *Potato virus S* (PVS). We deem it could indicate contamination of these plants with *Lily symptomless virus* (LSV), which is serologically related to PVS [6].

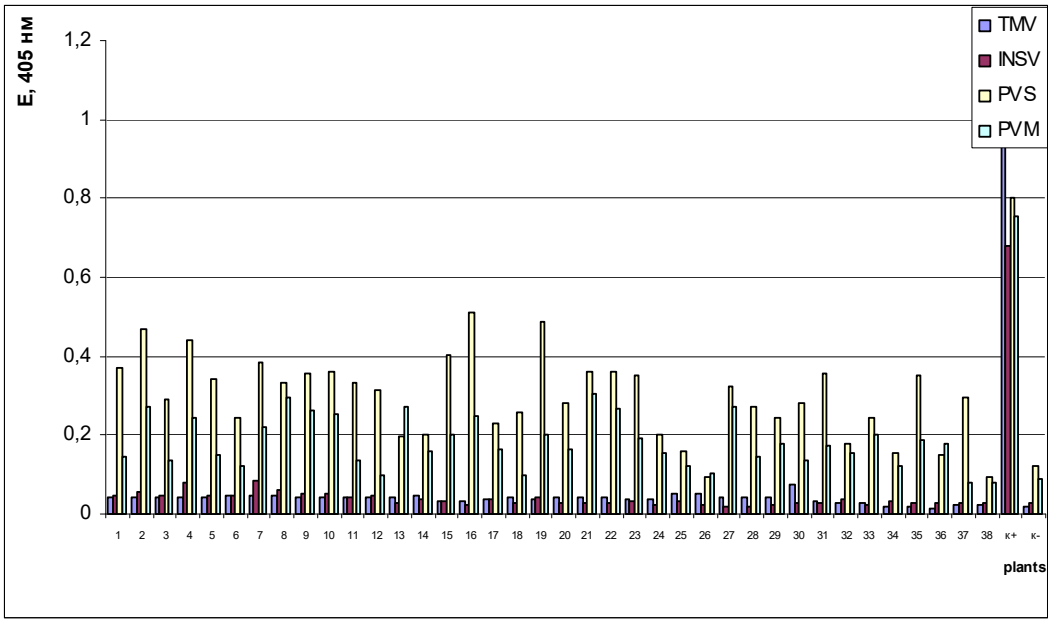

Fig.2. Results of ELISA testing of lily plants from the collection of M.M. Grichko' National Botanical Garden:
1 – Red Alert; 2 – Black Beauty; 3 – Elite (3n); 4 – Sunburst.; 5 – Heroes of Bres fortress; 6 – Magi; 7 – Krema-1; 8 – Kalinka; 9 – Black out; 10 – Conca D'or; 11 – Adelaide; 12 – Ce 6 Dazzle; 13 – Anastasia -2; 14 – Pink Perfection; 15 – Royal Gold; 16 – H-Dawn; 17 – Dream; 18 – Lilium henryi; 19 – Krema-2; 20 – Nymph; 21 – Rondo; 22 – Pagoda Bells; 23 – Touching; 24 – Conca D'or; 25 – Albung; 26 – H-9; 27 – Brigita; 28 – Anniversary; 29 – Anastasia; 30 – Criesdach Tetra Pink; 31 – Corona white; 32 – Yellow Planet; 33 – H-27; 34 – Saltarello; 35 – Mister Cas; 36 – hybrid Schenk; 37 – Kentucky; 38 – Original love; К+ – positive control; К- – negative control (normal plant)

Filamentous virus particles about 650 x 20 nm in size were observed in the sap of the plants. In addition, icosahedral particles were also observed, with a mean diameter of approximately 30 nm (Fig.3).

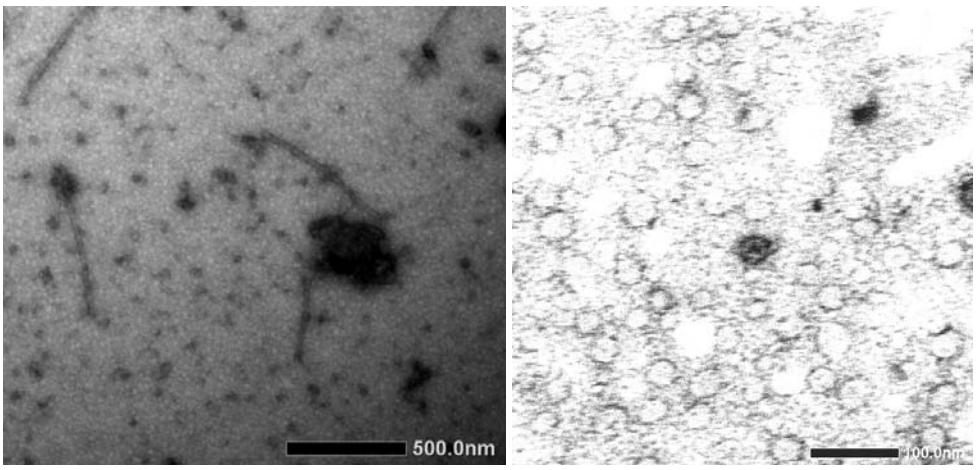

Fig. 3. Electron micrograph of viral particles in plant sap

Basing on serological, biological and morphological properties, we suggest that filamentous virus is related to *Lily symptomless virus*. Another virus is not completely identified, but symptoms induced on indicator plants suggested that it was *Tomato aspermy virus*.

Virus infections, especially latent diseases, are very dangerous because of the extensive exchange of untested plants among different botanical gardens and private collections. Additionally the incidence of such viruses as LSV which are generally symptomless in field-grown plants may cause problems while plants infected by other viruses. Besides, vegetative propagation of lilies without virus monitoring leads to uncontrolled distribution of viral infections within the collection.

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Г. Коротєєва, канд. біол. наук, Г. Петрова, студ.
Київський національний університет імені Тараса Шевченка, Київ, Украиїна,
Л. Кикоть, канд. біол. наук
Національний ботанічний сад імені М.М. Гришко НАН України, Київ, Україна

КАРЛАВІРУС ЛІЛІЇ В КОЛЕКЦІЇ НАЦІОНАЛЬНОГО БОТАНІЧНОГО САДУ ІМ. М.М. ГРИШКА НАН УКРАЇНИ

Проведено обстеження ліліейних колекцій Національного ботанічнго саду ім. М.М. Гришка на наявність вірусного ураження. За серологічними, біологічними та морфологічними властивостями виявлений вірус, подібний до безсимптомгого вірусу лілій. Другий – остаточно не ідентифіковано, але за морфологічними характеристиками, реакцією рослин індикаторів можна припустити, що це вірус аспермії томатів.

Ключові слова: вірус лілії, вірус декоративних рослин.

Г. Коротеева, канд. биол. наук, Г. Петрова, студ.
Киевский национальный университет имени Тараса Шевченко, Киев, Украина,
Л. Кыкоть, канд. биол. наук
Национальный ботанический сад имени Н.Н. Гришко НАН Украины, Киев, Украина

КАРЛАВИРУС ЛИЛИИ В КОЛЛЕКЦИИ НАЦИОНАЛЬНОГО БОТАНИЧЕСКОГО САДА ИМ. Н.Н. ГРИШКО НАН УКРАИНЫ

Проведено обследование лилиейных в коллекции Национального ботанического сада им. Н.Н. Гришко на наличие вирусного поражения. Опираясь на серологические, биологические и морфологические свойства изолированый вирус является безсимптомым вирусом лилий. Второй вирус окончательно не идентифицирован, но по морфологическим характеристикам, реакцией растений индикаторов можно предположить, что это вирус аспермии томатов.

Ключевые слова: вирус лилии, вирус декоративных растений.

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L. Mishchenko, Dr. Sci. Biol, Prof., A. Dunich, PhD
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
O. Taran, PhD
National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine,
L. Glushchenko, PhD
Experimental Station of Medicinal Plants of the Institute Agroecology and Environmental of National Academy of Agrarian Sciences of Ukraine, Poltava region, Ukraine

DETECTION OF THE PATHOGEN OF VIRAL DISEASE IN *SAMBUCUS NIGRA* PLANTS

For the first time viral disease of elderberry (Sambucus nigra L.) was detected in Ukraine. Symptomatology of the disease and morphological properties of the virus are studied. Based on scientific literature data, screening of viruses that can infect elderberry plants in Ukraine is conducted. Antigens of PVY, PVM, SMV, AMV, and BYMV in elderberry plants with symptoms of viral disease were not detected.

Key words: elderberry, Sambucus spp., viral diseases.

Introduction. Elderberry (*Sambucus nigra* L., family *Adoxaceae*) is widely used in scientific and traditional medicine [Chekman]. Extracts of elder berry flowers are part of complex drugs as "Novo Passit", "Sinupret", "Atma", etc [27]. It is known that the therapeutic activity of elderberry fruit is second only to chokeberry, which superior elderberry fruit for antioxidant effect. Biologically active compounds of elderberry fruit, flowers, leaves have antiviral, antibacterial, anti-inflammatory, analgesic, immunomodulatory and antiproliferative effects. Traditional medicine recommends taking elderberry fruit with damage to the mucosa of the stomach, liver and pancreas. Tincture of the flowers and leaves of black elderberry has anti-inflammatory, antioxidant and hepatoprotective activity [29].

Sambucus nigra L. plants are sensitive to atmospheric drought. Elderberry propagated mostly by seeds. Elderberry flowers contain up to 82 mg% ascorbic acid, glycoside sambunigrin, rutin, essential oils, organic acids, anthocyanins, phenolic compounds, coumarin, triterpenoids, micro elements, etc [27]. It is known that the content of ascorbic acid and essential oil in the raw material depends on the illumination of elderberry growth place. Ascorbic acid content in the raw material collected at cutting down was higher by 20% and amounted to 75-82 mg% compared with raw materials collected from plants undergrowth. Essential oil content indicators were also higher – by 10-15%. Essential oil content within the same version of the raw material varies depending on the lighting stage, from which were collected materials. Essential oil content can vary from 0.03% to 0.14% depending on the placement of flowers on the plant. Wild elderberry plants are good spring and summer honey. One flower provides 0.16 mg nectar containing 23% sugar. One hectare of continuous planting in open, well-lit areas allocates about 85 kg of nectar. The fruits of elderberry have a unique sweet-sour flavor and fresh not edible. But collected at the stage of full ripeness used as industrial raw materials for processing and manufacture of confectionery products, juices, in wine production, textile industry etc.

On the chemical composition and content of biologically active substances in medicinal plants significantly affect pests and diseases, including viral [17]. It is known that various elderberry species infected by viruses that affect the metabolism of plants, reduce productivity and can degrade the quality of medicinal raw materials.

Viral diseases of elderberry plants first described back in 1925 [15]. Many viruses are known to cause detrimental symptoms in both American and European elderberry including members of the family ***Bromoviridae***: *Cucumber mosaic virus* [19]; ***Secoviridae***: *Arabis mosaic virus, Cherry leaf roll virus, Cherry rasp leaf virus, Strawberry latent ringspot virus, Tobacco ringspot virus, Tomato black ring virus, Tomato ringspot virus* [5, 6, 10, 14, 19, 20, 21, 22, 24, 28, 30]; ***Virgaviridae****: Tobacco mosaic virus* [18], and ***Tombusviridae***: *Elderberry latent virus, Tobacco necrosis virus, Tomato bushy stunt virus* [8, 9, 19, 25].

Most reports of elderberry infecting are about *Cherry leaf roll virus* and carlaviruses. *Blueberry scorch virus* (BlScV), *Elderberry symptomless virus* (ElBSV) and several other putative members of the genus ***Carlavirus*** **(family *Betaflexiviridae*)** have also been reported in elderberry [1, 3, 4, 11, 26]. There is report about infecting of *Sambucus canadensis* plants by filamentous virus which similar on morphological features to carlaviruses [9]. Subsequently, the virus was detected in the Netherlands and was named *Elderberry virus A* [26]. Recent studies of elderberry samples (*Sambucus* spp.) from Missouri (USA) showed infecting of these plants with two different viruses, which also belong to the genus *Carlavirus* [12]. Five novel carlaviruses tentatively named as Elderberry virus A–E (ElVA–ElVE, respectively) were discovered [7, 8]. Elderberry carlavirus group 1 (ElVA, ElVB, ElVD)and group 2 (ElVC and ElVE) appear to have emerged from two distinct lineages, containing closely related viruses that infect the same host, indicative of sympatric speciation [Ho et al, 2016]. This, in addition to the recombination analysis, imply that elderberry, along with hop, phlox and potato (respectively infected by *Hop latent virus* and *Hop mosaic virus; Phlox virus B* and *Phlox virus S; Potato virus P* and *Potato virus S*), are major contributors of the carlaviruses evolution.

Despite the considerable amount of the studies of elderberry viruses in the world and particularly in Europe, such investigations in Ukraine haven't been conducted.

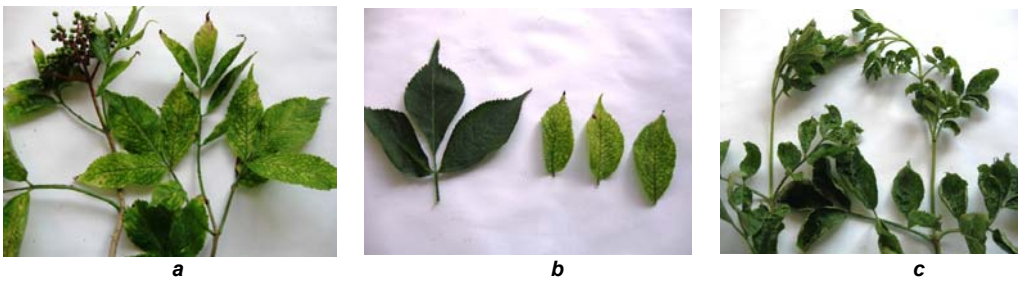
That's why the **aim of the research** was to obtain the *Sambucus nigra* plants on the presence of viral diseases.

Materials and methods. For diagnostics of viruses in the plants applied the methods of visual diagnostics, ELISA and transmission electronic microscopy (EM). Contrasting has been made with 2% solution of phosphorus – tungstic acid. Virions are investigated using electron microscope JEM 1230 (JEOL, Japan).

Detection and identification of viruses has been carried out with enzyme-linked immunosorbent assay (DAS-modification) using commercial test-systems of firm LOEWE (Germany). The results of reaction registered on the rider Termo Labsystems Opsis MR(THE USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 нм. All samples showing values three times higher than the negative controls are assumed as virus positive.

The extinction values (the optical density) of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski [13]. The confidential intervals were at a significance rate of $P \leq 0.05$ of Student's criterion.

Results and discussion. Under observations of wild elderberry plants in Poltava (2015-2016) and Kyiv (2016) regions we detected plants with chlorotic symptoms (a, b) and rolling of leaf tops (c) and twisting up the edges of the leaves (Fig.1).



a *b* *c*

Figure. 1. Symptoms of viral infection on *Sambucus nigra* plants:
a,b – chlorotic foliage symptoms; c – leaf rolling

Number of affected plants accounted for over 20% of surveyed wild elderberry.

It should be noted that analysis of world scientific literature on elderberry viruses showed that the most common symptom is chlorotic mottling ('blotching') and induced by many viruses [3, 20, 22]. But the chlorotic foliage symptom is not found in any report.

Filamentous virions 650±50 × 12 nm were found in the elderberry leaves conducting the transmission electron microscopy method. It was marked higher concentration of virions in plants with leaf rolling symptom compared with chlorotic. In addition, earlier PVM and PVY were identified by us in tomatoes with leaf rolling symptoms for the same agroecological conditions (in Poltava and Kiev regions) [16]. Such morphology is characteristic for viruses from the genus *Potyvirus* (*Potyviridae)* and *Carlavirus (Betaflexiviridae*). It is known that these genuses have a large number of representatives. So in our research, we settled on poty- and carlaviruses that are wide spread in Ukraine and have a wide range of host plants.

Based on the our results and on the data of other scientists we tested elderberry plants with mentioned above symptoms on the presence of carlaviruses *(Potato virus M)*, potyviruses (*Potato virus Y, Soybean mosaic virus, Bean yellow mosaic virus)* and *Alfalfa mosaic virus*. *Alfalfa mosaic virus* was detected in guelder rose (*Viburnum*), belonging to the same family with the elderberry [3, 23].

According to the ELISA results antigens of PVY, PVM, SMV, AMV, and BYMV in the tested elderberry samples were not found (tabl.).

***Table*. Content of the viruses antigens in *Sambucus nigra* plants, optical density at 405 nm**

sample / antiserum	chlorotic foliage symptoms	leaf rolling	Positive control (commercial)	Negative control (sap of healthy elderberry plants)
PVY	0,039±0,006	0,040±0,005	1,560±0,009	0,042±0,003
PVM	0,038±0,006	0,042±0,005	1,428±0,005	0,039±0,003
SMV	0,042±0,004	0,046±0,001	1,394±0,015	0,046±0,003
AMV	0,026±0,005	0,024±0,005	1,045±0,012	0,034±0,005
BYMV	0,049±0,005	0,041±0,001	1,800±0,007	0,042±0,004

Thus, literature data indicate circulation of many viruses in elderberry plants, and other shrubs of this family. Elderberry viral disease was for the first time founded by us also in Ukraine that is potentially dangerous in epidemiological aspect. Shrubs are reservoirs of viruses and contribute to the viruses wintering and future spreading to economically important crops in this region.

Conclusions.

1. For the first time viral disease of elderberry (*Sambucus nigra* L.) was detected in Ukraine.

2. Symptomatology of the disease and morphological properties of the virus are studied.

3. Based on scientific literature data, screening of viruses that can infect elderberry plants in Ukraine is conducted. Antigens of PVY, PVM, SMV, AMV, and BYMV in elderberry plants with symptoms of viral disease were not detected.

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Л. Міщенко, д-р біол. наук, проф., А. Дуніч, канд. біол. наук
Київського національного університету імені Тараса Шевченка, Київ, **Україна**,
О. Таран, канд. біол. наук
Національний університет природокористування та біоресурсів України, Київ, Україна,
Л. Глущенко, канд. біол. наук
Дослідна станція лікарських рослин Інституту агроекології і природокористування,
НААНУ, Полтавська обл., Україна

ВИЯВЛЕННЯ ЗБУДНИКІВ ВІРУСНОГО ЗАХВОРЮВАННЯ У РОСЛИН БУЗИНИ ЧОРНОЇ

Вперше в Україні виявлено вірусне захворювання рослин бузини чорної (Sambucus nigra L.). Досліджено симптоматику хвороби та морфологію вірусу. Базуючись на даних наукової літератури, проведено скринінг вірусів, які можуть уражувати рослини бузини в Україні. Антигенів цих вірусів у рослинах бузини із симптомами вірусного захворювання не виявлено.

Ключові слова: бузина, Sambucus spp., вірусні хвороби.

Л. Мищенко, д-р биол. наук, проф., А. Дунич, канд. биол. наук
Киевского национального университета имени Тараса Шевченко, Киев, Украина,
О. Таран, канд. биол. наук
Национальный университет природопользования и биоресурсов Украины, Киев, Украина,
Л. Глущенко, канд. биол. наук
Опытная станция лекарственных растений Института агроэкологии и природопользования НААНУ, Полтавская обл, Украина

ВЫЯВЛЕНИЕ ВОЗБУДИТЕЛЯ ВИРУСНОГО ЗАБОЛЕВАНИЯ У РАСТЕНИЙ БУЗИНЫ ЧЕРНОЙ

Впервые в Украине выявлено вирусное заболевание растений бузины черной (Sambucus nigra L.). Исследована симптоматика болезни и морфология вируса. Базируясь на данных научной литературы, проведен скрининг вирусов, которые могут поражать бузину в Украине. Антигенов этих вирусов в растениях бузины с симптомами вирусного заболевания не выявлено.

Ключевые слова: бузина, Sambucus spp., вирусные болезни.

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K. Naumenko, PhD stud., A. Golovan, PhD.,
G. Baranova, sen. techn., S. Zagorognya, PhD., Yu. Shermolovytch, Dr. Sci.
D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kiev, Ukraine

ANTIVIRAL AND IMMUNOSTIMULATORY POTENTIAL OF FLUORINE CONTAINING TRIAZOLES

The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections. The purpose of this study was to investigate of antyherpetic activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in in vivo models and determine their immunomodulatory potential. Shown significant inhibition of virus reproduction under the influence of the compounds at concentrations of 0.4 and 0.5 mg/kg, which was more effective of acyclovir. Protection ratio amounted to 80%. Increasing level of IFN-γ and IL-2 in serum of animals, indicated available immunomodulatory effect fluorinated nucleoside compounds. Our studies indicated that there is antiherpetic, immunomodulatory activity of fluorine containing triazole and there is need to in-depth study of the mechanisms of this process.

Ключовые слова: HSV-1, fluorinated nucleoside, antiherpetic activity.

Introduction. Herpes simplex virus type 1 (HSV-1) is member of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* virus family [1]. HSV-1 is a common infection in developed countries where rates of seropositivity usually exceed 50%. In both humans and experimental animals, primary infection of skin or mucosa results in the local replication of virus, infection of sensory nerve ending, and spread via retrograde axonal transport to the ganglia of the peripheral nervous system (PNS) where a productive infection of neurons ensues. Although infectious virus is eventually cleared, a latent infection is established in neurons of the PNS ganglia [1,2]. In humans, HSV-1 is a common cause of sporadic viral encephalitis with mortality rates reaching 20-30% despite treatment [2]. Also the virus plays an important role in human infectious pathology, causing diseases such as keratoconjunctivitis, stomato gingivitis, congenital herpes and others [2].

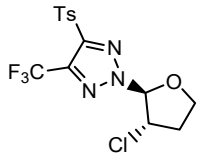
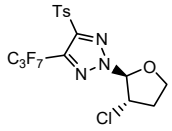
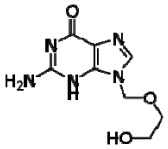
The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections accompanied by the development of protracted and chronic forms of severe consequences. In clinical practice for treating these diseases most frequently use nucleosides, modified in heterocyclic, phosphate or carbohydrate fragment of the molecule. Today discovered many anti-herpetic drugs. However, acyclovir and other acyclic nucleosides in it is purpose and mechanism of action inhibit only those herpesvirus actively replicate, so the virus will prevent a latent state, is one of the problems of treatment of HSV-1. Another issue that complicates treatment herpesvirus is the development of viral resistance to abnormal nucleosides. There are many compounds are promising system *in vitro*, but only a few remain active *in vivo*.

In response to a viral infection in the body is activation of cytokines that modulate the overall immune response. In this regard, one of the methods of treatment of viral infections is the use of various drugs – interferon inducers that stimulate the production of interferon in the body, providing thus strengthening antiviral response [3, 4].Interferon-gamma (IFN-γ) is a cytokine that plays physiologically important roles in promoting innate and adaptive immune responses. The absence of IFN-γ production or cellular responsiveness in humans and experimental animals significantly predisposes the host to viral infection, a result that validates the physiologic importance of this cytokine in preventing infectious disease [4]. Recently, an additional role for IFN-γ in preventing development of primary and transplanted tumors has been identified. Focusing on the data implicating IFN gamma as a critical immune system component that regulates antiviral immune response is important question for research [4, 6, 7]. Interleukin (IL)-4 and IL-2 are lymphokines synthesized primarily by activated T helper lymphocytes, and both are important regulators for development of T helper subsets (Th1-like vs. Th2-like) [8, 9]. Th1 cells are involved in cellular immunity (delayed type hypersensitivity and cellular cytotoxicity) and produce IL-2, tumor necrosis factor (TNF)-β, and IFN-γ. Th2 cells are involved in humoral (antibody-mediated) immunity and produce IL-4, IL-5 and IL-10 [10]. Il-4 is an important regulator of isotype switching, inducing IgE production in B lymphocytes and can exhibit anti-inflammatory effects [10, 11, 12]. Il-2 is important for *invitro* growth of cytotoxic T cell (CTL) lines and can enhance NK cell and B cell responses [13, 14]. The IFN-γ production is the most rapid reaction in response to a virus infecting cells, as determined immunomodulatory potential nucleoside compounds at the level of IFN-γ and two pro- and anti-inflammatory cytokine IL-2 and IL-4 [15].

The purpose of this study was to investigate of antyherpetic activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in *in vivo* models and determine their immunomodulatory potential.

Materials and methods. Herpes simplex virus type 1 (HSV-1, strain US1), obtained from the Institute of antiviral chemotherapy, The Center for Clinical and Theoretical Medicine (Germany). The compounds under study were G8 and G9 (they are the 2-N-substituted-4-tosyl-5-polyftoralkyl-1,2,3-triazoles). They were provided by the Institute of Organic Chemistry of Ukraine. The substance of acyclovir was used as a reference compound. Their structural formulas are given on table 1.

***Table* 1. Structure of studied compounds**

		
G8	G9	Acyclovir

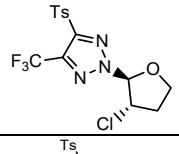
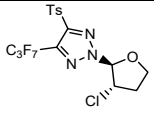
Animals. Inbread mice (3–4 weeks old) were obtained from vivarium of D.K. Zabolotny institute of microbiology and virology NAS of Ukraine. Animals were maintained under protocols approved by the Institutional Animal Use and Care Committee. Mice were inoculated with HSV by intracerebral inoculationwith 1,5-10^3 PFU HSV-1, which is a 50% lethal dose for mice. Acyclovir (ACV) at 0,1 μg/kg of body weight and G8 and G9 at 1 μg/kg of body weight as a control were administered by intraperitoneal injection.

Cytokines. Levels of cytokines were determined in blood serum and by isolating splenocytes using the "Pro immuno" protocol for preparation of murine splenocyte (BD Biosciences). The level of IFN- γ, IL-2 and IL-4 was investigated. The levels of cytokines were detected by using "Mouse INF-γ ELISA kit", "Mouse IL-2 ELISA kit", "Mouse IL-4 ELISA kit" (Thermo Scientific, USA).

Statistical analysis. Protective parameters and levels of cytokines were analyzed by Microsoft Excel. Results were considered statistically significant at $p < 0.05$.

Results and discussion. Previously at the system *in vitro* was determined cytotoxicity level and antiviral activity of the compounds. Cytotoxic concentration (CC_{50}), which was 887 and 990, effective concentration (EC_{50}) 50 and 7,6 μg/ml, was shown, respectively (table 2). Selective index of compounds G8 and G9 is 18 and 130.

***Table* 2. Cytotoxicity and antiviral activity of fluorinated nucleoside in *in vitro* system**

Code	The structural formula	Mol. mass of compounds	The cytotoxicity concentration (CC_{50}), μg/ml	The effective concentration (EC_{50}) μg/ml	IS
G8		395.78	887	50	18
G9		495.81	990	7,6	130

In vivo studies conducted fluorinated compounds on white outbred mice weighing 16-18 gram. The paper had 12 groups of 10 mice each. Animals were injected 30 ml intracerebral of virus, LD_{50} which was 1.5 * 10^3 PFU. The compounds were administered intraperitoneally at 200 ml, 3 concentrations, G8 – 40, 100 and 500 μg/ml, and G9 for 50, 100 and 500 μg/ml. As a reviewer of the drug was used acyclovir in concentrations of 10 μg/ml.

The dynamics of animal deaths were recorded daily for 21 days. In the control group, virus death of the animals took place on 4, 6, 10 and 14 days.

After analyzing the results, were identified 50% of the death of animals in the control group of HSV-1. In version 8 compounds at concentrations of 500 and 100 μg/ml recorded 10% and 20% of animals deaths (table 2).

***Table* 2. Analysis of animal deaths in the experimental group**

Groups of experimental animals	The dose, ml	Amount of mice	Animals death		Protection factor	Effectiveness Index
			Amount	%		
Controlof HSV-1	0,2	10	5	50	-	-
ControlG8, 100μg/ml	0,2	10	0	0	-	-
G8, 40μg/ml	0,2	10	0	0	-	-
G8, 100μg/ml	0,2	10	2	20	2,5	60
G8, 500μg/ml	0,2	10	1	10	-	-
ControlG9, 100μg/ml	0,2	10	0	0	-	-
G9, 50μg/ml	0,2	10	0	0	-	-
G9, 100μg/ml	0,2	10	1	10	5	80
G9, 500μg/ml	0,2	10	0	0	-	
Acyclovir, 10μg/ml	0,2	10	0	0		

The percentage of deaths of animals in the group G9 at a concentration of 100 μg/ml indicates high efficiency protection compound. Based on experimental data was determined protection ratio and the index of efficiency of the studied compounds. Effectiveness index amounted to 60% and 80% for G8 and G9 compouds, respectively. Our studies indicated that there is antiherpetic activity of fluorine containing triazole and there is need to in-depth study of the mechanisms of this process.

Previous studies had shown triazole derivatives of antiviral properties, but the impact of these compounds on the launch of major cytokine synthesis is still unknown. Therefore, was conducted a comparative study of production of

proinflammatory cytokines, activators of cellular immunity: IL-2 and IFN-γ and their antagonist IL-4.

The level of IFN and IL-2 was investigated in the blood serum of animals 14 days. In all experimental groups observed a significant increase in the level of IFN compared with the control virus. By adding the compound G9 indicators of interferon were increased with increasing concentration (fig.1).

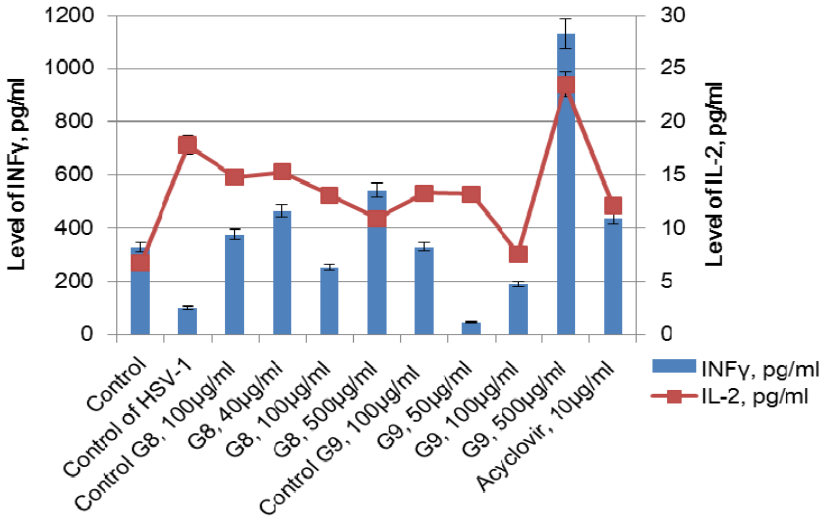


Fig. 1. The levels of interferon γ and interleukin-2 in the blood serum of experimental animals were determined

Interferon gamma suppresses viral replication in cells, my immunomodulatory properties. High levels INF compounds in samples from G8 and G9, may indicate immunostimulatory properties of the compound.

In the study of experimental data on levels of IL-2 was set pretty low. In the control group, HSV-1 levels of IL-2 was 17,8 pg/ml, while in other groups (G8 /1-3, G9/1-2, acyclovir) index were lower than control.Such data can be explained by one of the functions of IL-2 is to stimulate immune cells such as cytotoxic lymphocytes (for example, fast action in the early days of infection). Since the samples were selected on day 14, the level of IL-2 decreased in the groups of compounds. As a control virus observed high levels of IL-2, indicating that the active development of viral infection of inflammation (fig. 2).

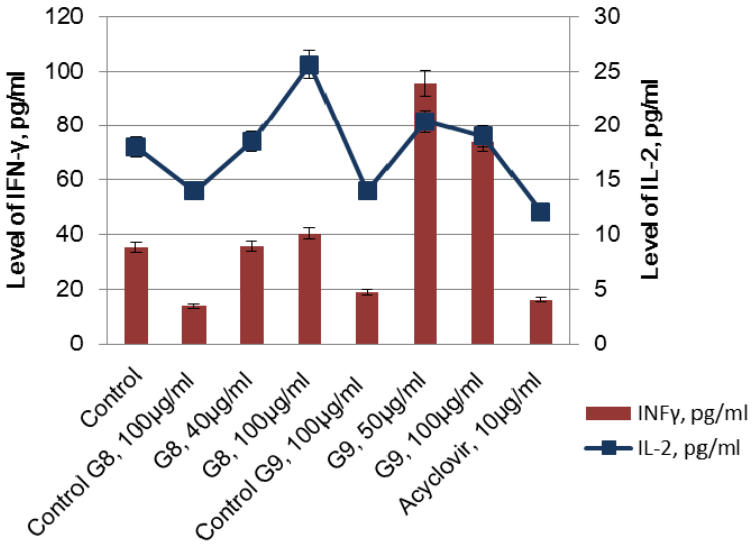


Fig. 2. Studying the levels of interferon γ and interleukin 2 secreted by isolated splenocytes

It was also determined activity of interferon producing by isolated splenocytes of mice under the influence of the studied compounds *in vivo*. Compared with controls, the compound G9 caused increased production INFγ, indicating that the interferon-inducing potential.

In the study of IL-2 secreted isolated splenocytes observed a significant increase in both compounds (G8, G9), as the level of IL-2 significantly higher than the control.

The data point to a slight activation of IL-4 isolated splenocytes, but this activation was not significant compared to the control. However, when examined serum of infected and control animals was detected slightly lower rates of IL-4 (fig. 3).

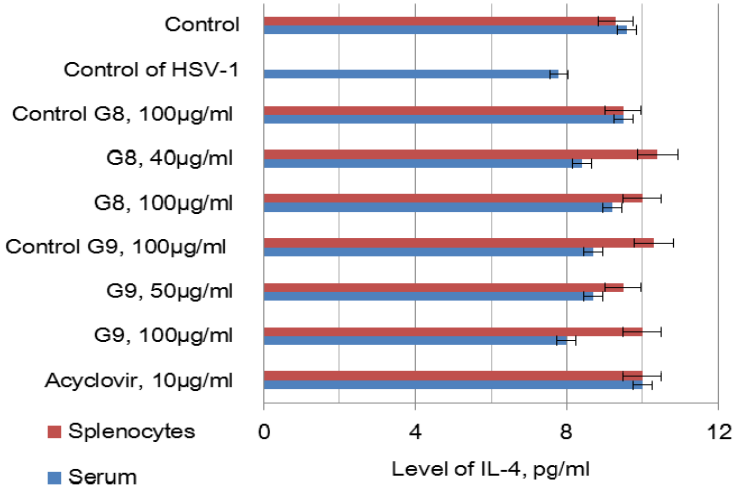


Fig. 3. Determining the level of interleukin-4 in the blood serum of animals and secreted by isolated splenocytes.

Thus, the compounds do not activate the production of IL-4. In turn, this cytokine enhances the proliferation and differentiation of B-lymphocytes, that contributes to the development of humoral immune response. Thus, the effect of these compounds is not directed at the development of humoral immune response.

In general, control of infection against viruses is linked to the induction of a Th1 response, while protection against extracellular pathogens correlates with a Th2 response [13]. IL-2 is a cytokine that exhibits an impressive number of different functions largely dictated by the biological context in which it operates. It is pivotal for cellular activation, important for primary T-cell responses and essential for secondary T-cell responses. Although, IL-2 specifically promotes T-cell activation and proliferation of only those cells that have been stimulated by cognate antigenic interaction, downregulation of T-cell responses occurs nonspecifically by facilitating a separate population T cell [10]. IL-4 induces the expression of class II major histocompatibility complex (MHC) molecules on macrophages and dendritic cells. IL-4 is a well-documented mediator of Th2 cell commitment, and induces Ig class switching to the Th2-associated isotypes IgG and IgE. However, IL-4 can exhibit anti-inflammatory effects, including suppression of macrophage function such as IL-1 and TNF production [12].

Also, the IFN-γ antiviral defense mechanism that occurs very early during the course of infection interferes both with the early steps of virus invasion and replication, and with the control of persistent infection. IFN- γ has immunomodulatory effects on T cells, macrophages, NK and B cells [5].

Analyzed data of the levels of cytokines indicate that significant immunostimulatory potential of the investigated compounds were determined. It is shown that the G8 and G9 affect at IFN-γ and IL-2, ie on the cellular immunity. Investigated that the compounds did not affect IL-4, ie on the humoral immunity. Our studies include compounds G8 and G9 to a relatively perspective antiviralsHSV-1 with immunomodulatory potential and can be used in further research.

Conclusions. The research activity anti-herpetic fluorinated nucleoside compounds in model *in vivo* were established. The models of HSV-1 herpes meningoencephalitis stimulated mice show antiviral activity of the compounds in minimally investigated concentrations of 0,4 and 0,5 mg/kg, they significantly inhibited the reproduction of the virus. Showing raising INFγ in the blood serum of animals when administered the compounds HSV-1 infected mice, which causes additional antiviral protection of animals. Increasing level of IFN-γ and IL-2 in serum of animals, indicated available immunomodulatory effect fluorinated nucleoside compounds. The results suggest the presence antiherpetic, immunomodulatory activity of fluorine containing triazole and the need for in-depth study of the mechanisms of this process.

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К. Науменко, студ., А. Головань, канд. біол. наук, Г. Баранова, пров. інж., С. Задорожна, канд. біол. наук, Ю. Шермолович, д-р біол. наук
Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, Київ, Україна

АНТИВІРУСНИЙ ТА ІМУНОСТИМУЛЮЮЧИЙ ПОТЕНЦІАЛ ФТОРВМІСНИХ ТРИАЗОЛІВ

Проблема пошуку ефективних противірусних препаратів зумовлена високою захворюваністю і широким розповсюдження вірусних інфекцій. Метою представленої роботи було дослідити антигерпетичну активність фторованих нуклеозидних сполук G8 та G9 (2-N-заміщені-4-тозил-5-поліфторалкіл-1,2,3-триазоли) на моделі invivo тавизначити їх імуномодулюючий потенціал.Показано значне інгібування репродукції вірусу під дією досліджуваних сполук в концентраціях 0,4 та 0,5 мг/кг, що було в рази ефективніше дії ацикловіру. Коефіцієнт захисту становив 80%. Встановлено збільшення рівня ІНФγ та ІЛ-2 в сироватці крові, що вказує на наявний імуномодулюючий ефект фторованих нуклеозидних сполук. Проведені дослідження дозволяють стверджувати про наявність антигерпетичної, імностимулюючої дії фторвмістних триазолів та необхідність поглибленого вивчення механізмів даного процесу.

Ключові слова: HSV-1, фторовані нуклеозидні сполуки, антигерпетична активність.

К. Науменко, студ., А. Головань, канд. биол. наук, Г. Баранова, вед. инж., С. Задорожная, канд. биол. наук, Ю. Шермолович, д-р биол. наук
Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, Киев, Украина

АНТИВИРУСНЫЙ И ИММУНОСТИМУЛИРУЮЩЕЕ ПОТЕНЦИАЛ ФТОРСОДЕРЖАЩИХ ТРИАЗОЛОВ

Проблема поиска эффективных противовирусных препаратов обусловлена высокой заболеваемостью и широким распространениэм вирусных инфекций. Целью представленной работы было исследовать антигерпетические активности фторированных нуклеозидных соединений G8 и G9 (2-N-замещены-4-тозил-5-полифторалкил-1,2,3-триазола) на модели invivo и определить их иммуномодулирующий потенциал. Показано значительное ингибирование репродукции вируса под действием исследуемых соединений в концентрациях 0,4 и 0,5 мг/кг, что было в разы эффективнее действия ацикловира. Коэффициент защиты составлял 80%. Установлено увеличение уровня ИФНγ и ИЛ-2 в сыворотке крови, что указывает на имеющийся иммуномодулирующий эффект фторированных нуклеозидных соединений. Проведенные исследования позволяют утверждать о наличии антигерпетические, имностимулюючои действия фторвмистних триазолов и необходимость углубленного изучения механизмов данного процесса.

Ключевые слова: HSV-1, фторированные нуклеозидные соединения, антигерпетическая активность.

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A. Pastyria, PhD stud., V. Polischuk, PhD
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
I. Sobko, headmaster of center
Center of Veterynary Diagnostics, Kyiv, Ukraine

GENETIC CHARACTERIZATION OF INFECTIOUS BURSAL DISEASE VIRUS ISOLATES IN UKRAINE

The objective of the investigation was to characterize infectious bursal disease viruses (IBDV) circulating in commercial poultry farms in Ukraine between 2014 and 2016. IBDV genetic material was amplified directly from bursa. The nucleotide sequence for VP2 hypervariable region of 16 IBDVs were determined by RT-PCR method, sequenced and compared to well characterised IBDV isolates worldwide. Neighbor-joining method was used for phylogenetic analyses. In result of the studyUkrainian IBDVs represented two genetic lineages: very virulent (vv) IBDVs and classical IBDV closely related to attenuated vaccine stains. The nucleotide identity among UkrainianvvIBDVs ranged between 87.2% and 99,8%. Ukrainian vvIBDV strains clustered together with very virulent strains from other counties like: United Kingdom, Egypt, China, Netherlands and Spain. In conclusion this report demonstrates the circulation of vvIBDV in commercial poultry farms in Ukraine.

Keywords: Infectious bursal disease virus, vvIBDV, VP2, RT-PCR, sequencing, phylogenetic analyses.

Introduction

Infectious bursal disease virus (IBDV) belongs to the Birnaviridae family Avibirnavirus genus. It has a non-enveloped, icosahedral capsid. Viral genome consists of two segments of double-stranded RNA. Virus replicates in immature IgM+ B-cells residing in the bursa of Fabricius of young chickens and causes infectious bursal disease or Gumboro disease. Two serotypes of the virus have been described. Serotype 1 IBDV strains arepathogenic to chickens, whereasserotype 2 strains are non-pathogenic [2, 5]. Serotype 1 IBDVisolates comprise the variant, classical virulent (cvIBDV) and very virulent (vvIBDV) strains, which greatlydiffer in their pathogenicity to chickens.VvIBDV strains were detected in Europe in 1986 and caused 70% mortality in susceptible chickens. These strains still cause great economical impact in poultry industry worldwide [3]. VvIBDV strains have been characterized in many countries, but there were no publications about these strains in Ukraine.

The IBDV genome is divided into segments A and B: segments A (3.4 kb) and B (2.8 kb). The large segment A encodes 4 viral proteins, the two capsid proteins VP2 (48 kDa) and VP3 (32–35 kDa), the viral protease VP4 (24 kDa), and a nonstructural protein VP5 (17–21 kDa), while the smaller segment B encodes VP1 (90 kDa), an RNA-dependent RNA polymerase. Expression/deletion studies have shown VP2 aa positions 206 to 350 to represent a major conformational, neutralizing antigenic domain called hyper variable region (HVR) [2], which includes the most variable region important for cell antigenic and pathogenic variation. Most exchanges of amino acid residues in VP2 occur in the four hydrophilic loops of the viral capsid [3]. These exchanges indicate that selective pressure for the evolution of IBDV is directly focused on the capsid regions that are immediately exposed to the immune system [4]. Most of the modern research has been focused on the VP2 gene [7,8,9].

Considering great variety and variability of IBDV strains and lack of described strains in Ukraine the objective of the investigation was to determine nucleotide sequence of hypervariable region of VP2 gene of different IBDV isolates circulating in poultry farms in Ukraine and compare obtained sequences with previously characterized and available in GenBank.

Materials and methods

For this research bursa samples were collected from infected chickens at the age of 24 – 44 days. RNA was extracted with silica-based method as recommended by supplier (Ribo-sorb, Amplisens). Reverse transcription was performed using a set of random primers (Reverta-L, Amplisens). The obtained cDNA has been used for the PCR. The oligonucleotide primers used in this work designated Bur1F (5'-TCACCGTCCTCAGCTTAC-3' nucleotide position 587-604) and Bur1R (5'-TCAGGATTTGGGATCAGC-3' nucleotide position 1212-1229) designed to amplify the hypervariable region of VP2 gene according to Bayliss, amplicon size – 643 bp [1]. To increase specificity and sensitivity of the reaction a second set of primers Bur2F (5'-CGCTATAGCGCTTGACCCAAAAA-3', nucleotide position 651 – 673) and Bur2R (5'-CTCACCCCAGCGACCGTAACGACG-3', nucleotide position 1179-1202) designed by Kataria et al [9] has been used which allows the amplification of the inner region of the first amplicon obtained after the first round of the amplification using Bur1F and Bur1R primers. The resulting product had the length of 550 bp.First round of amplification was carried out for 1 cycle at 95 °C for 2 min, 36 cycles at 95°C for 30 s., 52 °C for 30 s., 72 °C for 30 s., and 1 cycle at 72 °C for 2 min. Amplicons obtained from the first reaction have been diluted in 20 times and used for second reaction. Thermal profile for the second reaction was similar except the primer annealing temperature, which was 63 °C. PCR products were visualized in 1,5agarose gel. For sequencing 552 bpamplicons which contained hypervariable region of VP2 gene were separated from reaction components using the Thermo Scientific GeneJET Gel Extraction Kit. Purified amplicons were sequenced using Bur2F primer by Institute of Molecular Biology and Genetics (NAS, Ukraine). Sequences were analyzed using Mega 6 software.Nucleotide alignment was performed using ClustalW instrument.For phylogenetic analyses partial VP2 sequences of well characterized IBDV strains have been used. Phylogenetic analysis was performed usingneighbour-joining method.

Results and discussion

The nucleotide sequence of the VP2 HVR was determined for 16 Ukrainian IBDV isolates. Nucleotide identity between the 16 isolates ranged between 87.2% and 99,8%. Characteristics of the isolates in table 1.

***Table* 1.Description of IBDV isolates included in this study**

Virus isolate	Date of collection	Region of sample collection	Bird age (days)	Phylogenetic group
Ukraine 1517	17.04.2014	Lviv	44	VV
Ukraine 1853	24.04.2014	Cherkasy	48	CV
Ukraine 38_1943	29.05.2014	Cherkasy	38	CV
Ukraine 43_1943	29.05.2014	Cherkasy	43	CV
Ukraine 2045	29.05.2014	Lutsk	34	CV
Ukraine 58	12.06.2014	Ternopil	10	CV
Ukraine 55	17.06.2014	Kiev	26	VV
Ukraine 691_24	23.09.2014	Lviv	24	CV
Ukraine 691_35_4	23.09.2014	Lviv	35	VV
Ukraine 691_35_5	23.09.2014	Lviv	35	VV
Ukraine 760_45_4	23.09.2014	Lviv	45	VV
Ukraine 760_45_5	23.09.2014	Lviv	45	CV
Ukraine 1147	07.08.2015	Lutsk	37	CV
Ukraine 2065	13.11.2015	Kiev	38	VV
Ukraine 934	04.06.2016	Kiev	36	VV
Ukraine 964	04.06.2016	Kiev	36	VV

The phylogenetic relationship between Ukrainian IBDV isolates sequenced in this study and several well characterisedvvIBDVs, classical IBDVs, variant strains and attenuated vaccine strains was inferred using the neighbour-joining method (fig. 1). The Ukrainian IBDV isolates sequenced in this study represent two distinct genetic lineages (1) vvIBDV and (2) classical IBDV strains. EightIBDV isolates were characterized as very virulent, and eight as classical virulent strains (fig.1). Ukrainian vvIBDV strains were clustered together with very virulent strains from other counties like: United Kingdom, UK661 (AJ878898); Egypt, K406/89 (AF159218); China, HK46 (AF051838); Netherlands, 1986 (Z25482); Spain, SP/31/02 (AY770593). Detected isolates were also genetically related to attenuated vvIBDV strains MB and MB/3. Very virulent isolates formed two subgroups (VV-1 and VV-2) which indicates on different origin of this strains. There is not enough data to conclude direct correlation between phylogenetic distance and geographical distribution of analyzed strains, but vvIBDV stains have been only identified in Kiev and Lviv region. Similar heterogeneity among vvIBDV strains have been shown by Jenberie et al. [7] in Ethiopia. Nucleotide identity among vvIBDV strains detected in Ukraine varied from 99,8% (between strains 934 and 964) to 94,4% (between strains 1517 and 964). Isolates 760_45_5, 691_24 and 1147 were closely related with referent strains CU-1 (AF362771), Croatia.Cro-Pa/98

(EU184689) and classical attenuated vaccine strains. Isolates 58, 43_1943, 38_1943, 2045 and 1853 formed separate group with vaccines strains V877 and MB/5. This data indicates that these strains were isolated from chickens that have been previously vaccinated with IBDV vaccines that contain V877 or MB/5 strain.

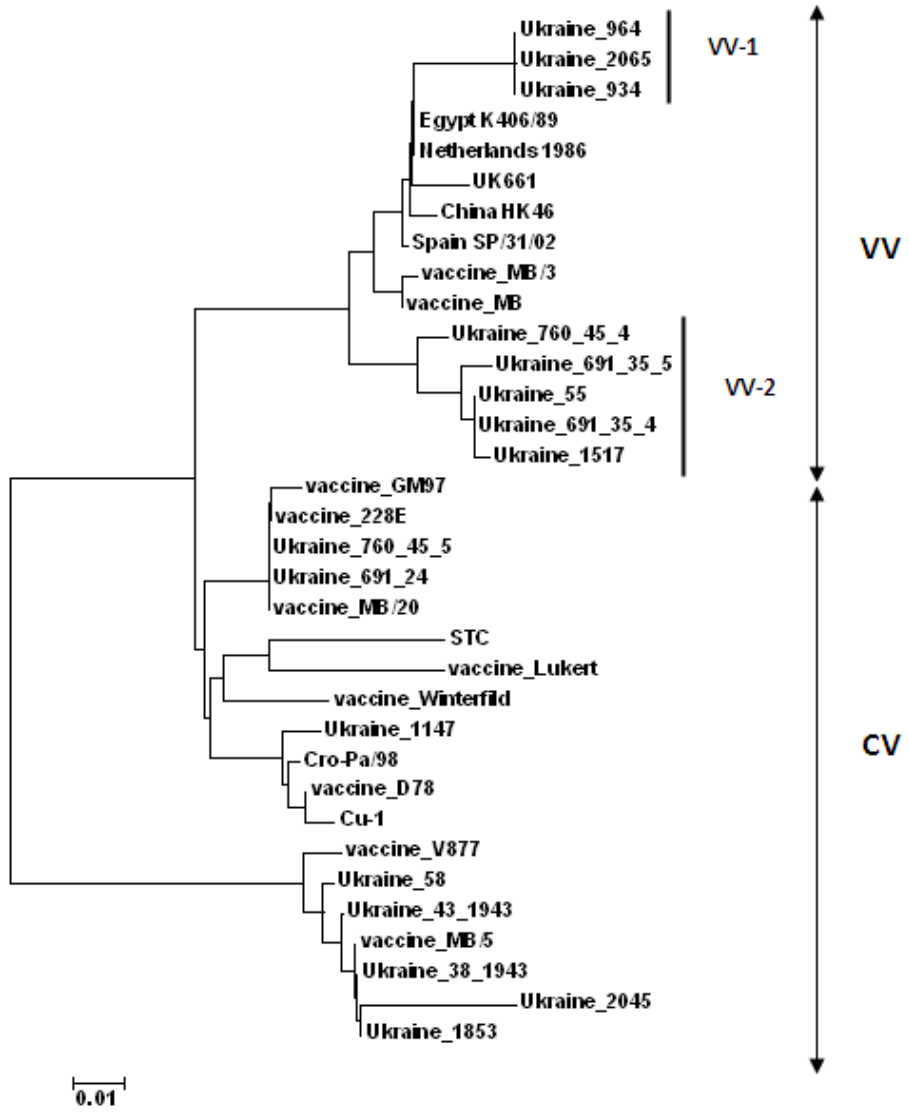

Fig. 1. Phylogenetic analysis of the VP2 hypervariable coding sequence of 34 IBDV isolates. The neighbour-joining consensus tree is shown

In this study we also used sequences of several attenuated vaccine strains to compare nucleotide identity with field isolates. There are three types of vaccine strains: intermediate-plus (or "hot"), intermediate and mild. Despite vaccination outbreaks still occure, that's why it is very important to choose vaccine which is phylogeneticall closer to field strain [11,12,13]. The most similar to Ukrainian vvIBDV was MB and MB/3 vaccine strain. This data suggest that level of protection against vvIBDV will be higher than with other vaccine strains used in this study.

Conclusion

In this study we have reported vvIBDV isolates circulating in Ukraine.UkrainianvvIBDVs cluster phylogenetically with previously characterized vvIBDVs from other countries and attenuated intermediate plus or "hot" vaccine strains. Classical Ukrainian strains were closely related with intermediate attenuated vaccine strains. Amino acid sequence analyses is needed for feather investigation.Information from this study could be used to guide IBDV vaccine selection in Ukraine.

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А. Пастиря, асп., В. Полищук, д-р біол. наук
Київський національний університет імені Тараса Шевченко, Київ, Україна,
І. Собко, директор центру
Центр ветеринарної діагностики, Київ, Україна

ГЕНЕТИЧНА ХАРАКТЕРИСТИКА ІЗОЛЯТІВ ВІРУСУ БУРСАЛЬНОЇ ХВОРОБИ НА УКРАЇНІ

Метою дослідження було охарактеризувати ізоляти вірус інфекційної бурсальної хвороби, що циркулюють у птахофабриках України з 2014 по 2016. Генетичний матеріал було виявлено і ампліфіковано з бурс. Послідовності вірусних РНК було вивалено за допомогою методу ЗП-ПЛР, секвеновано та порівняно із раніше охарактеризованими штамами вірусу ІБХ. Для філогенетичного аналізу було використано метод зближення сусідів. В результаті дослідження віруси ІБХ були представлені двома гілками: високовірулентні штами та класичні віруси ІБХ, близько споріднені з атенуйованими штамами. Гомологія між штамами складала від 87.2% до 99,8%. Високовірулентні штами були близькоспорідненими із такми виявленими в Великобританії, Єгипті, Китаї, Нідерландах та Іспанії. Висновком даного дослідження є підтвердження циркуляції високо вірулентних штамів вірусу ІБХ в Україні.

Ключові слова: вірус інфекційної бурсальної хвороби, ввВІБХ, ЗТ-ПЛР, секвенування, філогенетичний аналіз.

А. Пастыря, асп., В. Полищук, д-р биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина,
И. Собко, директор центра
Цент ветеринарной диагностики, Киев, Украина

ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ИЗОЛЯТОВ ВИРУСА ИНФЕКЦИОННОЙ БУРСАЛЬНОЙ БОЛЕЗНИ НА УКРАИНЕ

Целью исследования было охарактеризовать изоляты вируса инфекционной бурсальной болезни, циркулирующих в птицефабриках Украины с 2014 по 2016. Генетический материал был обнаружен и амплифицирован с бурс. Последовательности вирусных РНК были обнаружены с помощью метода ОП-ПЦР, секвенированы и сравнены с ранее охарактеризованными штаммами вируса ИББ. Для филогенетического анализа был использован метод сближения соседей. В результате исследования вирусы ИБХ были представлены двумя ветвями: высоковирулентные штаммы и классическими вирусами ИББ, которые были близкородственными атенуйоваными штаммами. Гомология между штаммами составляла от 87.2% до 99,8%. Высоковирулентные штаммы были близкородственными с такми выявленным в Великобритании, Египте, Китае, Нидерландах и Испании. Выводом данного исследования является подтверждение циркуляции высоковирулентных штаммов вируса ИББ в Украине.

Ключевые слова: вирус инфекционной бурсальной болезни, ввВИББ, ОТ-ПЦР, секвенирование, филогенетический анализ.

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S. Pavlova, PhD stud.,
Experimental station of grapes and fruit crops quarantine
Institute of Plant Protection National academy of agrarian sciences of Ukraine, Odessa, Ukraine,
O. Stakhurska, PhD stud., I. Budzanivska, DSc. Biol, Prof.,
V. Polischuk, DSc., Biol, Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

GISTECHNOLOGY FOR THE MONITORING OF SHARKA DISEASE IN THE ODESSA REGION

Plant virus causes many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world, and consequently, agronomists and plant pathologists have devoted considerable effort toward controlling virus diseases. One the most important virus on many Prunus species, causing great economic losses is Plum pox virus (PPV),casual agent of Sharka disease. Since its discovery, Sharka has been considered as a calamity in stone orchards. The virus has been detected in almost every country where any significant commercial stone fruit cultivation occurs [1]. The virus is entered into the list of regulated pests common in Ukraine. In Ukraine, the total area of PPV spread totals 4013,2764 ha. In Odessa region, 18.5 ha districts are in PPV quarantine. Six hotbeds of PPV infection totalling 28 hectares were found in Odessa region. For the first time in Odessa region, PPV was found on cherry trees. Peach and plum trees are hit equally. In this study, we use geographic information systems technology to identify potential locations in a Odessa region for controlling the spread of Plum pox virus. To our knowledge, this is the first attempt to employ GIS technology for controlling plant diseases in Ukraine. Provided it is properly maintained, the geospatial data, and the ability to generate detailed maps with it, is key to the success of PPV containment. Information management will be a key to improving for controlling the spread of Plum pox virus.

Keywords: Plumpoxpotyvirus, Sharkadisease, plantdiseasemonitoring, hotbeds, GIS technology.

Introduction. Plant viruses are group of pathogens that cause important loses in different fruit croups and they have great economic importance. This is especially true for diseases associated with vectored pathogens such as PPV, an aphid transmitted virus.

Plum pox virus(PPV) is one of the most important pathogens causing destructive viral diseases in stone fruit trees such as peach, plum, apricot and cherry. Sharka was first reported in plum trees in Bulgaria in 1917–1918 and was recognized as a viral disease by Atanasoff (1932) [2]. Since then, the virus has spread progressively to most of Europe, around the Mediterranean basin and the Near and Middle East. It has also spread to South and North America and Asia [3].

To understand the scope of PPV infection, regular surveys are necessary to determine the presence of the disease. PPV symptoms may appear on leaves, shoots, bark, petals, fruits and even stones (Fig. 1) They are usually distinct on leaves early in the growing season and include mild light-green discoloration, chlorotic spots, bands or rings, vein clearing or yellowing and leaf deformation. Flower symptoms can occur on petals (discoloration) of some cultivars. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape, and may develop brown or necrotic areas under the discolouredrings[4].

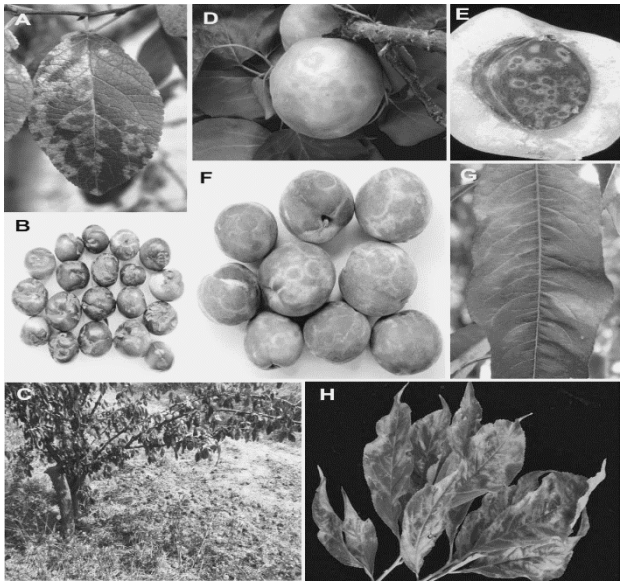

Figure. 1. Typical symptoms induced by *Plum pox virus* on a domestic plum leaf (A), domestic plum fruits (B), premature domestic plum fruit drop (C), an apricot fruit (D), an apricot stone (E), peach fruits (F), a peach leaf (G) and Japanese plum leaves (H) [5]

Visual inspection of trees is not a reliable detection method because of the variability in the expression of symptoms and many years may pass before an infected host manifests symptoms. To avoid PPV spread over long distances by the movement of plant material, reliable detection methods are needed for the accurate detection of the virus in symptomless nursery plants and propagative material. Two official and validated international protocols for the detection and characterization of PPV strains have been developed [6,7].

We had used enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) remain the preferred lab tests to screen for and confirm the presence of PPV in collected samples.

The climate in Odessa region is influenced by the vicinity of the Black Sea and fluctuates from year to year but typically is characterized as having higher summer temperatures, warmer winters and relatively rare rainfalls. Here, warm spring normally begins in mid-April followed by dry and hot summer months. Therefore, monitoring of stone fruit orchards in Odessa region is conducted in spring and autumn, when the symptoms are more obvious and plant tissues contain more virus.

This work was aimed at conducting Sharka disease monitoring of planted stone fruit crops in Odessa region using traditional visual, serological and molecular methods, and subsequent coupling of obtained data on virus spread with geographical information system (GIS) technology for further use by plant quarantine services, virologists and producers

Materials and methods. For the 3 years (2014-2016), plant disease monitoring of 185 hectares of stone fruit orchards in Odessa region was conducted, including 68 hectares in Belyaev and 43 hectares in Ovidiopol districts confirmed as the areas under PPV quarantine.

Visual diagnostics of PPV-specific symptoms was followed by serological analysis in the laboratory. Collected samples were tested for PPV by double antibody sandwich enzyme-linkedimmunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977) [8], using specific polyclonal antibodies purchased from Bioreba (Switzerland) following the manufacturer's recommendations. RT-PCR was mainly used as a confirmatory test for selected samples using probes specific to PPV-D and PPV-M strains.

Portable navigation system Garmin GPS 60 was used for accurate recording of geographical coordinates of the sample collection spots and locations where the Sharka symptoms were evident.

Results and discussion. In 2014-2015, for the first time in Odessa region, PPV was found on cherry trees (Fig. 2) in Khlebodarskoye village of Ovidiopol district (area 2,5 ha). Strain PPV M identified in 2015. Prior to this, surveys of cherry orchards have not been conducted due to the fact the PPV has never been detected there.

Figure. 2. Symptoms of PPV on cherry trees of Ovidiopol district, Odessa region

Also, new PPV hotbeds were found in Doslidnoe village (plum orchard, area 2,3 ha) and in Mirnoje village (peach orchard, area of 5 hectares; the total area of 80 ha) in Belyaev district. As of today, we have confirmed six hotbeds of Sharka disease totaling to 28 hectares in Odessa region(Fig. 3).

Therefore, the results of 3-year PPV monitoring in the affected areas of Odessa region revealed new hotbeds of virus spread.

Figure. 3. Pilot GIS-aided map showing plotted quarantine areas for PPV in Odessa region

In turn, these results underline the need for a) regular screening of stone fruit orchards (commercial, suspect/endangered, and quarantined ones as minimum) for PPV using EPPO-approved serological and/or molecular

techniques, and b) a database system combining virus monitoring, crop, vector, climate and map data.

Plant disease management practices can be improved by putting epidemiological information in the same format as other plant pathologistsinformation using a geographic information system (GIS). The integration of GIS provides a mean for the refined analysis of traditional and contemporary biological/ecological information on plant diseases. GIS has been applied in agriculture for the spatial analysis of insect pests, weed infestations, and plant diseases, which is particularly true for Sharka disease[8].

To our knowledge, this is the first attempt to employ GIS technology for controlling plant diseases in Ukraine. Provided it is properly maintained, the geospatial data, and the ability to generate detailed maps with it, is key to the success of PPV containment allowing for the ease of navigation to known sites each survey season but, most importantly, for decision making regarding the:

1) Efficient development of buffer zones and quarantine boundaries when a PPV-positive tree was identified;

2) Calculation of acreage to predict number of samples and prepare sample labels before field survey;

3) Calculations and verification of acreage for destruction of diseased trees;

Another advantage of interest to Odessa region is that such approach may also combine climate and vector data – both are of importance for PPV. Altogether, use of GIS technology for controlling PPV spread allows better planning of activities, personnel and limited funds

Conclusions.

1. For today six pestholes of PPV, total area of twenty-eight hectares, is found in Odessa region. Most of infected trees have peaches and plums.

2. For the first time in Odessa region, PPV was found on cherry trees: all the pestholes in plum and peach orchards the Odessa region identified strain PPV – D; in the pesthole in sweet cherry orchard the Odessa region identified strain PPV – M.

3. Modern technology phytosanitary monitoring of viral diseases, including PPV should be based on the integrated use of methods that allow you to get information about the spread of viral diseases in Ukraine and to determine the general trend of development of the pathological process, to inform the selection institutions of new invasive races of pathogens to develop reliable forecast for the development of the disease.

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С. Павлова, асп.
Опытная станция карантина винограда и плодовых культур
Института защиты растений национальной аграрной академии наук Украины, Одесса, Украина,
О. Стахурская, асп.
И. Будзанивская, д-р биол. наук, проф., В. Полищук д-р биол. наук, проф.
Киевскоий национальный университет имени Тараса Шевченко, Киев, Украина

ПРИМЕНЕНИЕ ГИС-ТЕХНОЛОГИИ ДЛЯ МОНИТОРИНГА ШАРКИ СЛИВЫ НА ТЕРРИТОРИИ ОДЕССКОЙ ОБЛАСТИ

Вирусы растений вызывают много различных заболеваний растений, что приводит к большим потям и качества урожая во всем мире, и поэтому, агрономы и фитопатологии посвящают значительные усилия для контроля за вирусными заболеваниями. Одним из самых важных вирусов вызывающих большие экономические потери у рода Prunus это Plumpoxvirus (PPV), возбудитель шарки сливы. С момента своего открытия шарка является настоящим бедствием для косточковых садов. Этот вирус есть в каждой стране, в которой есть промышленное выращивание косточковых деревьев[1]. Вирус относится к списку регулируемых вредных организмов Украины. На территории Украины зараженные вирусом сады занимают площадь в 4013,2764 га. В Одесской области эта площадь карантинных очагов составляет 18,5 га. За последнее время здесь было найдено 6 новых очагов общей площадью 28га. Впервые в Одесской области вирус PPV обнаружен на деревьях черешни. Персиковые и сливовые деревья поражаются в одинаковой степени. На сколько нам известно, мы впервые использовали ГИС-технологии для контроля вирусных болезней растений в Украине. Правильного использование геопространственных данных является ключом к успеху контроля распространения PPV.

Ключевые слова: вирус шарки сливы, фитосанитаный мониторинг, карантинный очаг, ГИС технологии.

С. Павлова асп.
Дослідна станція карантину винограду і плодових культур
Інституту захисту рослин національної аграрної академії наук України, Одеса, Україна,
О. Стахурська, асп.
І. Будзанівська, д-р біол. наук, проф., В. Поліщук, д-р біол. наук, проф.
Київський національный університет імені Тараса Шевченка, Київ, Україна

ЗАСТОСУВАННЯ ГІС-ТЕХНОЛОГІЇ ДЛЯ МОНІТОРИНГУ ШАРКИ СЛИВИ НА ТЕРИТОРІЇ ОДЕСЬКОЇ ОБЛАСТІ

Віруси рослин викликають багато важливих хвороби рослин і несуть відповідальність за великі втрати і якість врожаю у всьому світі, і тому, агрономи і фітопатологи докладають значних зусилль для контролю за вірусними захворюваннями. Одиним з найбільш поширених вірусів, який викликає значні економічні втрати у роду Prunus це Plumpoxvirus (PPV), збудник шарки сливи. З моменту свого відкриття шарка є справжнім лихом для кісточкових садів Цей вірус присутній в кожній країні, в якій є промислове вирощування кісточкових дерев[1]. Вірусвідноситься до перелікурегульованихшкідливихорганізмів України. На територіїУкраїнизараженівірусом сади займаютьплощу в 4013,2764 га. В Одеськійобластіплощакарантиннихвогнищ становить 18,5 га. За останній час тут булознайдено 6 новихвогнищзагальноюплощею 28 га. Вперше в Одеськійобластівірус PPV був виявлений на деревах черешні. Персикові і сливові дерева уражаютьсяоднаковоюмірою. На скільки нам відомо, ми впершевикористали ГІС-технологии для контролю за вірусними хворобамирослин в Україні. Правильневикористаннягеопросторовихданих є ключем до успіху контролю поширення PPV.

Ключові слова: Вірус віспи сливи, шарка сливи, фітосанітарний моніторинг, карантинне вогнище, ГІС технології.

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O. Shevchenko, PhD., S. Petrenko, lead ing., O. Iutynska, PhD, A. Bysov, PhD, T. Shevchenko, PhD, Ass.Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

SPREAD OF *TURNIP MOSAIC VIRUS* IN SUSCEPTIBLE CROPS IS STRONGLY EFFECTED BY DIFFERENT CULTIVATION PRACTICES

Samples of plants showing symptoms of Turnip mosaic virus (TuMV) were collected from fields planted to Brassicaceae crops in Kyiv region and different locations in the city of Kyiv. TuMV was detected in the main brassica-crop fields, private gardens and urban locations of Ukraine, with a high overall incidence of 50%. This paper describes the effects of different cultivation approaches on the incidence rate of viral infection in susceptible crops and confirms the importance of preventive measures for disease control.

Key words: Turnip mosaic virus, cultivation practices.

Introduction. *Turnip mosaic virus* (TuMV) is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses [1]. As many potyviruses, TuMV has an extremely wide host range but infects mostly plant species from the *Brassicaceae* family and induces persistent symptoms (mosaics, mottling, chlorotic lesions, etc.). For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses [2]. TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America. In Europe, TuMV was reported from the UK, Spain, Italy, Greece, Germany, The Netherlands, Czech Republic, Hungary, Bulgaria, Poland, and Russia [3-9]. Despite Ukraine's geographical location and wide cultivation of different *Brassica* crops for centuries, it's only recently that the authors have registered TuMV in our country (unpublished data). In the study reported here, we describe the importance of preventive measures for the control of wide-spread and damaging pathogen of brassicas.

Materials and methods. Sampling was restricted to crop-producing areas in Kyiv region and different locations in the city of Kyiv where Brassicaceae plants were growing/cultivated. In Kyiv, sampling locations included two botanical gardens, the city center, Museum of Folk Architecture and Life of Ukraine (open-air location w/o agricultural activity), and private gardens where different brassica plants were regularly cultivated. Several large fields in Luka and Gorenychi villages used for commercial cabbage cultivation were chosen for sampling in Kyiv region. Brassica plants were visually examined, samples were collected from plants with TuMV-like symptoms typically including mosaics, mottling, vein banding and/or leaf deformation.

Collected samples were tested for TuMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977) [10], using specific polyclonal antibodies purchased from Loewe (Germany). Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Maxisorb, NUNC, Denmark) were coated with TuMV-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of TuMV in the samples was detected in 200 µL homogenate by TuMV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Multiscan-334 microtitre plate reader (Labsystem, Finland). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

Results and discussion. A total of 54 plant samples with TuMV-like mosaic and mottling symptoms were collected in different districts of the city of Kyiv and Kyiv region. Sampling areas included both agricultural sites (two cabbage producing fields and private gardens) and urban locations where no agricultural activity was carried out (different sites in the City of Kyiv, two botanical gardens and open-air Museum of Folk Architecture and Life of Ukraine).

Using ELISA, TuMV was detected in samples from cabbage, red radish, mustard, radish, white mustard, gold of pleasure, weed species (hill mustard), etc. (Table 1).

***Table* 1. Double-antibody enzyme-linked immunosorbent assay for the detection of *Turnip mosaic virus* by hosts**

Plant	No of samples	Positives	Incidence of TuMV infection (%)
Brassica oleracea (**cabbage**) *Brassica sp.*	23	8	35
Brassica juncea (**mustard**)	4	2	50
Sinapis alba (**white mustard**)	3	3	100
Raphanus sativus (**red radish**) *Raphanus sp.*	12	11	92
Other brassicas	5	3	60
Other non-brassicas (*Asteraceae*, *Primulaceae*, *Papaveraceae*, *Malvaceae*)	7	0	0
TOTAL	**54**	**27**	**50**

TuMV has been detected in 27 samples of plants (overall 50% incidence rate in symptomatic hosts) including *B. oleracea* var. capitata, *R. sativus*, *Raphanus* sp., *S. alba*, *B. juncea*, *C. sativa*, *Brassica* sp., and *Bunias orientalis*. On cabbage plants, TuMV typically induced systemic mosaics, vein banding and leaf deformation (Fig.1), whereas systemic mosaics and mottling were common for naturally infected radish and mustard plants.

Figure 1. TuMV-positive cabbage plant (*B. oleracea* var. capitata) showing virus-like symptoms of vein banding/clearing

TuMV was found in the main brassica-crop fields, private gardens and urban locations of Ukraine, with a high overall incidence of 50%. Importantly, the agricultural sites used for plant sampling were characterized with different level of incidence of TuMV infection varying from 17% and 42% for two crop fields, and to as much as 58% for private gardens (Table 2).

***Table* 2. DAS-ELISA detection of *Turnip mosaic virus* by sampling sites continuously used for crop cultivation**

Sampling site	No of samples	Positives	Incidence of TuMV infection (%)
Commercial cabbage producing field 1	6	1	17
Commercial cabbage producing field 2	12	5	42
Private gardens	12	7	58
Total for agricultural sites	**30**	**13**	**39**

Several sampling sites within the Kyiv city (i.e. where no agricultural activity was carried out) demonstrated even higher incidence rate of TuMV with the minimum value of 33% for symptomatic plants. These results suggest that TuMV is probably widespread in both agricultural and urban locations but remained undetected for a long time.

Expectedly, different locations demonstrated high but varying level of TuMV occurrence. However, several aspects were of special interest in this regard. For the two fields used for commercial cabbage production in Kyiv region and situated in neighboring villages just 5 km apart, the TuMV incidence rate varied from 17% to 42%. This probably reflects the efficiency of the confirmed regular eradication of diseased plants in the former case (field 1) and underpins the significance of long-known simple approach – elimination of virus inocula – for the disease control.

In turn, rather high rate of TuMV infection in private gardens (58%) may be explained by both growing of infected plants and repeated cultivation of susceptible crops, as reported by the landowners. Another approach allowing to limit virus spread – crop rotation – was also missing in this case.

Obtained results clearly demonstrate that trivial measures for crop cultivation (known for decades but often thoroughly disregarded) remain highly efficient in controlling the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

Conclusions. In summary, the survey indicated high occurrence of TuMV in urban and agricultural regions in Ukraine where average infection incidence rate reached 50%. Wide range of infected plant species and high incidence rate in surveyed areas obviously demonstrates both the lack of virus screening and important role of efficient cultivation approaches for disease control in Ukraine. Obtained data suggests a long-term coexistence of the virus and the hosts in Ukraine.

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О. Шевченко, канд. біол. наук, С. Петренко, пров. інж.,
О. Іутинська, канд. біол. наук, А. Бисов, канд. біол. наук. Т. Шевченко, канд. біол. наук
Київський національний університет ім. Тараса Шевченка, Київ, Україна

ПОШИРЕННЯ ВІРУСУ МОЗАЇКИ ТУРНЕПСУ У СПРИЙНЯТЛИВИХ КУЛЬТУРНИХ РОСЛИНАХ СИЛЬНО ЗАЛЕЖИТЬ ВІД РІЗНИХ ПІДХОДІВ ДО ВИРОЩУВАННЯ

Зразки рослин с симптомами вірусу мозаїки турнепсу (TuMV) відбиралися с промислових полів вирощування хрестоцвітих культур у Київській обл. та на різних ділянках у місті Києв. TuMV був знайдений на всіх промислових полях, приватних присадибних ділянках та міських ділянках, а сумарний рівень інфекцій становив 50%. У даній роботі описуються наслідки застосування різних агроекологічних прийомів для поширення вірусу у сприйнятливих культурах та підтверджена важливість профілактичних заходів у боротьбі з вірусними хворобами.

Ключові слова: вірусу мозаїки турнепсу, вирощування хрестоцвітих культур.

А. Шевченко, канд. биол. наук, С. Петренко, вед. инж., О. Иутинская, канд. биол. наук,
А. Бисов, канд. биол. наук, Т. Шевченко, канд. биол. Наук
Киевский национальный университет им. Тараса Шевченко, Киев, Україна

РАСПРОСТРАНЕНИЕ ВИРУСА МОЗАИКИ ТУРНЕПС У ВОСПРИИМЧИВЫХ КУЛЬТУРНЫХ РАСТЕНИЯХ СИЛЬНО ЗАВИСИТ ОТ РАЗНЫХ ПОДХОДОВ К ВЫРАЩИВАНИЮ

Образцы растений с симптомами вируса мозаики турнепса (TuMV) отбирались с промышленных полей выращивания крестоцветных культур в Киевской обл. и на различных участках в городе Киев. TuMV был найден на всех промышленных полях, частных приусадебных участках и городских участках, а суммарный уровень инфекций составил 50%. В данной работе описываются последствия применения различных агроэкологических приемов для распространения вируса в восприимчивых культурах и подтверждено важность профилактических мероприятий в борьбе с вирусными болезнями.

Ключевые слова: вируса мозаики турнепса, выращивание крестоцветных культур.

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V. Solomakha, DSc., N. Smoliar, PhD.,
O. Smagliuk, PhD stud.
Taras Schevchenko National University of Kyiv, Kyiv, Ukraine

FLORISTIC CLASSIFICATION OF THE FLOODPLAIN ALDER, WILLOW AND POPLAR FORESTS IN THE BASIN OF THE LOWER SULA (UKRAINE)

The floodplain alder (Alnus glutinosa), willow (Salix alba, rarely S. fragilis) and poplar (Populus nigra, P. alba, outliers of Populus x canescens) forests in the basin of the lower Sula were investigated. Mesohygrophilous forests of European black alder were referred to Alno-Ulmion alliance Querco-Fagetea class (com. Aegopodium podagraria-Alnus glutinosa, D. c. Acer negundo-Alnus glutinosa). Swamp forests of European black alder of Alnetea class are mostly common in the floodplains of small rivers and are represented by two associations (Carici ripariae-Alnetum glutinosae and Carici acutiformis-Alnetum glutinosae). The floodplain willow and poplar forests were referred to class Populetea albae (order Populetalia albae). Willow forests of floodplains of the river Sula and its tributaries and also waterlogged gully talwegs and rarely outliers belong to Salicion albae alliance and Salicetum albae association. Lower reach poplar forests of the river Sula floodplain belong to Calamagrostio epigei-Populion nigrae alliance and are divided into two associations that we propose to change in accordance with the requirements of the International Code of Phytosociological Nomenclature for Galio veri-Populetum nigrae and Strophiostomo sparsiflorae-Populetum albae. It is emphasized that the studied groups don't contain the species from the Red Data Book of Ukraine. The alder, willow and poplar forests of each association that are least transformed, largest in area and oldest require the nature reserve creation, that is proved by their significant water conservation role.

Key words: Querco-Fagetea (Alno-Ulmion), Alnetea, Populetea albae, Ukraine, Dnieper left-bank Forest-Steppe, basin of the lower Sula, syntaxonomy.

Introduction. The floodplain alder (*Alnus glutinosa* (L.) P. Gaertn.), willow (*Salix alba* L., rarely *S. fragilis* L.) and poplar (Po*pulus nigra* L., *P. alba* L.*, Populus* x *canescens* (Ait.) Smith. forests are located throughout the whole region of our research of the floodplain of Sula river and its brances, however on the left bench they are less numerous. Accumulation and generalization the data about its phytocoenotic diversity according to the methodology of

G.Brown-Blanche school have been conducted for the last decades [1, 4-5, 6, 9, 12-14]. The results were included and critically processed in the monograph dedicated to the classification of the Northern Black Sea area forests [10].

Concerning the basin of Sula river, floristic and syntaxonomic content of flooded forest is not studied enough. We continued and detalized the study of flooded forests according to the methodology of G.Brown-Blanche school. These researches are important considering the fact that such forests have significant water preservation value and make up important resource of its ecological network. Also, the most part of the forests has been cut down or transformed. Integrating new phytocoenotic material into scientific use will help to solve the problems of ecology – floristic classification of willow, poplar and alder forests of the Forest-Steppe region of Ukraine.

Physical and geographical features of the researched object. The lower Sula basin is situated in Pridniprovskiy lowlands between Poltava and Cherkasy administrative regions. It spreads from the confluence of Udai river (northern part of Lubny city) into the mouth of Sula. Now it is flooded by the waters of Kremenchug reservoir. The main branches of this part of the river are Sliporid and Orgytsa and both of them flow into Sula from the right side. Basin of the Sula river is situated within Obolon-Gradyz physical-geography region.

The main feature of lower Sula basin climate is gradual decrease of the amount of precipitations in eastern and southern directions, which is about 500 mm per year. Average year temperature estimated about +6ºC. The relief is represented by reduced plain with integrated left tributaries of the Dnieper throughout the area. Flooded poplar, willow and alder forests are located throughout the whole region of our ivestigation of the floodplain of Sula river and its brances, however on the left bench they are less numerous. They are characterized by mostly short term flood regime, however during the last decade, the flood has became a rare phenomenon for this area. Their hydrological regime significantly impacted drainage reclamation, which covered most of the floodplains of small rivers and flood waters Kremenchug reservoir, resulting in decreased fluctuation amplitude of the water level and alluvium deposits.

Materials and methods. 74 geobotanical descriptions were used in this work, 53 of them were made by O.Yu. Smagliuk in the valley of lower Sula and its branches in 2014-2015 and 21 descriptions of N.O.Smoliar during shared expedition in 2015.

The descriptions were performed and processed according to the methodology of G.Brown-Blanche school [8]. The size of descriptive area was about 25x25m, sometimes the area was within natural phytocenotic boundaries if the area was small or striped. During the processing, part of descriptions were discarded as transitions between different sub-associations. Abundance points given in the table correspond to the following indexes of projective cover: + – < 1%, 1 – 1-5%, 2 – 6-15%, 3 – 16-25%, 4 – 26-50%, 5 – 51-100%. Points of permanence denote: + – < 10%, I – 10-20%, II – 21-40%, III – 41-60%, IV – 61-80%, V – 81-100%. Layering is shown in parentheses after the name of species of trees and bushes: a – the top wood tier; b – shrub tier; c – grass tier. The names of the species are given according to S.L.Mosyakin and M.M. Fedoronchuk [15].

Results and discussions

Syntaxon layout of flooded willow, poplar and alder forests of the lower Sula basin

Cl. **Querco-Fagetea** Br.-Bl. et Vlieger 1937
Ord. **Fagetalia sylvaticae** Pawl. in Pawl., Sokol. et Wall. 1928
All. **Alno-Ulmion** Br.-Bl. et R.Tx. 1943
Com. **Aegopodium podagraria-Alnus glutinosa** (Alno-Ulmion)
D.c. **Acer negundo-Alnus glutinosa** (Alno-Ulmion)
Cl. **Alnetea glutinosae** Br.-Bl. et R.Tx. 1943 em Mull. et Gors 1958
Ord. **Alnetalia glutinosae** R.Tx. 1937 em Mull. et Gors 1958
All. **Sio latifolii-Alnion glutinosae** Vorobyov & I. Solomakha in I. Solomakha 2015
Ass. **Carici ripariae-Alnetum glutinosae** Weisser 1970
Ass. **Carici acutiformis-Alnetum glutinosae** Scamoni 1935
Cl. **Populetea albae** Br.-Bl. 1962
Ord. **Populetalia albae** Br.-Bl. 1931
All. **Salicion albae** Klika 1955
Ass. **Salicetum albae** Klika 1955
Subass. **S.a. aegopodietosum podagrariae** subass.prov.
Subass. **S.a. urticetosum galeopsifoliae** subass.prov.
Subass. **S.a. caricetosum acutiformis** subass.prov.
Com. **Ulmus glabra-Salix alba** (Salicion albae)
All. **Calamagrostio epigei-Populion nigrae** (Shevchyk et Solomakha 1996) Shevchyk et V.Solomakha in I.Solomakha et. al. 2015 in nomen novum (Nomenkl. synon. Rubo caesii-Amorphion fruticosae Shevchyk et V.Solomakha 1996; syntax. synon. Galio veri-Aristolochion clematidis Shevchyk et V.Solomakha 1996)
Ass. **Galio veri-Populetum nigrae** nom. nov. prov. (Syn. Galio veri-Aristolochietum clematidis Shevchyk et V.Sl. 1996)
Ass. **Strophiostomo sparsiflorae-Populetum albae** nom. nov. prov. (Syn. Strophiostomo sparsiflorae-Amorphetum Shevchyk et V.Sl. 1996)
Com. **Swida sanguinea-Populus x canescens** (Calamagrostio epigei-Populion nigrae)
Com. **Carex hirta-Populus balsaminus** (Calamagrostio epigei-Populion nigrae)

Characteristic of marked syntaxons

All descriptions of willow forests in the lower Sula basin are summarized in one table (Table 1). They are divided into two classes – **Querco-Fagetea** and **Alnetea glutinosae.** The first one includes mostly drained alder populations of nemorial type. Because of its defect, the associations were not identified and only two groups were separated. Swampy black-alder forests classified as **Alnetea glutinosae**, which includes two associations. Overall, associations of alder forests are richer than willow forests in terms of floristic content but less rich than poplar forests.

***Table* 1. Phytocenotic characteristic of alder forests of lower Sula basin**

Desnity of wood tier	09	07	06	10	07	06	07	06	08	07	09	09	08	06	03	05	–
Density of shrub tier	01	01	03	01	02	+	04	04	+	+	+	02	+	+	+	+	04
Projective cover of grass tier	90	80	75	25	95	30	20	65	85	85	90	85	95	55	90	25	90
Amount of species described	16	29	24	15	21	13	14	25	19	12	27	15	16	14	20	23	24
Number of description	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D.s. Com. Aegopodium podagraria-Alnus glutinosa																	
Corylus avellana (b)	1	.	3	.	.	.	.	.	.	.	.	.	.	.	.	+	+
Corylus avellana (c)	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euonymus europaea (c)	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Aegopodium podagraria	5	5	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Asarum europaeum	1	+	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stellaria holostea	.	+	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pulmonaria obscura	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stachys sylvatica	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D.s. D.c. Acer negundo-Alnus glutinosa																	
Acer negundo (a)	.	.	.	5	2	.	.	.	.	.	.	.	.	.	.	.	.
Acer negundo (b)	.	.	.	1	+	+	.	3	1	.	.	+	.	.	.	.	+
Acer negundo (c)	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Ulmus laevis (a)	1	.	.	.	3	2	.	.	.	.	.	.	.	.	.	.	.
Ulmus laevis (b)	.	.	.	.	.	+	1	2	.	.	.	.	.	.	.	+	+
Acer platanoides (b)	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	.	+
Geum urbanum	.	.	.	+	+	+	.	+	.	.	+	.	.	.	.	.	+
Anthriscus sylvestris	.	.	.	1	.	.	.	+	.	.	.	.	+	.	.	.	.
Poa nemoralis	.	.	.	.	2	+	.	.	.	.	.	.	.	.	.	.	.
Festuca gigantea	.	.	.	+	+	.	.	.	+	.	.	.	.	.	.	.	.
D.s. All. Alno-Ulmion (Cl. Querco-Fagetea)																	
Salix alba (a)	.	1	.	.	.	1	.	1	.	.	.	.	.	.	.	1	.
Fraxinus excelsior (a)	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Fraxinus excelsior (c)	.	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Quercus robur (a)	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Quercus robur (c)	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ulmus glabra (a)	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ulmus glabra (b)	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ulmus carpinifolia (c)	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Tilia cordata (b)	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	+	.
Tilia cordata (c)	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Padus avium (b)	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Acer campestre (b)	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Acer tataricum (b)	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Swida sanguinea (b)	.	.	1	+	2	.	.	.	.	.	1	.	.	+	.	.	.
Viburnum opulus (b)	.	.	.	.	+	.	.	.	.	.	+	.	.	.	.	.	.
Acer platanoides (c)	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rubus caesius	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Ficaria verna	+	1	1	1	5	.	+	.	.	.	.	.	.	.	.	.	.
Adoxa moschatellina	2	.	1	.	.	2	.	.	.	.	.	.	.	.	.	.	.
Lamium maculatum	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gagea minima	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anemonoides ranunculoides	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Corydalis cava	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Corydalis solida	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gagea lutea	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Scilla siberica	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Paris quadrifolia	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Cardamine amara	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Crysosplenium alternifolium	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Geum rivale	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Polygonatum multiflorum	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Millium effusum	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Urtica dioica	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+	.
Frangula alnus (b)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Scrophularia umbrosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
D.s. Ass. Carici ripariae-Alnetum glutinosae																	
Sambucus nigra (b)	1	1	.	.	.	.	+	1	1	.	.	3	1	.	.	.	.
Sambucus nigra (c)	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Glechoma hederacea	+	.	+	1	.	+	.	.	1	.	1	+	2	.	.	.	.
Chelidonium majus	.	.	.	.	.	+	.	+	.	.	.	+	+	.	.	.	.
Geranium robertianum	.	.	.	.	.	+	.	+	.	.	.	1	.	.	.	.	.
Myosoton aquaticum	.	.	.	.	.	.	.	+	1	.	+	1	+	.	.	+	.
Eupatorium cannabinum	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	.
Cirsium oleraceum	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
Coccyganthe flos-cuculi	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Myosotis sparsiflora	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.

Ending tabl.1

Desnity of wood tier	09	07	06	10	07	06	07	06	08	07	09	09	08	06	03	05	–
Density of shrub tier	01	01	03	01	02	+	04	04	+	+	+	02	+	+	+	+	04
Projective cover of grass tier	90	80	75	25	95	30	20	65	85	85	90	85	95	55	90	25	90
Amount of species described	16	29	24	15	21	13	14	25	19	12	27	15	16	14	20	23	24
Number of description	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D.s. Ass. Carici acutiformis-Alnetum glutinosae																	
Salix cinerea (b)	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	+
Carex acutiformis	.	.	.	.	+	.	.	+	.	.	.	.	.	4	4	3	+
Lycopus exaltatus	.	.	.	.	.	.	.	+	.	.	.	.	.	+	+	+	.
Filipendula ulmaria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
Carex pseudocyperus	.	.	.	.	.	.	.	.	.	.	1	.	.	.	+	+	.
Stachys palustris	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.
D.s. All. Sio latifolii-Alnetum glutinosae																	
Carex riparia	+	.	.	.	.	.	+	.	3	1	2	+	1	2	+	.	5
Galium aparine	.	.	1	.	+	2	+	5	2	1	2	5	5	.	.	.	+
Humulus lupulus	+	.	+	.	.	+	+	.	1	+	1	1	1	1	+	+	+
Symphytum officinale	.	.	.	.	.	.	.	+	+	.	+	+	+	.	+	+	.
D.s. Cl. Alnetea glutinosae																	
Alnus glutinosa (a)	5	5	4	2	3	4	5	4	5	5	5	5	5	4	3	4	.
Alnus glutinosa (b)	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	3
Ribes nigrum (b)	.	.	.	.	1	.	3	.	.	+	+	1	.	.	.	.	2
Ribes nigrum (c)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Salix pentandra (b)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Urtica galeopsifolia	3	3	5	1	+	.	2	+	2	5	3	2	1	1	+	.	3
Impatiens noli-tangere	2	.	2	+	4	.	2	.	5	1	5	.	3	+	+	.	.
Thelypteris palustris	.	.	+	.	+	.	.	.	2	1	.	.	+	1	5	1	.
Equisetum fluviatile	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.	1	+
Lysimachia vulgaris	.	.	.	.	.	.	.	+	+	.	.	+	.	.	+	+	.
Dryopteris carthusiana	+	.	+	.	+	.	+	.	.	.	.	.	.	+	+	.	.
Phragmites australis	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Solanum dulcamara	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Caltha palustris	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
Galium palustre	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.

Rarely occurred species: *Acorus calamus* (8 – +), *Agrostis canina* (11 – 1), *Alopecurus ventricosus* (8 – +), *Angelica sylvestris* (5, 17 – +), *Arctium lappa* (11 – 1), *A. minus* (8 – +), *Ballota nigra* (8 – 1), *Betula pubescens* (a) (16 – 1), *Carex acuta* (11 – +), *Carex appropiquata* (11 – +), *Chaerophyllum temulum* (4 – +), *Cirsium acanthoides* (5 – +), *C. arvense* (17 – 1), *Dipsacus strigosus* (5, 16 – +), *Elytrigia repens* (11 – +), *Epilobium roseum* (17 – +), *Equisetum palustre* (9 – 1, 11 – +), *Euonymus europaea* (b) (17 – +), *Galeopsis bifida* (9 – 1), *Geum allepicum* (9 – +), *Glechoma hirsuta* (14 – +), *Impatiens parviflora* (10 – 1), *Lycopus europaeus* (11 – +), *Lysimachia nummularia* (16 – +), *Lythrum salicaria* (13, 17 – +), *Moehringia trinervia* (1 – +), *Poa remota* (10 – +), *Populus alba* (a) (16 – 1), *Ranunculus repens* (16 – +), *Salix aurita* (b) (10, 16 – +), *Salix fragilis* (a) (12 – 1), *Scirpus sylvaticus* (11 – 1), *Sorbus aucuparia* (c) (11 – 1), *Sium latifolium* (2 – +), *Typha latifolia* (17 -1), *Ulmus carpinifolia* (a) (11 – 1).

Legend for descriptions

Description 1. N.O. Smoliar 25.05.2015. Flooded forest between villages Bilousivka and Kozorizi (Cherkasy region Drabivsky district).

Description 2. O.Yu.Smagliuk 30.04.2015. Mixed deciduous forest in the "Morozivska dacha" in the outscirts of Lubny city in the valley of meandering stream. Diameter of alder 0.3 m, poplar – 0,5 m, willow – 0,6 m, height of 27 m.

Description 3. O.Yu.Smagliuk 25.05.2015. Alder on the floodplain of Chumgak between villages Bilousivka and Kozorizi. Alder diameter 0,6-0,7 m, height of 28 m.

Description 4-5. O.Yu.Smagliuk 23.05.2014. Transformed alder swamp in the lowland of floodplain of Orzhytsa river (Poltava region, village Zolotuhi, Orzhytsia destrict).

Description 6. O.Yu.Smagliuk 03.05.2014. In the terrace reduction of Sula river valley (Poltava region, Velykoburimske forestry).

Description 7. O.Yu.Smagliuk 08.05.2014. On the floodplain. Orzhitsa at the indigenous banks (Poltava region, Orzhytske forestry).

Description 8. O.Yu.Smagliuk 24.05.2014. On the floodplain of Sula (Poltava region, between the villages, Velykoselytske and Maloselytske, Orzhytsia destrict).

Description 9-12. O.Yu.Smagliuk 25.05.2015. In alder floodplain of Chumgak river near Bilousivka village. Alder diameter 0,35-0,4 m, height 20 m.

Description 13. O.Yu.Smagliuk 25.05.2015. In the alder forest on the floodplain of Gnyla river near village Rudka) (Poltava region, Hrebinka destrict). The diameter of alder 0,3 m, height is 23 m.

Description 14. O.Yu.Smagliuk 23.05.2014. In lowland with wet soil on the floodplain of Sliporid river (Poltava region, near the villages Novoselivka-Pryymivschyna-Voronyntsi).

Description 15. O.Yu.Smagliuk 08.05.2014. On floodplain of Orzhutsia river, far from the root bench (Poltava region, Orzhytske forestry)

Description 16. O.Yu.Smagliuk 03.05.2014. On the floodplain of Sula river near the root bench (Poltava region, Velykoburimske forestry).

Description 17. O.Yu.Smagliuk 08.05.2014. In the deforestation zone of the floodplain of Orzhytsia river (Poltava region, Orzhytske forestry).

Aggregation **Alno-Ulmion** includes hygrophyllic deciduous forests. On the research territory it is introduced by frequently transformed communities as a result alder drainage. As a result of peat decomposition and nutrients enrichment we can observe the increase of nemorial mesophytes and nitrophilic species, and also increasing of alder populations. Diagnostic list represents its differentiation from swampy alder forests.

Aggragation **Aegopodium podagraria-Alnus glutinosa** (Alno-Ulmion) represented by old natural forests with tree diameter 0,6-0,7 m 27-28 m heigh. Widely spread on the floodplains of the streams and small rivers among the mas-

sives of deciduous forests. Insufficient amount of descriptions does not allow to include the aggregation to a separate association but allows to classify them to associations of **Ficario-Ulmetum** Knapp ex Medwecka-Kornas 1952 **or Rubo caesii-Alnetum** Bajrak 1997 because of difference of its floristic content. Average density of wood tier is 0,7, shrub – 0,2, grass tier coverage – 80%. index of floristic diversity of the groups is the highest among alder forests.

Derivative group **Acer negundo-Alnus glutinosa** (Alno-Ulmion) distributed on the floodplains of Orzhetsia and Sula rivers, and introduced by drained alder forests (swamped before) where *Acer negundo* L. growed. Such groups are found in the valley of river Chorol [6]. Average density of wood tier estimates 0,7, shrub – 0,2, grass tier coverage – 50%.

Association of **Sio latifolii-Alnion glutinosae** is represented by alder swamps of Forest-Steppe and Steppe areas with the most nutrient rich soils [10]. On the researched area, aggregations contain many species of flooded alder and poplar forest class **Populetea albae**. On the research area there were no aggregations of **Alnion glutinosae** (Malc. 1929) Meijer Drees 1936 s. str., association which is typical for forest zone. Average density of wood tier is higher than alder – 0,75, shrub – less (0,1), grass tier coverage – 80%.

Association of **Carici ripariae-Alnetum glutinosae** is distributed on the floodplains of small rivers – right branches of Sula and represented by mild humidity alder forests with vivid features of nitrification. It occupies the largest territories of the alder forests of the region. Micro relief is often not observed Hygro- and hygrophytes are represented poorly. Average density of wood tier estimates 0,8, shrub – 0,1, grass tier coverage – 85%.

Association of **Carici acutiformis-Alnetum glutinosae** is distributed on the lowlands of river Sula and its right branches, floodplains represents well humidified and peated alder forests without signs of nitrification. Micro relief is expressed well enough. Association is rarely occurred and needs preservation because part of its aggregations have been transformed into previous association. Average density of wood tier is minor C – 0,45, shrub – 0,1, grass tier coverage is less than in the previous association – 65%.

Class **Populetea albae**

During the last decade, aggregation of flooded alder and poplar forests are considered to belong to the class **Salicetea purpureae** Moor 1958. But not much time ago alder and poplar forests were attributed to forgotten class **Populetea albae** Br.-Bl. 1962 [10]. We support the division of wood and shrub aggregations on the level of classes. Flooded alder and poplar forests of the researched region are divided into two big groups which correspond to the unions – meso hygrophyllic and more nutritious aggregations of loamy soil with predominance of *Salix alba* and xeromesophyllic short term flooded aggregations of loam and sandy soils with a predominance of *Populus* L. Description of white willow forests from **Salicion albae** union of the lower Sula basin are summarized in a separate table (Table 2)

***Table 2*. Phytocenotic characteristic of willow forests of lower Sula basin**

Desnity of wood tier	07	06	08	07	07	08	06	06	07	06		06	06	04	06	04	10	06	09
Density of shrub tier	–	05	06	–	03	02	03	03	+	+		02	03	+	+	02	+	03	08
Projective cover of grass tier	50	99	90	70	75	75	75	85	80	80		65	60	90	70	65	3	70	2
Amount of species described	17	20	20	10	17	14	29	11	21	26		25	17	16	18	9	25	25	22
Number of description	1	2	3	4	5	6	7	8	9	10		11	12	13	14	15	16	17	18
D.s. Subass. S.a. aegopodietosum podagrariae subass.prov.																			
Ficaria verna	4	+	5	4	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Aegopodium podagraria	.	5	4	3	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Urtica dioica	+	.	2	1	+	.	.	.	.	.		.	2	.	.	.	.	.	+
Geum urbanum	+	.	.	.	+	2	.	.	.	.		.	.	.	.	.	.	.	.
Anemonoides ranunculoides	.	1	3	+	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Pulmonaria obscura	.	+	+	.	+	.	.	.	.	.		.	.	.	.	.	.	.	.
Adoxa moschatellina	.	1	+	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Scilla bifolia	.	.	.	2	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Arctium lappa	+	.	+	+	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Asarum europaeum	.	+	+	.	+	.	.	.	.	.		.	.	.	.	.	.	.	.
Galeobdolon luteum	.	.	1	.	5	.	.	.	.	.		.	.	.	.	.	.	.	.
Lamium maculatum	.	+	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Festuca gigantea	+	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.
Stellaria holostea	.	+	.	.	+	.	.	.	.	.		.	.	.	.	.	.	.	.
D.s. Subass. S.a. urticetosum galeopsifoliae subass.prov																			
Salix triandra (b)	.	.	.	.	.	4	.	.	.	.		.	.	.	.	.	.	.	.
Acer negundo (c)	.	.	.	.	.	.	1	.	.	+		.	.	.	.	.	.	.	.
Fraxinus excelsior (c)	.	.	.	.	.	.	+	.	+	+		.	.	.	.	.	.	.	.
Rubus caesius	.	.	.	.	.	.	3	+	.	+		+	.	.	.	.	1	5	1
Urtica galeopsifolia	.	1	.	.	.	3	5	5	5	4		.	.	.	.	.	.	.	.
Impatiens noli-tangere	.	1	.	.	.	4	1	2	1	1		.	.	.	.	.	.	.	.
Symphytum officinale	.	.	.	.	.	.	+	+	1	2		.	.	.	.	.	+	+	.
Caltha palustris	.	.	.	.	.	.	+	+	3	1		.	.	.	.	.	.	.	.
Carex pseudocyperus	.	.	.	.	.	.	+	+	.	1		.	.	.	.	.	.	.	.
Iris pseudacorus	.	.	.	.	.	.	+	+	+	+		.	.	.	.	+	.	.	.
Thelypteris palustris	.	.	.	.	.	.	+	.	1	.		.	.	.	.	.	.	.	.
Glechoma hederacea	.	.	.	.	.	+	+	.	.	.		+	.	.	.	.	+	2	+
Humulus lupulus	.	.	.	.	.	.	+	.	.	1		.	.	.	.	.	.	.	.
Filipendula ulmaria	.	.	.	.	.	.	+	.	.	1		.	.	.	.	.	.	.	.
Urtica kioviensis	.	.	.	.	.	.	.	.	2	4		.	.	.	.	.	.	.	.
Lycopus exaltatus	.	.	.	.	.	.	.	.	1	+		.	.	.	.	.	.	.	.
Phalaroides arundinacea	.	.	.	.	.	.	.	.	+	1		.	.	.	.	.	.	+	+
Mentha pulegium	.	.	.	.	.	.	.	.	+	+		.	.	.	.	.	.	.	.
Galium palustre	.	.	.	.	.	.	+	.	.	.		.	.	.	.	+	.	.	.

Ending tabl. 2

Desnity of wood tier	07	06	08	07	07	08	06	06	07	06		06	06	04	06	04	10	06	09
Density of shrub tier	–	05	06	–	03	02	03	03	+	+		02	03	+	+	02	+	03	08
Projective cover of grass tier	50	99	90	70	75	75	75	85	80	80		65	60	90	70	65	3	70	2
Amount of species described	17	20	20	10	17	14	29	11	21	26		25	17	16	18	9	25	25	22
Number of description	1	2	3	4	5	6	7	8	9	10		11	12	13	14	15	16	17	18
D.s. Subass. S.a. caricetosum acutiformis subass.prov.																			
Carex acutiformis	.	.	.	.	.	.	.	.	2		2	4	3	5	4	5	.	.	.
Phragmites australis	.	.	.	.	.	.	.	.	.		+	2	4	1	+	.	.	+	.
Scutellaria galericulata	.	.	.	.	.	.	.	.	+		+	.	.	+	1	.	.	.	.
Lythrum salicaria	.	.	.	.	.	.	.	.	1		.	+	.	.	+	1	.	.	+
Lysimachia vulgaris	.	.	.	.	.	.	.	.	.		.	.	.	+	+	.	.	+	+
Ranunculus repens	.	.	.	.	.	.	+	.	+		+	+	.	+	.	.	.	.	.
Vicia cracca	.	.	.	.	.	.	.	.	.		.	+	.	+	+	.	.	.	.
Eupatorium cannabinum	.	.	.	.	.	.	.	.	.		.	2	+	.	.	.	.	+	.
Lycopus europaeus	.	.	.	.	.	.	.	.	.		.	.	+	2	1	.	.	.	.
Calystegia sepium	.	.	.	.	.	.	.	.	.		.	+	.	2	2	.	.	.	.
Agrostis stolonifera	.	.	.	.	.	.	.	.	.		.	.	2	1	1	.	+	+	.
Glyceria maxima	.	.	.	.	.	.	.	.	.		.	.	.	2	+	.	.	.	.
Bidens tripartite	.	.	.	.	.	.	.	.	.		.	+	2	.	.	.	.	.	.
Mentha arvensis	.	.	.	.	.	.	.	.	.		.	.	.	2	2	.	.	.	.
Cirsium arvense ssp. *Setosum*	.	.	.	.	.	.	.	.	.		.	.	.	.	1	.	.	+	.
Teucrium scordium	.	.	.	.	.	.	.	.	.		.	.	.	.	.	1	.	.	.
D.s. Subass. S.a. aegopodietosum podagrariae & S.a. urticetosum galeopsifoliae																			
Ulmus laevis (a)	1	.	.	2	.	.	1	.	.		.	.	.	.	.	.	.	.	.
Ulmus laevis (b)	.	.	.	.	.	.	.	+	.		.	.	.	.	.	.	1	.	.
Acer negundo (a)	4	.	2	.	.	.	.	.	.		1	.	.	.	.	.	.	2	.
Acer negundo (b)	.	2	4	.	.	1	.	.	.		.	.	.	.	.	.	.	1	1
Sambucus nigra (b)	.	3	2	.	.	+	+	.	.		.	.	2	.	.	.	+	.	.
Sambucus nigra (c)	.	.	.	.	.	.	.	.	.		.	.	+	.	.	.	.	.	.
Corylus avellana (b)	.	3	.	.	+	.	.	.	.		.	.	.	.	.	.	.	.	.
Galium aparine	+	2	1	.	.	3	1	.	+		+	.	.	.	.	.	1	+	+
Myosoton aquaticum	.	+	.	.	.	+	.	.	.		.	.	.	.	.	.	.	.	.
Myosotis sparsiflora	+	.	.	.	.	+	.	.	.		.	.	.	.	.	.	.	.	.
D.s. Com. Ulmus glabra-Salix alba (Salicion albae)																			
Ulmus glabra (a)	.	2	.	.	.	.	.	.	.		.	.	.	1	1	.	5	3	.
Ulmus glabra (b)	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	1	4
Ulmus glabra (c)	.	.	.	.	.	.	.	.	.		.	.	.	.	.	+	.	.	.
Swida sanguinea (b)	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	1	.	2
Swida sanguinea (c)	.	.	.	.	.	.	.	.	.		.	.	+	.	.	.	.	.	.
Viburnum opulus (b)	.	.	.	.	.	.	1	.	.		.	.	.	.	.	.	+	2	.
Rhamnus cathartica (b)	.	.	.	.	.	.	.	.	.		.	.	+	.	.	.	+	.	.
Elytrigia repens	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	+	.	.
Carex hirta	.	.	.	.	.	.	.	.	.		.	.	.	1	1	.	.	2	+
Artemisia vurgaris	.	.	.	.	.	.	.	.	.		.	+	.	.	.	.	.	+	.
Arctium nemorosum	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	+
Taraxacum officinale	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	+	.	.
Cucubalis baccifer	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	+	.	.
D.s. Ass. Salicetum albae & All. Salicion albae (Cl. Populetea albae)																			
Salix alba (a)	2	5	5	4	1	4	5	5	5		4	3	5	4	4	4	2	.	5
Salix fragilis (a)	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	2	.
Populus nigra (a)	3	.	.	.	5	.	.	.	.		.	.	.	.	.	.	2	.	.
Populus alba (a)	.	.	.	.	.	1	.	.	.		.	.	.	.	.	.	.	.	.
Populus alba (b)	.	.	.	.	.	.	.	.	.		.	+	.	.	.	.	.	.	.
Populus alba (c)	.	.	.	.	.	.	.	+	.		.	.	.	.	.	+	.	.	+
Lisimachia nummularia	.	.	.	.	.	.	+	.	.		+	+	.	.	+	.	+	.	.
Scutellaria hastifolia	.	.	.	.	.	.	+	+	.		.	+	.	.	.	.	.	.	+
Myosoton aquaticum	.	.	.	.	+	.	.	.	.		.	.	.	.	.	.	.	.	.
Althaea officinalis	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	+	.

Rarely occurred species: *Acer campestre* (b) (5 – 1), *A. platanoides* (c) (2, 9, 18 – +), *A. tataricum* (b) (5 – +, 7 – 1), *Alliaria petiolata* (1 – 2), *Alnus glutinosa* (a) (9, 10 – 1), *A. glutinosa* (c) (7 – +), *Alopecurus pratensis* (17 – +), *Anthriscus sylvestris* (4 – +), *Armeniaca vulgaris* (c) (16 – +), *Calamangrostis canescens* (12 – 3), *Carex acuta* (17 – +), *C. contigua* (14 – 1), *C. praecox* (16 – 1), *Carpinus betulus* (b) (5 – +), *Cirsium heterophyllum* (17 – +), *C. oleraceum* (12 – +), *C. palustre* (18 – +), *Chelidonium majus* (1 – +), *Chenopodium album* (11 – +), *Corydalis solida* (3 – +), *Daucus carota* (11 – +), *Dryopteris filix-max* (3 – +), *Epilobium roseum* (11 – +), *Euonymus europaea* (b) (6 – +), *E. europaea* (c) (5,6 – +), *Frangula alnus* (b) (7 – 1, 11 – +), *F. alnus* (c) (16 – +), *Fraxinus excelsior* (b) (10 –

+), *Gagea lutea* (3 – +), *Galium boreale* (16 – +), *Geranium robertianum* (18 – +), *Glechoma hirsuta (3 – 1), Grossularia reclinata* (b) (18- +), *Heracleum sibiricum (11 – +), Lactuca serriola (11 – 2), L. stricta* (12 – +), *L. tatarica* (12 – +), *Lapsana communis* (11 – +), *Lonicera tatarica* (b) (17 – +), *Lythrum virgatum* (16 – +), *Malus sylvestris* (a) (1 – 1), *Mentha longifolia* (14 – +), *Morus nigra* (c) (18 – 1), *Moehringia trinervia* (7 – +), *Omphalodes scorpioides (10 – +), Padus avium* (b) (9 – +), *Polygonatum multiflorum* (3 – +), *Polygonum amphibium* (7 – +), *Populus tremula* (a) (6,121 – 1), *P. tremula* (b) (11 – +), *P. tremula* (c) (18 – 1), *Prunus divaricata* (b) (17 – +), *Ptarmica carthilaginea* (13 – +), *Pyrus communis* (a) (1 – 3), *Quercus robur* (b) (16 – 1), *Ranunculus lingua* (15 – 1), *Rumex aquatica* (7 – +), *R. confertus* (13 – +), *Ribes nigrum* (b) (7 – 1), *Robinia pseudoacacia* (a) (1 – 1), *R. pseudoacacia* (b) (5 – +), *Rosa sp.* (c) (16 – +), *Salix caprea* (b) (16 – +), *S. cinerea* (b) (11 – 2), *Scilla siberica* (3 – +), *Senecio erucifolium* (11 – +), *Sonchus oleraceus* (12 – +), *S. palustris* (17 – +), *Stachys sylvatica (2 – 1), Stellaria neglecta (1 – 2), Tilia cordata* (b) (5 – 2), *Torylis japonica* (17 – +), *Typha latifolia* (17 – +), *Ulmus carpinifolia* (a) (3 – 2, 10 – 1), *U. carpinifolia* (b) (9 – +), *Viola hirta (1 – +), V. odorata* (5 – +).

Legends for descriptions

Description 1. O.Yu. Smagliuk 03.05.2014. In reduced beams on the floodplain of Sula river, near Lyaschivka (Chornobaivsky area Cherkasky district 29 block about 30 quarter).

Description 2. O.Yu. Smagliuk 25.05.2015. In poplar forest of river Chumgak valley, near Bilousivka (I Prokhorov-in Zolotoniyskiy forestry). At the foot of the slope of the indigenous banks plain area. The diameter 0,3-0,8 m, height 22 m.

Description 3. O.Yu. Smagliuk 29.04.2015. In osier bed in thalweg beams Lubenskyi Forestry AIC (tract Vynnytsia 23 quarter). The diameter 0.4 m, height 30 m.

Description 4. O.Yu. Smagliuk 03.05.2014. In the floodplain of Sula river near Mokhnach (Chernobaevsky area Cherkasy region).

Description 5. O.Yu. Smagliuk 23.05.2014. In the plantation of poplar and willow in broadleaf forests around Aleksandrovka (Lubny Poltava region, 35 quarter 2 division).

Description 6. N.O. Smoliar 25.05.2015. In willow riparian forests near river Chumgak around. Bilousivka. In description present green moss – 2% of coverage.

Descriptions 7, 10. N.O. Smoliar 30.04.3015. In willow forests of botanical nature monument "Mgarska dacha" (outskirts of Lubni).

Description 8. O.Yu. Smagliuk 26.05.2015. In a young osier bed floodplain of Chumgak river between villages Bilousivka and Kozorizi. The diameter 0,1-0,35 m, height – 16 m.

Description 9-10. O.Yu. Smagliuk 30.04.2015. In a large array willow forests of "Mgarska dacha". Water is squeezed. The diameter of 0.4 m, height – 26 m.

Description 11. N.O. Smoliar 26.07.2015. Trench among the fields to the right from the highway from Lypove to Kryva Ruda (Poltava region, Semyonov district).

Description 12. N.O. Smoliar 27.07.2015. Trench near the village. Demianivka (Poltava region, Semyonov district).

Descriptions 13, 14. O.Yu. Smagliuk 27.07.2015. On the island Vysokyi of Sula river. Implanted willows, diameter 0,2-0,4m, height 25 m.

Descriptions 15. O.Yu. Smagliuk 27.07.2015. On the island Horbivka of Sula river. Diameter 0,2-0,3 m, height 13 m.

Descriptions 16. N.O. Smoliar 26.07.2015. Trench on the north-east from village Demyanivka poplar diameter 0,2-0,3m, height – 18 m.

Description 17. O.Yu. Smagliuk 26.07.201. Trench on the north-east of Tukalo village (Poltava region, Semyonov district). 0.5 m elm diameter, willow – up to 1 m, height – 12 m.

Description 18. O.Yu. Smagliuk 26.07.2015. In the floodplain of Sula near the ferry of Tarasivka village (Poltava region, Orzhytsia destrict). Willow diameter 0.3 meters, height – 24 m.

The union **Salicion albae** includes more humid aggregations of the class which are represented by white willow forests of the floodplain of Sula and its right branches, wetland thalweg ravines on its left bank and, occasionally, branches of the left bank. They are clearly divided into three groups – mesophyllic, meso-hygrophyllic and hygrophyllic. They do not have much in common in terms of species content. We have marked them within the rank of provisor sub-associations of the **Salicetum albae** association. After some time, as the phytocenotic material of related areas will be accumulated, their syntaxonic status must be updated. It is also typical that these three sub-associations show complete similarity of species and ecotype conditions towards listed above syntaxons of alder forests – union **Alno-Ulmion** and associations of **Carici ripariae-Alnetum** and **Carici acutiformis-Alnetum.** Average density of wood tier estimates 0,65, shrub – 0,2, garss tier coverage – 75%. Willow forests in terms of floristic diversity are inferior to alber and poplar forests.

Sub-association of **S.a. aegopodietosum podagrariae** represents natural and cultivated white willow forestries mainly in moist thalwegs, sometimes on the higher creek's areas of Sula and Sula's tributaries that have a lot of mineral and organic compounds. Diameter of willow is in range of 0,1-0,8 m and height is 22-30 m. Scrubbery and herbaceous layers mostly consist of nemoralis (forest) species. Some of the sub-association's territories are used as pastures. Medium tree crown density is 0,7, medium density of scrubbery layer is 0,3, grass layer – 80%.

Sub-association of **S.a. urticetosum galeopsifoliae** represents meso hygrophyllic natural white willow forests in moist thalweg, in particular on the territory of botanical nature monument "Mgarska dacha" (outskirts of Lubny), the lower parts of the floodplain Sula and its tributaries (mostly river Chumgak), on rich mineral and organic nutrients, somewhat nitrified soils. In summer water lies on the depth of 0,2-0,3 m or almost near the ground surface; in spring and after rains flowing water is above the ground level. Diameter of willow rages from 0,1-0,7 m, height – 16-27 м. Scrubbery and herbaceous layers include typical species of swampy alder forests, excluding typical species of **Salicion albae union.** In the aggregations of sub-associations of "Mgarska dacha" was found a very rare species for Forest-Steppe – *Urtica kioviensis.* Average density of wood tier estimates 0,7, scrubbery – 0,2, grass tier coverage – 80%. Floristic index is high for the willow forests of the region.

Sub-association of **S.a. caricetosum acutiformis** represents hygrophyllic, periodically flooded but mostly drained natural and planted white willow forests on the floodplains of lower Sula area islands and in the fields on the left bank, and also in the lower basin of Sula. Diameter of willow ranges from 0,2-0,4 m, height – 13-25 m. Among shrub and grass tiers, typical species of **Salicion albae** union are dominant. Average density of wood tier estimates 0,5, shrub – 0,15, grass tier coverage – 70%, it means that density of all levels

is the lowest for willow forests. In terms of floristic content it is inferior to other groups of willow forests.

Aggregation **Ulmus glabra-Salix alba (Salicion albae)** represent mild transformed white willow-elm groves among the fields in the outskirts of Demianivka, Tukalo, Obolon, Semenov district of Poltava region, occasionally flooded willow forests near Tarasovka village of Orzhyts region. Diameter of elm ranges from 0,5 m, willor 0,2-1 m, height 12-24 m. Average density of wood and shrub tier is the highest for willow groups and estimates: wood (0,8), shrub – (0,4), grass tier coverage – 40% – the lowest index. The floristic content of the aggregation is the highest among willow forests. Syntaxonic position of the aggregation requires detalization and according to floristic content it is related to the union **Calamagrostio epigei-Populion nigrae**.

Twenty yers ago the communities of mesophillic union **Rubo caesii-Amorphion fruticosae** were described for Kaniv Natural Preserve [14] and xeromesophyllic union **Galio veri-Aristolochion clematidis** from flooded island of Forest-Steppe region of Dnieper [14]. Comparsion of these syntaxons with the aggregations of flooded areas of Steppe region have shown that there is only one allocated union [3, 7, 11]. It may be determined as the driest (mesoxerophyllic, xeromesophyllic and alsp mesophyllic) flooded poplar forests of the Forest-Steppe and Steppe areas on sand and sandy soils. Therefore, new name of the union was chosen in monograph about forest plants classification of the Northern Black Sea region [10], which is very informative and reflects the environmental and geographical specificity of the unity.

In wood tier of flooded poplar forests of **Calamagrostio epigei-Populion nigrae** union, *Populus nigra* L. Is a typical dominant, in the cover – *Rubus caesius* L., *Carex praecox* Schreb., *Calamagrostis epigeios* (L.) Roth., *Glechoma hederacea* L., *Elytrigia repens* (L.) Nevski. Description of the union from lower Sula basin is summarized in table 3. Overall, the aggregation of poplar forests are richer than alder and willow forests. The highest amount of trees (as seedlings) and shrubs species comparing to the entire region was indicated in the aggregations within unity.

***Table* 3. Phytocenotic characteristic of poplar forests of lower Sula basin**

Desnity of wood tier	06	08	04	03	07	04	07	07	07	10	09	07	08	08	06	07	04
Density of shrub tier	01	02	04	+	01	01	04	06	07	02	01	02	02	03	03	08	06
Projective cover of grass tier	75	30	80	3	25	3	45	20	25	1	85	55	15	65	75	60	60
Amount of species described	22	24	17	12	15	14	14	17	38	10	20	35	24	20	19	37	26
Number of description	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D.s. Ass. Galio veri-Populetum nigrae nom. Nov																	
Populus nigra (a)	5	5	2	2	5	4	3	4	3	.	.	.	.	.	.	.	.
Populus nigra (b)	.	+	.	+	2	2	.	1	+	.	.	.	.	.	.	.	.
Populus nigra (c)	+	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.
Salix alba (a)	.	.	3	2	.	.	.	.	1	.	.	.	.	.	.	.	.
Salix alba (b)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium verum	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D.s. Ass. Strophiostomo sparsiflorae-Populetum albae nom. nov.																	
Amorpha fruticosa(b)	.	.	.	.	.	.	4	4	3	.	.	.	.	.	.	.	.
Amorpha fruticosa (c)	.	.	.	.	.	.	1	1	+	.	.	.	.	+	.	.	.
Achillea millefolium	.	.	.	.	.	.	+	+	+	.	.	.	.	.	.	.	.
Populus alba (a)	.	.	1	.	.	.	4	3	2	4	5	.	.	.	.	.	.
Populus alba (b)	.	.	.	.	.	.	+	1	+	+	.	+	.	.	.	+	.
Populus alba (c)	.	.	.	.	.	.	.	.	+	+	+	.	.	+	.	.	.
Humulus lupulus	.	.	.	.	.	.	.	.	2	+	3	.	.	.	+	.	+
D.s. Com. Swida sanguinea-Populus x canescens																	
Populus x *canescens* (a)	.	.	.	.	.	.	.	.	.	.	.	5	5	5	5	5	.
Populus x *canescens* (b)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Populus x *canescens* (c)	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.
Swida sanguinea (b)	.	+	.	.	.	.	.	.	.	+	.	+	2	2	2	1	.
Swida sanguinea (c)	+	+	.	.	.	.	.	.	+	.	.	+	+	+	.	.	+
Viburnum opulus (b)	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+	.	.
Rosa sp. (c)	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.
Populus tremula (c)	.	.	.	.	.	+	.	.	+	.	+	+	.	.	.	1	.
Quercus robur (c)	.	.	.	.	.	.	.	+	.	.	.	.	+	+	.	+	.
Lysimachia vulgaris	.	.	.	.	.	+	.	.	.	.	.	+	+	.	.	+	.
Galium aparine	.	1	+	.	+	.	.	.	.	.	.	.	.	.	+	+	.
Geum urbanum	.	.	.	.	.	.	.	.	2	+	.	.	+	+	.	+	.
Moehringia trinervia	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	.
Taraxacum officinale	.	.	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.
Artemisia vurgaris	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	+	.
Cucubalis baccifer	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.
Equisetum arvense	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	1
Galium boreale	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.
Phragmites australis	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Carex otrubae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Arctium nemorosum	.	+	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.
D.s. Com. Carex hirta-Populus balsaminus																	
Populus balsaminus (a)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
Populus balsaminus (c)	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+

Ending tabl. 3

Desnity of wood tier	06	08	04	03	07	04	07	07	07	10	09	07	08	08	06	07	04
Density of shrub tier	01	02	04	+	01	01	04	06	07	02	01	02	02	03	03	08	06
Projective cover of grass tier	75	30	80	3	25	3	45	20	25	1	85	55	15	65	75	60	60
Amount of species described	22	24	17	12	15	14	14	17	38	10	20	35	24	20	19	37	26
Number of description	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D.s. All. Calamagrostio epigei-Populion nigrae																	
Rubus caesius	.	.	5	.	.	.	.	.	.	+	1	.	1	.	.	5	1
Elytrigia repens	3	.	.	.	1	+	.	+	.	.	4	3	.	2	.	.	+
Poa pratensis	3	.	.	.	+	.	.	.	.	.	3	2	.	.	.	+	2
Agrostis stolonifera	.	.	.	.	.	.	1	2	.	.	2	.	.	1	.	+	.
Dactylis glomerata	.	+	.	.	.	.	.	.	2	.	.	.	.	2	.	+	.
Calamangrostis epigeios	5	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.
Carex hirta	.	.	.	+	3	.	4	2	3	.	3	.	2	.	.	+	5
Carex praecox	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+
Lactuca serriola	.	.	.	+	+	.	+	.	.	.	+	.	.	+	.	.	+
Glechoma hederacea	.	1	+	.	+	.	.	+	.	.	1	2	.	.	+	+	+
Anthriscus sylvestris	.	+	.	.	+	.	.	.	+	.	.	1	.	+	.	1	.
Fallopia convolvulus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Lactuca tatarica	+	.	.	.	.	.	.	.	2	.	.	.	+	.	.	.	.
Aristolochia clematitis	.	.	+	.	.	.	.	.	1	.	.	.	.	.	.	.	.
Sonchus arvensis	.	.	.	.	.	.	+	+	.	.	.	+	.	.	.	.	.
Alopecurus pratensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D.s. Cl. Populetea albae																	
Salix fragilis (a)	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
Acer negundo (a)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Acer negundo (b)	.	+	4	.	.	.	+	+	1	1	.	.	+	.	.	4	+
Acer negundo (c)	+	+	.	.	+	.	.	.	+	.	.	+	+	.	.	+	.
Urtica dioica	.	1	+	.	+	.	.	.	1	.	2	.	+	1	.	+	.
Lysimachia nummularia	.	.	.	+	.	+	.	.	.	+	+	.	.	.	.	+	+
Symphytum officinale	.	.	+	.	.	.	+	+	.	.	.	.	.	.	.	+	.
Carex acutiformis	.	2	+	.	.	.	.	.	.	.	.	4	1	.	.	.	.
Lythrum salicaria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+
Ranunculus repens	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Mentha arvensis	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	+
Myosoton aquaticum	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Scutellaria hastifolia	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+
Stachys palustris	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lycopus europaeus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Solanum dulcamara	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Calystegia sepium	.	+	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.
Myosoton aquaticum	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+
Ptarmica carthilaginea	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Potentilla reptans	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Thalictrum flavum	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Asparagus officimalis	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
Sonchus palustris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Althaea officinalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Eupatorium cannabinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium palustre	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Rarely occurred species: *Acer campestre (a) (15 – 1), A. campestre (b) (15 – 1), A. campestre (c) (15 – +), A. platanoides (b) (2 – 3, 13 – +), A. platanoides (c) (12 – +), A. tataricum (c) (2, 15 – +), Achillea inundata (1 – 1), Alliaria petiolata (12 -1, 15 – +), Armeniaca vulgaris (b) (9 – +), Artemisia absinthium (5 – +), Atriplex lucens (17 – +), Ballota nigra (14 – 3), Brachypodium sylvaticum (9 -1), Calamangrostis canescens (14 – 1), Campanula rotundifolia (1 – +), Carduus crispus (11 – +), Carex acuta (3 – +), C. caespitosa (6 – +), C. contigua (12 – +), C. melanostachya (4, 6 – +), C. vulpina (6 – +), Chaiturus marrubiastrum (3 – +), Chamaecytisus ruthenicus (7 – +), Chelidonium majus (2, 12 – 3, 8 – +), Clinopodium vulgare (12 – +), Convallaria majalis (15 – 5), Corylus avellana (c) (15 – +), Crataegus sp. (b) (2, 12, 16 – +), C. sp. (c) (1, 2,12 – +), Cynoglossum officinale (5 – +), Daucus carota (11 – +), Dryopteris carthusiana (10 – +), Eleagnus argentea (b) (13, 14 – +), Conyza canadensis (17 – +), Euonymus europaea (b) (15 -1), E. europaea (c) (2, 15 – +), Fragaria moschata (9 – 3), Frangula alnus (b) (9 – +, 13, 16 – 1), F. alnus (c) (2 – +), Fraxinus excelsior (b) (2- +), F. excelsior (c) (2, 15 – +), F. viridis (a) (4 -1), F. viridis (b) (3, 4 – +), F. viridis (c) (6 – +), Galium palustre (15 – +), Geranium robertianum (2 – 2, 12 – 3), Heracleum sibiricum (14 – +), Hieracium umbellatum (9 – +), Hypericum perforatum (1, 16 – +), Iris pseudacorus (15 – +), Ligustrum vulgare (b) (9 – +), Linaria vulgaris (1 – +), Lythrum salicaria (6 – +), Malus praecox (b) (7 – +), Melandrium album (5, 11- +), Morus nigra (b) (1, 2, 4 – +, 9 – 2), M. nigra (c) (1, 2 – +), Padus racemosa (b) (1, 12 – +), Parthenocissus quinquefolius (8 – +), Pinus sylvestris (a) (9 -1), P. sylvestris (b) (7 – +), Poa nemoralis (6 – +), Populus tremula (b) (6, 9, 11 – +), Prunus divaricata (c) (16 – +), P. spinosa (b) (9, 17 – +), Pyrola rotundifolia (9 – 2), Pyrus communis (b) (1,2 – +), Quercus robur (b) (1, 4, 13 – +), Rhamnus cathartica (b) (1 – 2), R. cathartica (c) (1, 9, 12 – +), Ribes nigrum (b) (15 – 2), R. nigrum (c) (16 – +), Robinia pseudoacacia (b) (1, 9, 12 – +), Rumex confertus (12 -+), Salix cinerea (b) (9 – 2, 13 – +), Sambucus nigra (b) (1, 2, 9 – +, 11 – 2, 13,14 – 1), Sambucus nigra (c) (1, 2, 9, 11, 13, 16 – +), S. racemosa (c) (12 – +), Setaria viridis (17 – +), Sorbus aucuparia (c) (12, 13 – +), Stellaria media (1 – +), Steris viscaria (9 – 1), Torylis japonica (16 – +), Trifolium alpesre (17 – +), Trifolium medium (17 -+), Tussilago farfara (9 – +), Ulmus carpinifolia (b) (9 – +), U. glabra (a) (10 – 5), U. glabra (b) (12, 16 – +), U. glabra (c)*

(12 – +), Ulmus laevis (a) (15 – 1), U. laevis (b) (3, 10 – +), U. laevis (c) (15 – +), Veronica longifolia (17 – +), Viburnum lantana (b) (9 – +), V. opulus (c) (7 – +), Vicia cracca (5, 17 – +), Viola canina (16 – +), Viola elatior (6 – +).

Legends for descriptions

Description 1. N.O. Smoliar 27.07.2015. In the tract "Horbivka" on Romanov Horb Island in the gulf of Sula.

Description 2. N.O. Smoliar 27.07.2015. In the tract "Dubina" on the island Lyaschivka in Sula lough. Implanted poplars in the peripheral zone of the island.

Description 3. O.Yu.Smagliuk 24.07.2014. Areas of floodplain of Sula river near the bridge across Sula.

Description 4-6. O.Yu.Smagliuk 23.05.2014. Poplar plantations on the southern part of village. Nesen-Irzhavets (Poltava region, Orzhytsia district).

Description 7-10. O.Yu. Smagliuk 26.07.2015. Forest area of the floodplain near Sula upland terraces near Lypove village. The diameter of poplar 0,3-0,4 m, height – 23 m.

Description 11. N.O. Smoliar 27.07.2015. Trench in the center of the field, right to the highway from Kryva Ruda to Lypove.

Description 12. N.O. Smoliar 27.07.2015. On the island Vysokiy in the gulf of Sula.

Description 13. N.O. Smoliar 26.07.2015. Trench system on the fields near Demyanivka village.

Description 14. N.O. Smoliar 26.07.2015. Trench in the center of the field near Kryva Ruda village (Poltava region, Semyonov district).

Description 15. N.O. Smoliar 30.04.3015. On the territory of "Mgarska dacha" near the city Lubny.

Description 16. O.Yu.Smagliuk 26.07.2015. Trench in the outskirts of Kukoba village (Poltava region, Semyonov district).

Description 17. N.O. Smoliar 26.07.2015. Trench system in the center of the field in the outskirts of Demyanivka vallage.

Association **Galio veri-Populetum nigrae** nom. nov. prov.

Diagnostic species: *Populus nigra* (dom.), *Salix alba* (dom.), *Calamangrostis epigeios, Carex praecox, Galium verum.*

The majority of poplar forests of the lower Sula basin are close to association, described as a variant of **Populus nigra** (nomenclature type of this association belongs to this variant) association **Galio veri-Aristolochietum clematidis** Shevchyk et V.Sl. 1996 [14]. But this name contradicts to the International Code of Phytosociological Nomenclature [17], because it does not contain any type of dominant tier (trees) in its name, so it must be changed. So, we propose the new name – **Galio veri-Populetum nigrae.**

Aggregations of association in the territory of experimental region are natural aggragations of black poplar, black poplar's croppers aging up to 50 years and spontaneous forests in the former agricultural lands. They are detected on the Sula's flood plains, frequently on the islands of Sula's creeks, sometimes in the form of small forestries among the fields on Sula's left shore. These are the driest aggregations on the experimental territory. Even though humidity varies according to season, floods in the experimental region are rare. Diameter of black poplar is in range of 0,1-0,5 m and height is 6-28 m. Medium density of tree crowns is lowest among poplars' aggragations (0,55) and scrubbery (0,1), covering of grass layer is considerable – 0.55. These aggregations are the poorest in floristical contingent among poplars' aggragetions.

Association **Strophiostomo sparsiflorae-Populetum albae** nom. nov. prov.

Diagnostic species: *Populus alba* (dom.), *P. nigra* (dom.), *Amorpha fruticosa, Humulus lupulus.*

Most of white poplar forests of the lower Sula basin are close to association, described as **Strophiostomo sparsiflorae-Amorphetum** Shevchyk et V.Sl. 1996 [14]. But this name contradicts to the International Code of Phytosociological Nomenclature [17], because it does not contain any type of dominant tier (trees) in its name, so it must be changed. What we offer here is previously called **Strophiostomo sparsiflorae-Populetum albae.** Association represents flooded and trench poplar forests of the valley of Sula river. Diameter of poplars ranges between 0,3-0,5 m, height – 23-27 m. Average density of wood tier estimates 0,8, shrub – 0,4, grass tier coverage – 35%.

Aggregations of **Swida sanguinea-Populus x canescens (Calamagrostio epigei-Populion nigrae)** diagnosed in trenches throughout the fields. They have been formed by hybrid species (*Populus alba* x *P. tremula*) and noticed in the outskirts of villages Kryva Ruda, Demyanivka, Kukoba, Semenov district of Poltava region. Also, indicated on the territory of botanical natural monument "Mgarska dacha" near Lubny and on the Vysoky island in Sula lough. Diameter of the poplar ranges from 0,07-0,4 m, height – 15-18 m. Syntaxonomical position of the aggregation requires more researches, probably it should be refereed to recently described class **Dactylido glomeratae-Populetea tremulae** Vorobyov et I. Solomakha in I.Solomakha et al. 2015 [10]. Average density of wood tier is 0,8, shrub – 0,35, grass tier coverage – 45%. In terms of floristic content these communities are the richest among poplar forests.

Aggregations of **Carex hirta-Populus balsaminus (Calamagrostio epigei-Populion nigrae)** was found in one of the trenches in the fields, left from the highway from Lypove to Gradyzk near Demyanivka villagte, Semenov district of Poltava region. Density of wood tier is low – 0,4, shrub and seedings – 0,6, grass tier coverage – 60%.

Conclusion. Flooded alder (*Alnus glutinosa*), willow (*Salix alba,* зрідка *S. fragilis*) and poplar (*Populus nigra, P. alba, Populus* x *canescens*) forests in the basin of lower Sula occupy a lot of space and characterized by significant level of cenotic diversity. In particular, meso-hygrophyllic black alder forests have been assigned to the **Alno-Ulmion** union of the **Querco-Fagetea** class, which is divided into two groups. Swampy black poplar forests, which are distributed mainly on the floodplains of small rivers, have been assigned to **Alnetea glutinosae** class, within which two associations were identified. One of them – is the most common among alder forests of the region association **Carici ripariae-Alnetum glutinosae** – three variants were selected. Flooded forests with the domination of *Salicaceae* family species have been assigned to **Populetea albae** and order **Populetalia albae**. Willow forests of the floodplains of river Sula and its branches, and also moist thalweg ravines and, occasionally, trenches, belong to the union **Salicion albae** and association **Salicetum albae**. Within the association, three widespread but very different sub-associations have been previously allocated (its rank must be obviously increased), homologous to syntaxons of alder forests and one community of unidentified status. Poplar forests of Sula lower course floodplains including those which are situated on the islands of the Sula lough, occupy less area comparing to alder and willow forests, most part of them was implanted. They belong to the union **Calamagrostio epigei-Populion nigrae**, and, according to the dominant tree layer is divided into two associations, which name we offer to change according to the International Code of Phytosociological Nomenclature [17] – **Galio veri-Populetum nigrae** and **Strophiostomo sparsiflorae-Populetum albae** (two options selected). Trench aggregations of the left bench of Sula lower course **Swida sanguinea-Populus x canescens** turned out to be

very interesting, but, for now, they are described as non-ranked. Its syntaxonomical status must be specified. Community of **Carex hirta-Populus balsaminus** was found only in one locality. It should be noted that species from the Red Book were not found in alder, willow and poplar forests of the lower basin of Sula river, however a number of regionally rare species were revealed and a separate publication will be dedicated to this theme.

Considering the risks of damages and adventisation of the flora, the least transformed, the largest and the oldest poplar, alder and willow forests of each association require preservation. Significant water preservation role is the indisputable argument for prohibition of its total deforestation.

References

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В. Соломаха, д-р біол. наук, Н.Смоляр, канд. біол. наук, О. Смаглюк, асп.
Київський національний університет імені Тараса Шевченка, Київ, Україна

ФЛОРИСТИЧНА КЛАСИФІКАЦІЯ ЗАПЛАВНИХ ВІЛЬХОВИХ, ВЕРБОВИХ І ТОПОЛЕВИХ ЛІСІВ У БАСЕЙНІ НИЖНЬОЇ СУЛИ (УКРАЇНА)

Досліджено заплавні вільхові (Alnus glutinosa), вербові (Salix alba, зрідка S. fragilis) та тополеві (Populus nigra, P. alba, колки з Populus x canescens) ліси у басейні нижньої Сули. Мезогігрофільні чорновільхові ліси віднесено до союзу Alno-Ulmion класу Querco-Fagetea (com. Aegopodium podagraria-Alnus glutinosa, D.c. Acer negundo-Alnus glutinosa). Заболочені чорновільхові ліси класу Alnetea поширені переважно на заплавах малих річок і представлені двома асоціаціями (Carici ripariae-Alnetum glutinosae та Carici acutiformis-Alnetum glutinosae). Заплавні вербові та тополеві ліси віднесено до класу Populetea albae (порядок Populetalia albae). Вербові ліси заплав Сули та її приток, а також перезволожених тальвегів балок та зрідка колків належать до союзу Salicion albae і асоціації Salicetum albae. Тополеві ліси пониззя заплави Сули належать до союзу Calamagrostio epigei-Populion nigrae і розділяються на дві асоціації, назву яких пропонуємо змінити згідно з вимогами Міжнародного кодексу фітосоціологічної номенклатури – Galio veri-Populetum nigrae та Strophiostomo sparsifloraе-Populetum albae. Наголошується на відсутності в досліджених угрупованнях видів із Червоної книги України. Найменш трансформовані, найбільші за площею та найстаріші вільхові, вербові та тополеві ліси кожної з асоціацій потребують заповідання, на користь чого свідчить також їх значна водоохоронна роль.

Ключові слова: Querco-Fagetea (Alno-Ulmion), Alnetea, Populetea albae, Україна, Лівобережний Лісостеп, басейн нижньої Сули, синтаксономія.

В. Соломаха, д-р биол. наук, Н.Смоляр, канд. биол. наук, Е. Смаглюк, асп.
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

ФЛОРИСТИЧЕСКАЯ КЛАССИФИКАЦИЯ ПОЙМЕННЫХ ОЛЬХОВЫХ, ИВОВЫХ И ТОПОЛЕВЫХ ЛЕСОВ В БАССЕЙНЕ НИЖНЕЙ СУЛЫ (УКРАИНА)

Исследованы пойменные ольховые (Alnus glutinosa), ивовые (Salix alba, зрідка S. fragilis) и тополевые (Populus nigra, P. alba, колки с Populus x canescens) леса в бассейне нижней Сулы. Мезогигрофильные ольховые леса отнесены к союзуAlno-Ulmion классу Querco-Fagetea (com. Aegopodium podagraria-Alnus glutinosa, D.c. Acer negundo-Alnus glutinosa). Заболоченные ольховые леса класса Alnetea glutinosae распространены преимущественно в поймах малых рек и представлены двумя ассоциациями (Carici ripariae-Alnetum glutinosae и Carici acutiformis-Alnetum glutinosae). Пойменные ивовые и тополевые леса отнесены к классу Populetea albae (порядок Populetalia albae). Ивовые леса пойм Сулы и ее притоков, а также переувлажненных тальвегов балок и изредка колков принадлежат к союзу Salicion albae

и асоциации Salicetum albae. Тополевые леса низовий поймы Сулы отнесены к союзу Calamagrostio epigei-Populion nigrae и распределяются на две ассоциации, название которых предлагаем изменить согласно требований Международного кодекса фитосоциологической номенклатуры – на Galio veri-Populetum nigrae и Strophiostomo sparsiflorae-Populetum albae. Указывается на отсутствие в исследуемых сообществах видов растений из Красной книги Украины. Наименее трансформированные, наибольшие по площади и более старые ольховые, ивовые и тополевые леса каждой из ассоциаций требуют заповедания, в пользу чего свидетельствует их значительная водоохранная роль.

Ключевые слова: Querco-Fagetea (Alno-Ulmion), Alnetea glutinosae, Populetea albae, Украина, Левобережная Лесостепь, бассейн нижней Сулы, синтаксономия.

UDK 575.28+578.832.1

O. Smutko, PhD stud
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
L. Radchenko, JS, A. Mironenko, MD.
SI "Gromashevsky L.V. Institute of epidemiology and infectious diseases, NAMS of Ukraine", Kyiv, Ukraine

MOLECULAR AND GENETIC CHARACTERISTICS OF SURFACE AND NONSTRUCTURE PROTEINS OF PANDEMIC INFLUENZA VIRUSES A(H1N1)PDM09 IN 2015-2016 EPIDEMIC SEASON

The aim of the present study was identifying of molecular and genetic changes in hemaglutinin (HA), neuraminidase (NA) and non-structure protein (NS1) genes of pandemic influenza A(H1N1)pdm09 strains, that circulated in Ukraine during 2015-2016 epidemic season. Samples (nasopharyngeal swabs from patients) were analyzed using real-time polymerase chain reaction (RT-PCR). Phylogenetic trees were constructed using MEGA 7 software. 3D structures were constructed in Chimera 1.11.2rc software.Viruses were collected in 2015-2016 season fell into genetic group 6B and in two emerging subgroups, 6B.1 and 6B.2 by gene of HA and NA. Subgroups 6B.1 and 6B.2 are defined by the following amino acid substitutions. In the NS1 protein were identified new amino acid substitutions D2E, N48S, and E125D in 2015-2016 epidemic season. Specific changes were observed in HA protein antigenic sites, but viruses saved similarity to vaccine strain. NS1 protein acquired substitution associated with increased virulence of the influenza virus.

Key words: A(H1N1)pdm09 influenza virus, amino acid substitution, antigenic site, non-structure protein.

Introduction. Influenza viruses are antigenicaly variable pathogens, capable of continuously evading immune response. Accumulation of mutations in the antigenic sites is called the "antigenic drift". In circulating influenza viruses this antigenic drift is a major process, accumulating mutations at the antibody binding sites of receptor proteins, and enabling the virus to evade recognition by hosts' antibodies, which often translates into periodic epidemics of influenza. To tame the influenza spread a flexible vaccination WHO's program, based on periodic production of novel versions of vaccine, is adapted to the actually prevalent stain(s). For such programs the data on phylogenesis of circulating versions of pathogens, and genetic stability of their hemagglutinin (HA) sets data, could help to rationalize possible epidemiological measures [1].

This year's seasonal influenza risk assessment identifies type A viruses, in particular A(H1N1)pdm09, as dominant thus far in EU/EEA countries. There are strong indications from some EU/EEA countries that the A(H1N1)pdm09 virus is responsible for the hospitalization of a large number of severe cases. This includes hospitalizations for severe outcomes for both risk groups and otherwise healthy young adults. A similar pattern of severity is likely to be observed in other countries as the season progresses [2].

Matherials and methods. Samples were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses subtype A(H1N1)pdm09 were isolated in MDCK and MDCK-SIAT cell culture from samples positive in PCR. Hemagglutinin (HA), neuraminidase (NA) and non-structure protein (NS1) gene sequences of Ukrainian isolates were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 7 software [3]. The influenza A(H1N1)pdm09 sequences are characterized in a neighbor-joining phylogenetic tree with reference strains rooted from the current vaccine strain, A/California/07/2009-like virus. 3D structures were constructed in Chimera 1.11.2rc software [4].

Results and discussion. In this study we compared nucleotide sequences of influenza viruses HA, NA and NS1 proteins.

Comparison of neuraminidase (HA) genes.

Over the last five years the HA genes have evolved and eight genetic groups have been designated, with A/California/7/2009 representing group 1, and viruses in group 6 have formed clusters designated groups 6A, 6B and 6C. Viruses collected in 2015-2016 season fell into genetic group 6B and in two emerging subgroups, 6B.1 and 6B.2. Subgroup 6B and subgroups 6B.1 and 6B.2 are defined by the following amino acid substitutions in HA1 and HA2.

Most of the viruses had amino acid substitutions that define the new group of viruses in genetic group 6B, now called group 6B.1. Isolates had a substitution at one of these sites N162K resulting in loss of glycosylation site, acquired by the 6B.1 viruses (fig.1).

Also Ukrainian viruses had substitutions S84N and I216T. Three isolates from Khmelnitsky, Kiev and Ternopol had unique mutation in HA2 – I91V. New substitution S83P was observed in the majority of viruses from 6B.1 group. Four isolates from Odessa belonged to group 6B.2. Its HA protein had a substitution at residue 152 of HA1, V152T. Substitutions in this region, as well as at residue 152, are often selected in culture and known to affect the antigenicity of the virus. Viruses from group 6B.2 also had substitutions R113K D127E (gain of glycosylation site) and E47Q (HA2).

Gain or loss of *N*-linked glycosylation sites has been shown to alter HA protein surface topology. A gain in glycosylation could be advantageous to the virus by virtue of a masking effect on important antibody recognition sites, thus potentially modulating viral antigenicity [5]. Observations are based solely on sequence motifs. For the influenza A(H1N1)pdm09 specimens characterized in this report, two mutations, S162N (serine to asparagine) and D127E (asparagine acid to glycine acid), were observed that could cause a gain of a glycosylation motif.

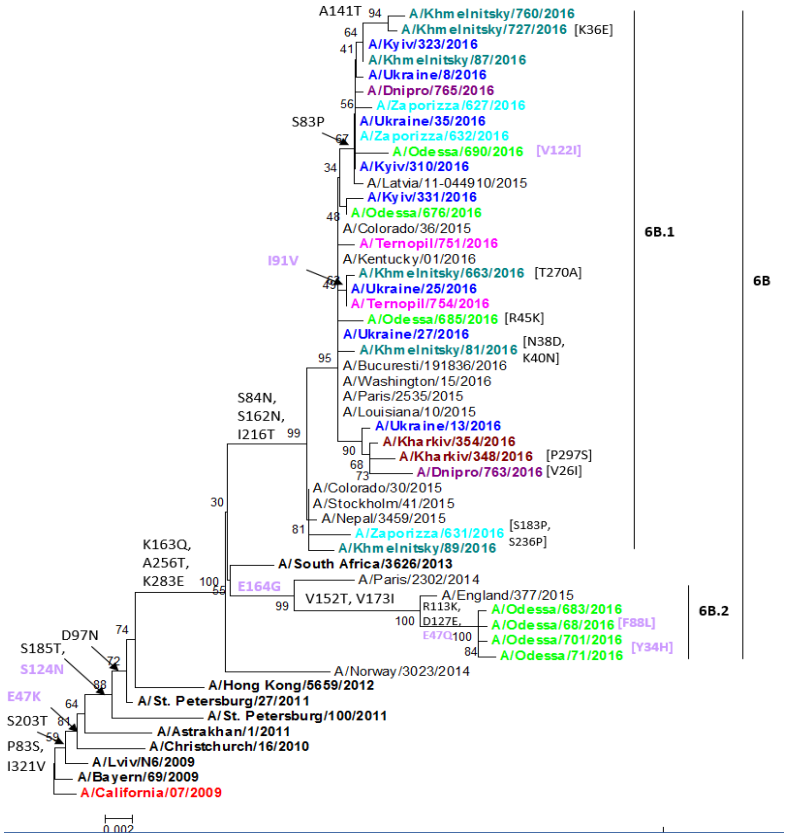

Fig.1. Phylogenetic tree of the HA gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

It is known that the H1 HA molecules have four distinct antigenic sites: Sa, Sb, Ca, and Cb [6]. As a result, these sites consist of the most variable amino acids in the HA molecule of the seasonal human H1N1 viruses that have been subjected to antibody-mediated immune pressure. Notably, the Sa and Sb sites that contain many amino acids involved in neutralizing epitopes near the receptor binding pockets [6].

In Ukrainian isolates were observed mutations in antigenic sites, which emerged in 2015-2016 epidemic season. The main substitution S162N emerged in Sa antigenic site and was observed in all isolates from group 6B.1 (fig.2).

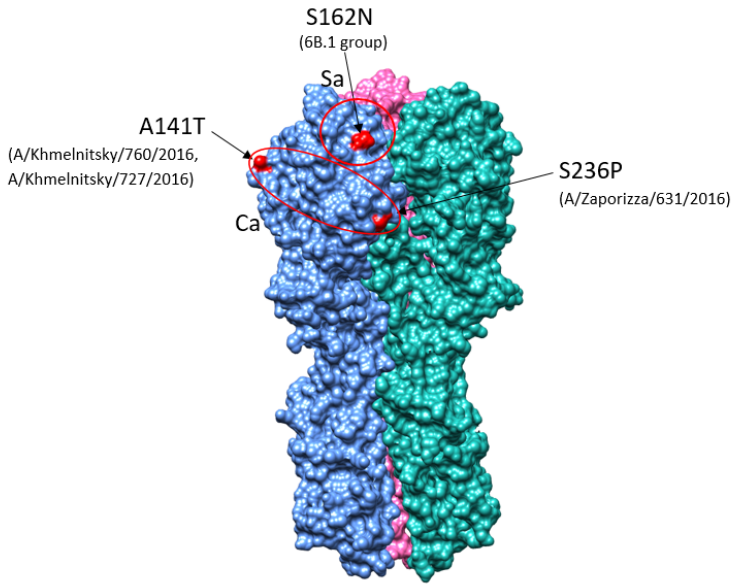

Fig.2. 3D structure of antigenic sites on the HA molecule of Ukrainian isolates

Two substitutions were observed in antigenic site Ca, A141T – had isolates №727 and №760 from Khmelnitsky, S236P – in A/Zaporizza/631/2016. Information about changes in antigenic sites very important for prediction next dominant strains. It is well-documented that antigenic changes of HA occasionally result in the acquisition of carbohydrate side chains on the HA molecule [7]. Since the carbohydrate side chains in the vicinity of antigenic sites mask the neutralizing

epitopes on the HA surface, amino acid substitutions associated with acquisition of carbohydrate chains are believed to efficiently generate antigenic variants.

Comparison of neuraminidase (NA) genes.

Genetic comparison of influenza virus A(H1N1)pdm neuraminidase genes shown that all investigated isolates were genetically related to reference strain A/South Africa/3626/2013 and saved high genetic similarity to vaccines strain A/California/07/2009. On phylogenetic tree of NA genes was shown that viruses also divided into two subgroups – 6B.1 and 6B.2, as on phylogenetic tree of HA genes (fig.3).

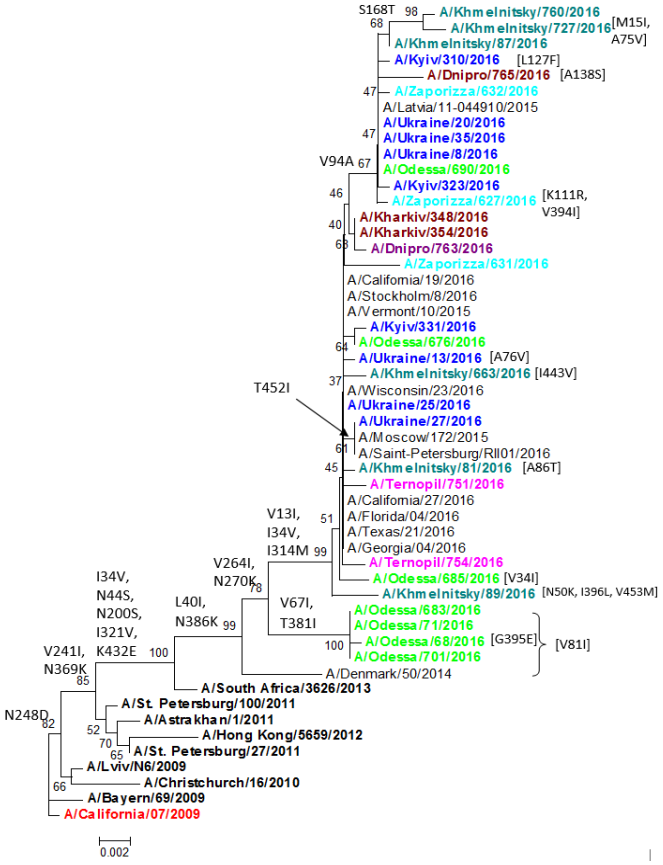

Fig.3. Phylogenetic tree of the NA gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

All discovered isolates had amino acid substitutions – I34V, N44S, N200S, I321V, K432E, except reference strains. Most of Ukrainian isolates belonged to group 6B.1 and acquired substitutions – V13I, I34V, V264I, N270K, I314M. Part of these viruses had mutation V94A.

Group 6B.2 included isolates from Odessa, viruses acquired substitutions V67I (valine to isoleucine), T381I (threonine to isoleucine). Isolate A/Odessa/68/2016 had unique acid substitution G395E.

All discovered viruses retain susceptibility to oseltamivir and zanamivir.

Comparison of non-structure protein (NS1) genes.

Viral NS1 protein plays a central role in counteracting host cell processes that try to interfere with viral replication.

In 2015-2016 epidemic season in Ukrainian isolates amino acid substitutions D2E, N48S, and E125D were identified in the NS1 protein. These mutations were absent in isolates in 2014-2015 epidemic season. Substitutions D2E and E125D occurred in 70% Ukrainian viruses and N48S in 12,5% of sequenced viruses.

Ukrainian isolates 2015-2016 season have been divided into two groups. The second group included 6 isolates from Odessa and 1 isolate from Dnepropetrovsk. In these group substitutions D2E, N48S, and E125D were absent, but isolates had unique point substitutions – I18V, V129I, I182V (fig.4).

An EpiFlu database search revealed that the frequency of substitutions D2E and E125D in NS1 protein of influenza A(H1N1)pdm09 viruses drastically increased in less than 1 year from 10% in 2015 in the Southern Hemisphere epidemic season to 74% in 2015/2016 in the Northern Hemisphere epidemic season [8].

Conclusions. Genetic analysis of influenza A(H1N1)pdm09 viruses circulating in Ukraine in the 2015/2016 epidemic season showed that all of them were similar to the vaccine strain recommended by WHO. Viruses had acquired amino acid substitutions in HA molecule antigenic sites, which can lead to antigenic changes at the next epidemic seasons. Although new genetic subgroups have emerged in 2015-2016 epidemic season, the A(H1N1)pdm09 viruses received were antigenically similar to the vaccine virus A/California/7/2009 and retain susceptibility to oseltamivir and zanamivir

Detailed analysis of substitutions in the protein encoded by internal gene NS1 showed that most of Ukrainian viruses acquired specific amino acid changes: D2E, N48S and E125D. E125D in NS1 is known to be one of the key substitutions involved in shutdown of host mRNA transport, restoring inherent disability of A(H1N1)pdm09 virus to efficiently control human cell gene expression. NS1 of all seasonal human influenza viruses (H1N1 seasonal and H3N2) contains D125 that interacts with cellular cleavage and

polyadenylation factor 30 (CPSF30)6. Interaction with CPSF30 is absent in most animal-adapted strains, so E125D substitution can be considered a milestone in host adaptation of influenza A(H1N1)pdm09 virus.

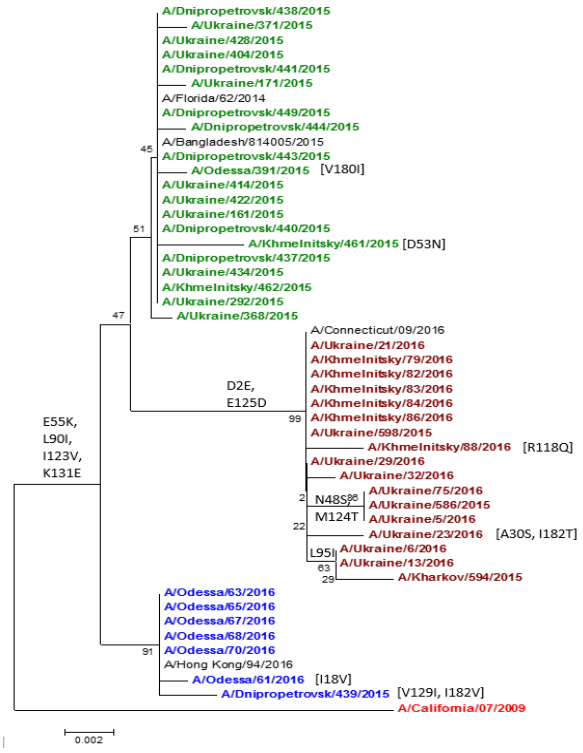

Fig.4. Phylogenetic analysis of the NS1 gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

The observed rapid spread of influenza A(H1N1)pdm09 viruses with no significant antigenic changes in HA can be speculatively explained by increased transmissibility, as well as by increased virulence or by combination of both. The possible link between transmissibility or virulence and described changes in NS1 internal gene in influenza A(H1N1) pdm09 viruses awaits experimental proof [8].

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О. Смутько, асп.
Київський національний університет імені Тараса Шевченка, Київ, Україна,
Л. Радченко, мол.наук. співроб., А. Мироненко, д-р мед. наук
ДУ "Інститут епідеміології та інфекційних хвороб імені Л.В.Громашевського, НАМН України", Київ, Україна

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.

О. Смутько, асп.
Киевский национальный университет имени Тараса Шевченко, Киев, Украина,
Л. Радченко, млад. научн. сотр., А. Мироненко, д-р мед. наук
ГУ "Институт эпидемиологии и инфекционных заболеваний имени Л.В. Громашевского, НАМН Украины", Киев, Украина

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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**T. Shevchenko, PhD., O. Tymchyshyn, MD stud.,
I. Kosenko, MD stud., I. Budzanivska, Dr.Sci., Prof.,
V. Polischuk, Dr.Sci., Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine**

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).

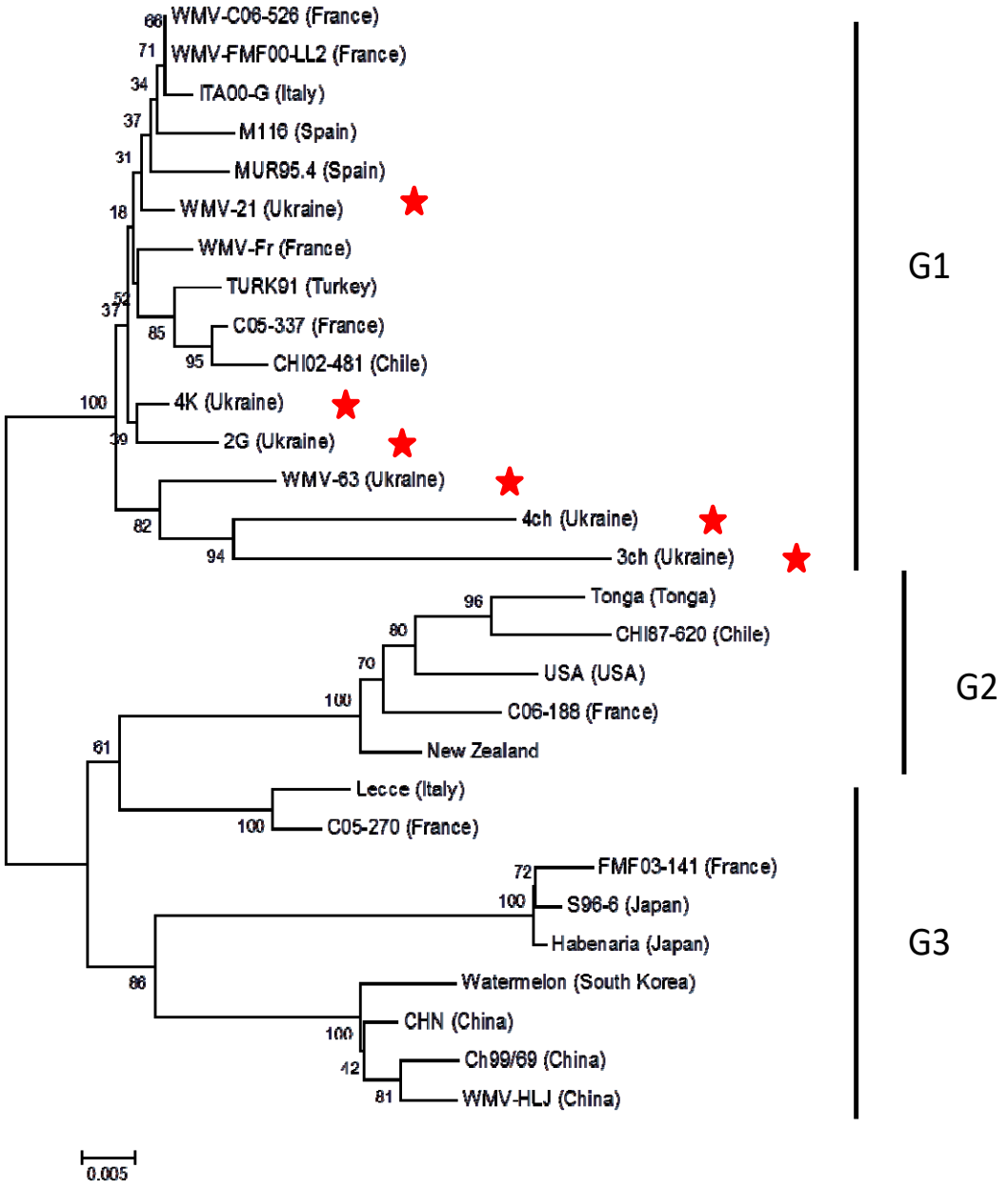

Figure 3. Phylogenetic tree of WMV-2 isolates constructed using Neighbor-Joining method

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).

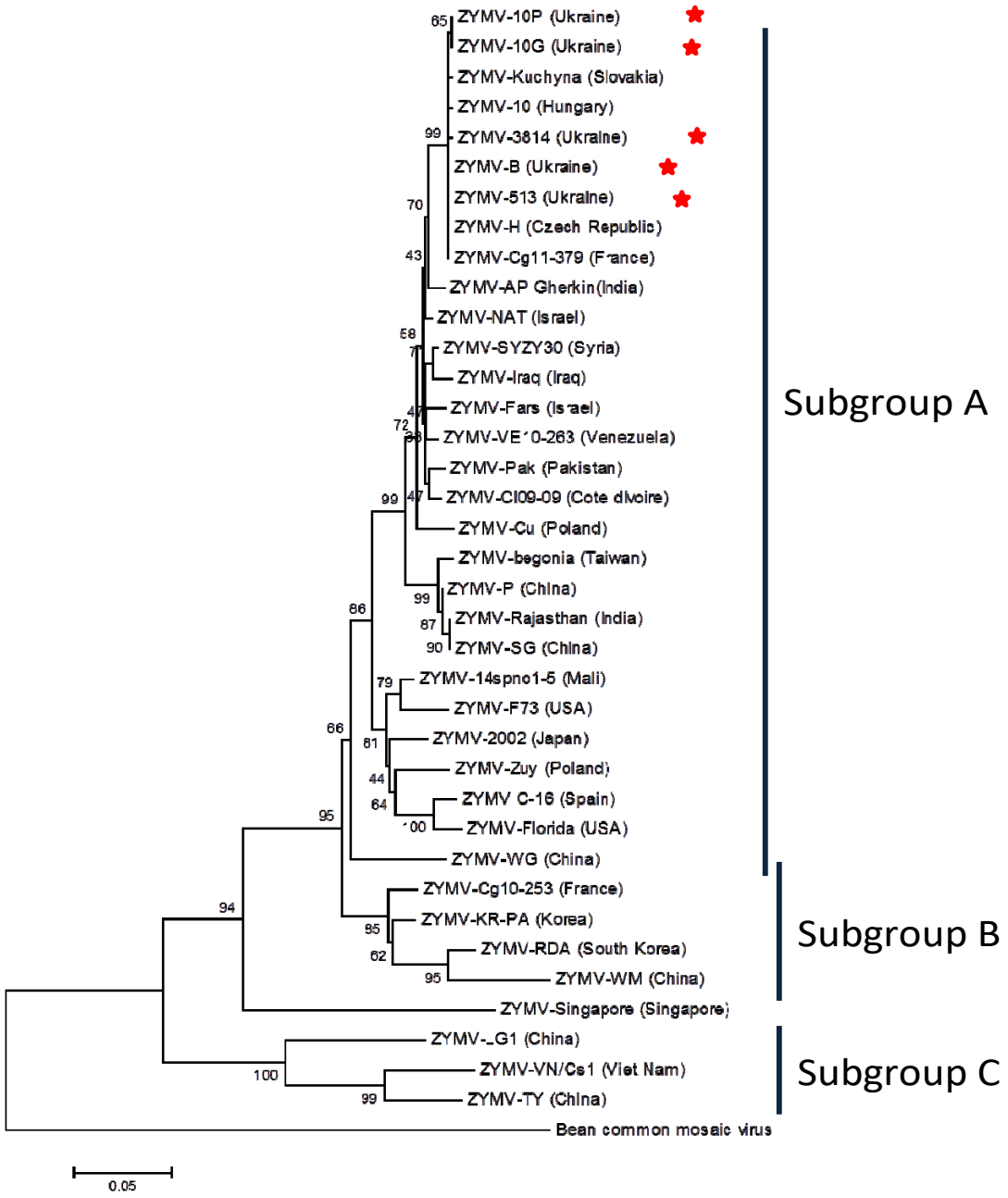

Figure 4. Phylogenetic tree of ZYMV isolates constructed using Neighbor-Joining method

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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Т. Шевченко, канд. біол. наук, О. Тимчишин, магістр, Ю. Косенко, магістр, І. Будзанівська, д-р біол. наук, В. Поліщук, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

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.

Т. Шевченко, канд. биол. наук, О. Тымчишин, магистр, Ю. Косенко, магистр, И. Будзанивская, д-р биол. наук, В. Полищук, д-р биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

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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T. Koval, engineer, T. Ischuk, PhD, Ya. Raetska, PhD
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

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

ammonium sulfate solution (final concentration 50%) and were left at 4 °C overnight. The precipitate was centrifuged at 3000 rpm/min for 30 min. The supernatant was withdrawn and the precipitate was dissolved in 1 ml of 0.05 M Na-phosphate buffer, pH 7.4 [5]. To remove ammonium residues, the solution of antibodies was applied to column G 25 equilibrated with 0.05 M Na-phosphate buffer, pH 7.4 (total column volume 50 ml). Samples containing protein were concentrated, then absorbance was measured and the antibody concentration was calculated. The obtained samples were stored at – 20 °C. The IgG fractions from serum were isolated on the 15th day of the experiment.

The level of antibodies in the blood of animals studied was evaluated by enzyme-linked immunosorbent reaction [4], which was carried out in 96-well microplate (Dynatech, Sweden). In the hole made microplate 100 ml goat IgG monoclonal antibodies against mouse (Sigma, USA).

CIC content in blood serum determined by precipitation of 4.5% solution of polyethylene glycol 6000 (PEG-6000) [8]. The method is based on different solubility Ig monomers composed of IC in the presence of PEG in the environment. Different concentrations of PEG (2.5%, 3.5%, 7%, 10%) cause precipitation of different molecular weight and size of CIC. Low concentrations of PEG precipitated complexes large, high concentrations cause precipitation of low molecular weight compounds. Results reaction photometrical determined using ELISA analyzer (Titertek Multiskan, Finland).

Statistical analysis of the results was performed using the program OriginLab 8.0. Changes considered reliable indicators at $p \leq 0,05$; at $p \geq 0,05$ changes into account as a trend.

Results: The degree of the pathological states by various organs and results of disease, are depended from severity of inflammation trauma, timely and accuracy of treatment, and age of victim human [5; 6]. In our researches principally was used of inflammation model, which would meet one, that are characteristically to children since 1 to 8 years.

Analysis and generalization of received research results on this model, are allowed reveal a not only factors, that determine the different level of means, and features of regulation mechanisms of the child immune system at the chemical inflammation of esophagus. Mortality from inflammation trauma, are dependent from spread, severity and degree of inflammation.

We were elected under shock and septic -toxemia of inflammation, because the biggest of interest of the dead there in period of septic -toxemia. Causes at the inflammation trauma: sepsis, pneumonia and on it background progressing the multiple organ failure [7].

Analysis of conducted researches, was viewed, that ABE causes of the humoral immunity link change, that consistent with the dates of references [13]. Was established decreased of IgG level, which are the most specific effector of humoral link, on the 1st day of ABE, that responsible of shock stage (Figure 1).

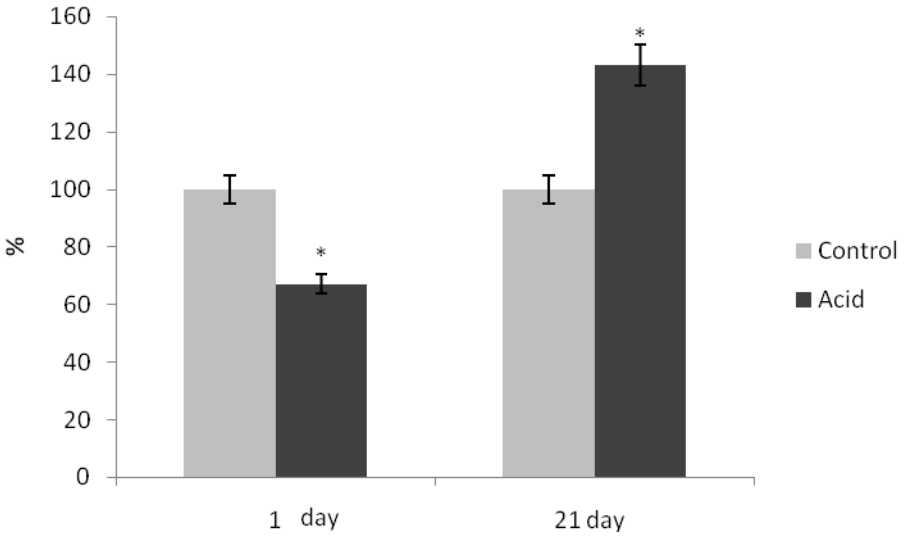

Figure 1. The level of IgG in serum of rats under the experimental simulation of acid burns the esophagus as a percentage relative to control (M ± m, n = 10)

* – $p < 0,05$ compared with control

The average level of antibodies in this group decreased by 1.48 times against the intact control. These changes may indicate that in a chemical burn is the failure of adaptation responses.

One of the indicators of immune status is the level of circulating immune complexes (CIC) in blood. In the process of immunocomplex are important dimensions of immune complexes because most pathogenic immune complexes are small and medium-sized, able to activate the complement system.

Determining the level of immune complexes useful in burn disease, however, problems related to the rationale for determination of circulating immune complexes at ABE have not yet received a clear decision.

We have found that the ABE has been a change of prices on the first day after the burn (Figure 2).

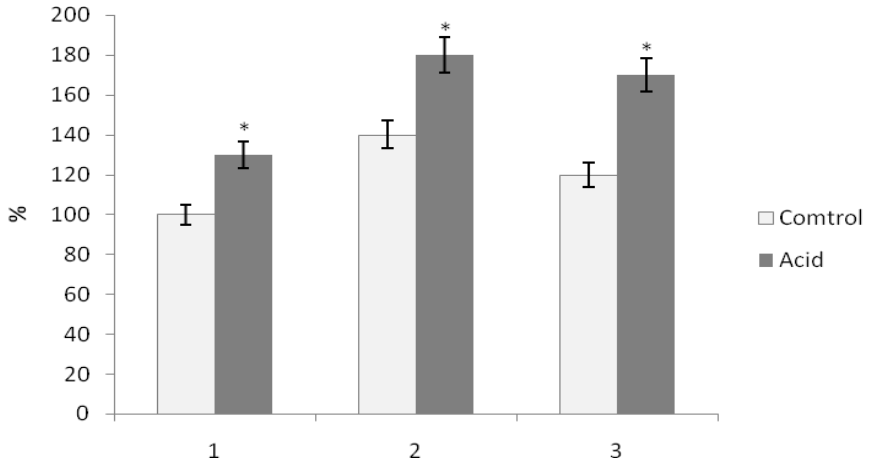

Fig. 2. The level of circulating immune complexes in the blood serum of rats under experimental modeling of acid burns the esophagus as a percentage relative to control at 1 day after the formation of burn wounds. (M ± m, n = 10)
1. macromolecular immune complexes
2. The average molecular immune complexes
3. low immune complexes

* – p <0,05 compared with control

In the study of high-CIC in animals which ABE induced the tendency to increase their level of performance compared to intact controls. Performance exceeded the reference value of 1.3 degrees in the animals treated with 30% solution of acetic acid.

The level of average molecular CIC also exceeded the reference value of 1.3 degrees, indicating the development of inflammation. At the same time increased the level of low-CIC group of animals with ABE. Performance exceeded the reference value of 1.4 degrees.

So, along with a decrease at the level of IgG in 1st day of ABE seen significant shifts in the molecular composition of immune complexes that manifested itself primarily increased concentration of toxigenic most – middle- and low-CIC.

Figure 1 presents data indicate that on the 21st day of burn disease in rats under experimental modeling of ABE was a significant increase the level of IgG antibodies in serum. It was found that in the group of animals with burns to 30% CCl_3COOH, antibody levels exceeded indicators in the control group 1.43 degrees.

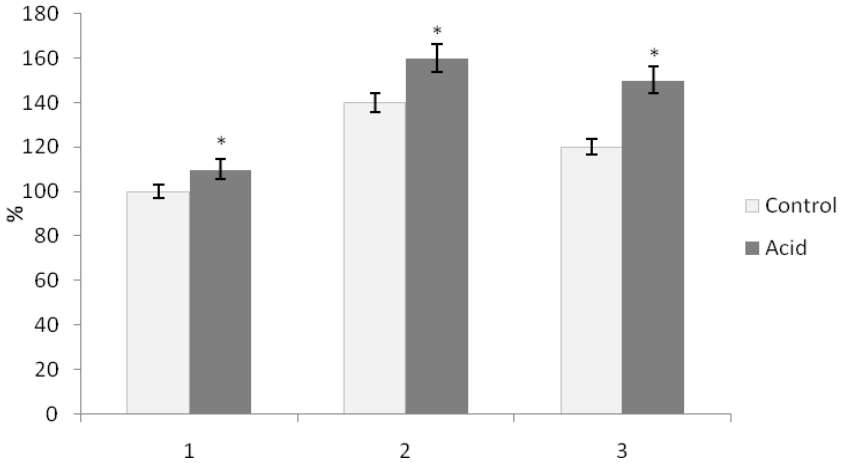

Fig. 3. The level of circulating immune complexes in the blood serum of rats under experimental modeling of acid burns the esophagus as a percentage relative to controls at 21 st day after the formation of burn wounds. (M ± m, n = 10)
1. macromolecular immune complexes
2. The average molecular immune complexes
3. low immune complexes

* – p <0,05 compared with control

At the research of high and average molecular CIC in the animals on the 21st day after inflammation (Figure 3) was observed trend to some magnification of this level, in compared with means of intact control.

The means of low molecular CIC in animals with ABE, were above than control in 1.25 degrees. In accordance with references, a long circulation of immune complexes in organism, even slight increase of this complexes results to appearance of savings of this complexes in tissues, to increase of aggregation and adhesion of thrombocytes, that results to violation of microcirculation of blood and necrosis [9].

At the development of immunocomplex process, important are the dimensions of immune complexes, because the most pathogenic immune complexes are small and medium size, which are able to activate the complement system, which causes the development of inflammation. Actually, these immune complexes interact with a number of regulatory systems, causing damage response.

Features of the immune system in the 21st day lies in the fact that during our investigation in immature rats (1-month) with ABE was activation of most indicators. Data from clinical observations [1] cannot be considered a change of immunological parameters as evidence of normalization or healing. This can be explained by high lability of most studied indicators and due to the immaturity of physiological systems.

Conclusion. So ABE accompanied by considerable changes in the levels of the humoral immune system. On the 1 st day of ABE in immature rats (1-month) took place reduction of IgG and were marked significant shifts in the molecular composition of immune complexes that manifested itself primarily in increased concentration of toxigenic most – middle- and low-CIC. On the 21 st day of burn disease in rats under experimental modeling of ABE was a significant increase in the level of IgG antibodies in serum. In animals that were burned by 30% solution CCl_3COOH medium and low CIC tended to increase.

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Т. Коваль, асп., Т. Іщук., канд. біол. наук, Я. Раєцька, канд. біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ОСОБЛИВОСТІ ІМУННОЇ ВІДПОВІДІ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ВІДТВОРЕННЯ МОДЕЛІ КИСЛОТНОГО ОПІКУ СТРАВОХОДУ

Добре відомо, що імунна система активно бере участь в регенерації і процесах загоєння опікових ран. Проте, без відповіді залишаються питання про роль гуморального імунітету в механізмах загоєння і розвитку ускладнень опікової рани. Ми розробили експериментальну модель кислотного опіку стравоходу (КОС), який відповідає опіку стравоходу у дітей 1-8 років. Ми вивчали особливості гуморального імунітету при КОС у щурів, при цьомуспостерігали зниження рівня IgG і підвищення рівнів середніх і низькомолекулярних циркулюючих імунних комплексів (ЦІК) в перший день після опіку стравоходу. На 21-й день після опіку, ми спостерігали збільшення концентрації IgG і незначне накопичення середньо- і низькомолекулярних ЦІК. Вивчені показники можуть бути використані для диференціації розвитку КОС.

Ключові слова: кислотний опік стравоходу, рівень IgG , рівень циркулюючих імунних комплексів (ЦІК).

Т. Коваль, асп., Т. Ищук, канд. биол. наук, Я. Раецкая, канд. биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

ОСОБЕННОСТИ ИММУННОГО ОТВЕТА В УСЛОВИЯХ ЭКСПЕРЕМЕНТАЛЬНОГО КИСЛОТНОГО ОЖОГА ПИЩЕВОДА

Хорошо известно, что иммунная система активно принимает участие в регенерации и процессах заживления ожоговых ран. Однако, без ответа остаются вопросы о роли гуморального иммунитета в механизмах заживлении и развитии осложнений ожоговой раны. Мы разработали экспериментальную модель кислотного ожога пищевода (КОП), который соответствует ожогу пищевода у детей 1-8 лет. Мы изучали особенности гуморального иммунитета при КОП у крыс, при этом наблюдали снижение уровня IgG и повышение уровня средних и низкомолекулярных циркулирующих иммунных комплексов (ЦИК) в первый день после ожога пищевода. На 21-й день после ожога, мы наблюдали увеличение концентрации IgG и незначительное накопление средне- и низкомолекулярных ЦИК. Изученые показатели могут быть использованы для дифференциации развития КОП.

Ключевые слова:кислотный ожог пищевода, уровень IgG , уровень циркулирующих иммунных комплексов (ЦИК).

УДК 574.583

Z. Burian, PhD stud., V. Trokhymets, PhD.
Taras Shevchenko National University of Kyiv, Kyiv, Україна

STRUCTURAL AND FAUNISTIC ORGANIZATION OF THE UDAY RIVER'S LITTORAL ZOOPLANKTON IN THE NATIONAL NATURE PARK "PYRIATYNSKIY"

Results of the investigation of the littoral zooplankton's structural and faunistic organization of the Uday river are presented. The research was conducted in the National Nature Park "Pyriatynskiy" in summer 2015. As the result of research 58 zooplankton's species were established. Their density, biomass, ecological spectrum of the community, type and way of the feeding were analyzed.

Key words: zooplankton, Uday river, National Nature Park "Pyriatynskiy", Ukraine.

Introduction. The influence of anthropogenic factor causes the significant changes and restructuring in the aquatic ecosystems. In turn, all these processes have a strong impact on the status of different taxonomical and ecological groups of hydrobionts. Thus these processes lead to the quantitative and qualitative changes in hydrobionts' communities and to the development of adaptive characteristics in different water organisms [1]. Such transformations are intense and proceed in a short time, what may lead to the considerable restructuring of the diversity of hydrobionts [2].

The special attention is attracted to the Uday river. Its valley is located in the National Park "Pyryatynsky" created in 2009. This pond was considered as one of the cleanest rivers in Ukraine a few decades ago. However, due to the active reclamation in recent time, many floodplains of tributaries that fed Uday were drained. Also, toxic substances extremely impact the ecosystem of the river. This led to a reduction in the diversity of aquatic organisms, siltation and overgrowing of the river. Therefore there is an urgent need for continuous monitoring of the Uday river to examine the state of hydrobiocenosis, which is a necessary component of the hydroecological studies.

Material and Methods

The objects of research were representatives of the three main groups of zooplankton: rotifers (class Eurotatoria), cladocerans (class Branchiopoda, order Cladocera), different age stages of copepods (class Copepoda). Also, ostracods (class Ostracoda) and larvae of bivalves (class Bivalvia) were investigated. Monogonont rotifers, copepods and crustaceans were identified to the species. For bdelloid rotifers (subclass Bdelloidea), ostracods and larvae of bivalves only the higher taxonomic groups were identified.

As the material for research was used zooplankton which was collected in late July 2015. Eight experimental stations in the intertidal zone of the Uday river were investigated (Fig. 1): outskirts of the Kroty village N $50^0$23.197' E $32^0$28.494'; Gurbentsi village N $50^0$21.314' E $32^0$28.612'; Leliaky village N $50^0$20.137' E $32^0$23.700'; Keibalivka village N $50^0$18.351' E $32^0$30.100'; Sumskiy bridge, Pyriatyn city N $50^0$13.636' E $32^0$33.324'; Masalskiy island, Pyriatyn city N $50^0$14.365' E $32^0$31.883'; Velyka Krucha village N $50^0$11.159' E $32^0$34.300'; Povstyn village N $50^0$11.183' E $32^0$40.283'. Zooplankton was studied within the overgrown habitat (in the following formations: common reed *Phragmites communis*, broadleaf cattail *Typha latifolia*, yellow water-lily *Nuphar lutea*) and within the freshwater ones. Collection of zooplankton was performed using conical nets [3]. 16 samples were collected and analyzed on the basis of generally accepted methods [3-10].

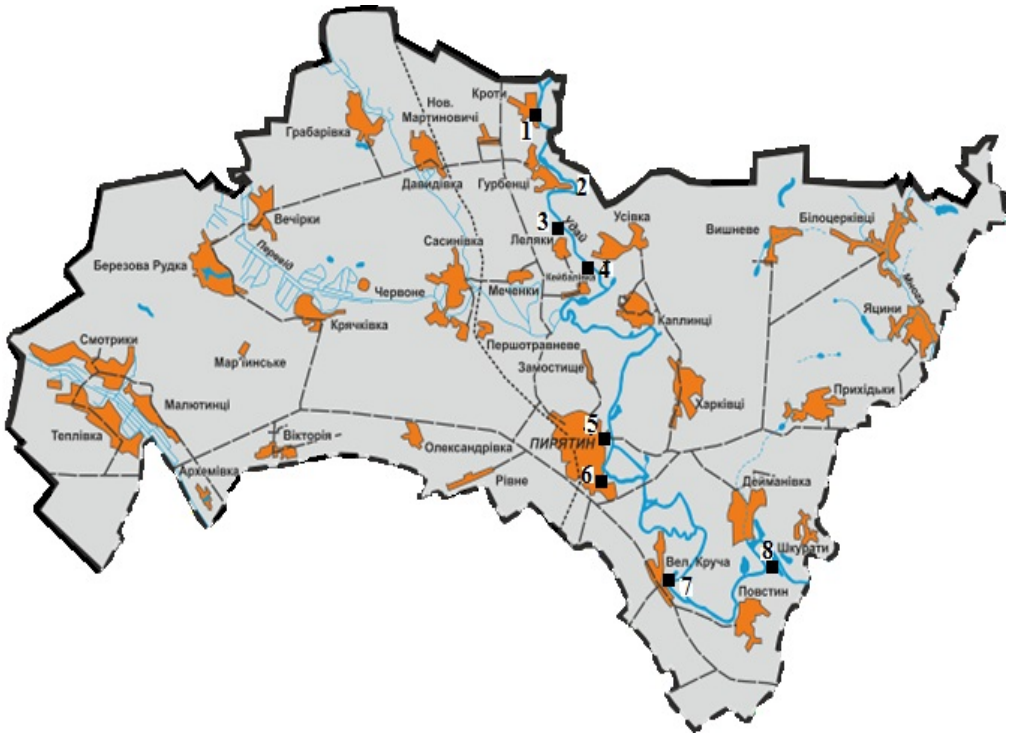

Figure 1. Stations of the littoral zooplankton sampling (Uday river, National Nature Park "Pyriatynskiy").
Marks: 1 – outskirts of the Kroty village; 2 – Gurbentsi village; 3 – Leliaky village; 4 – Keibalivka village; 5 – Sumskiy bridge, Pyriatyn city; 6 – Masalskiy island, Pyriatyn city; 7 – Velyka Krucha village; 8 – Povstyn village

Results and discussion

In the result of research, 58 zooplankton species were registered. The representatives of the rotatoria and cladocera complex were dominated.

23 species of monogonont rotifers were registered: *Anuraeopsis fissa fissa* Gosse, 1851; *Asplanchna priodonta* Gosse, 1850; *Brachionus angularis* Gosse, 1851; *Br. calyciflorus* Pallas, 1766; *Br. quadridentatus* Hermann, 1783; *Euchlanis deflexa* (Gosse, 1851); *E. dilatata* Ehrenberg, 1832; *E. lyra* Hudson, 1886; *E. triquetra* Ehrenberg, 1838; *Hexarthra mira* (Hudson, 1871); *Lecane bulla* (Gosse, 1851); *L. luna* (O.F.Müller, 1776); *L. lunaris* (Ehrenberg, 1832); *L. closterocerca* (Schmarda, 1859); *Lophocharis oxysternon* (Gosse, 1851); *Mytilina ventralis* (Ehrenberg, 1830); *Plationus patulus* (O.F.Müller, 1786); *Platyias quadricornis* (Ehrenberg, 1832); *Polyarthra dolicoptera* Idelson, 1925; *Synchaeta pectinata* Ehrenberg, 1832; *Testudinella patina* (Hermann, 1783); *Trichocerca rattus* (O.F.Müller, 1776); *Trichotria pocillum* (O.F.Müller, 1776). Also bdelloid rotifers (subclass Bdelloidea) were registered.

22 species of cladocerans were discovered: *Acroperus harpae* (Baird 1834); *Alona guttata* Sars, 1862; *A. rectangula* Sars, 1862; *Alonella nana* (Baird 1843); *Bosmina longirostris* (O.F.Müller, 1776); *Ceriodaphnia affinis* Lilljeborg, 1900; *C. pulchella* Sars, 1862; *C. quadrangula* (O.F.Müller, 1785); *Chydorus piger* Sars, 1862; *Ch. sphaericus* (O.F.Müller, 1785); *Daphnia cucullata* Sars, 1862; *D. longispina* (O.F.Müller, 1776); *Diaphanosoma brachyurum* (Lievin, 1848); *Disparalona rostrata* (Koch, 1841); *Eurycercus lamellatus* (O.F.Müller, 1776); *Graptoleberis testudinaria* (Fischer, 1848); *Lathonura rectirostris* (O.F.Müller, 1776); *Macrothrix hirsuticornis* Norman & Brady, 1867; *Pleuroxus aduncus* (Jurine, 1820); *Pseudochydorus globosus* (Baird, 1843); *Sida crystallina* (O.F.Müller, 1776); *Simocephalus vetulus* (O.F.Müller, 1776).

Copepods were represented by the 13 species: Веслоногі ракоподібні були представлені 13 видами: *Acanthocyclops americanus* (Marsh, 1893); *Cryptocyclops bicolor* (Sars, 1863); *Ectocyclops phaleratus* (Koch, 1838); *Eucyclops denticulatus* (Graeter,1903); *E. Macrurus* (Sars, 1863); *E. serrulatus* (Fischer, 1851); *Macrocyclops albidus* (Jurine, 1820); *Megacyclops viridis* (Jurine, 1820); *Mesocyclops leuckarti* (Claus, 1857); *Microcyclops varicans* (Sars, 1863); *Thermocyclops crassus* (Fischer, 1853); *Th. oithonoides* (Sars, 1863); *Eurytemora velox* (Lilljeborg, 1853).

Zooplankton species composition of different habitats and experimental stations had low similarity, as evidenced by Jaccard index (J = 4,7 – 44,5).

Littoral zooplankton was characterized by significant ecological diversity. Three environmental groups were observed in zooplankton composition: pelagic, demersal and phytophilous.

The ecological spectrum of zooplankton communities was characterized by a significant prevalence of phytophilous groups over pelagic and demersal. Pelagic representatives included 18 species (31%), demersal – 14 (24%) and phytophilous – 26 (45%). Rotifers and cladocerans dominated among the demersal group, while copepods constituted a significant percentage of pelagic and phytophilous groups (Fig. 2).

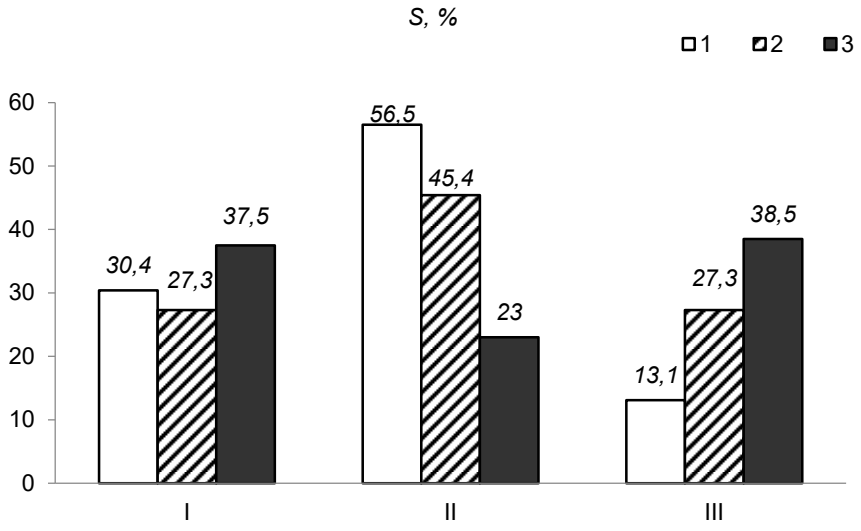

Figure 2. Environmental groups of zooplankton main communities within the district of Uday river.
Marks: I – pelagic, II – demersal, III – phytophilous; 1 – rotifers 2 – cladocerans, 3 – copepods

Zooplankton species are also distinguished by the type and method of feeding. The representatives of different zooplankton species are divided in three trophic groups depending on the type of feeding: peaceful – 44 species (75.9%), omnivorous – 6 (10.3%) and predators – 8 (13.8%). Among rotifers dominated peaceful species – 22 (95.7%) and only 1 (4.3%) omnivorous representative – *Asplanchna priodonta*. Cladocerans were a peaceful group – 22 (100%), while omnivorous copepods included 7 (53.8%) species and predators – 6 (46.2%).

By method of feeding planktonic invertebrates are divided on the following groups: verticators, suction feeders, primary and secondary filter-feeders, gatherers, active and passive hijackers, and parasites. Nine groups were noted during investigation of the Uday river: vertification – 15 species (25.9%), vertification and suction – 4 (6.9%), suction – 3 (5.2%), secondary filtration – 12 (20.7%), seizure – 8 (13.8%), gathering – 6 (10.3%), seizure and absorption – 1 (1.7%), capturing and filtering – 1 (1 7%), primary filtration – 8 (13.8%). Among rotifers dominated the representatives feeding by vertification – 15 species (65.2%),

vertification and absorption – 4 (17.4%), suction – 3 (13%), seizure and absorption – 1 (4.4%). Cladocerans were represented by 12 species (54.5%) of secondary and 8 (36.4%) of the primary filter feeders and 2 (9.1%) gatherers. Among the copepods dominated hijackers – 8 species (61.5%), gatherers – 4 (30.8%) and filtrators and hijackers – 1 (7.7%).

According to the standard classification [3], overall dencity parameters of overgrown habitats in the experimental waters were "very low" for stations Leliaki village (820 ind./m^3) and the island Masalskiy (1600 ind./m^3), "low" – Sumskiy bridge(24300 ind./m^3), Keybalivka village (26900 ind./m^3), Povstyn village (17,280 ind./m^3), Gurbentsi village (9160 ind./m^3), "below average" – Kroty village (111 600 ind./m^3) and Velyka Krucha village (54,080 ind./m^3). In the fresh waters these parameters can be described as "very low" – 200-2480 ind./m^3, except density parameters of the station Keybalivka village – "low" (11700 ind./m^3), and Kroty village – "below average" (109,260 ind./m^3).

Copepods dominated by the density within both habitats in the most of experimental stations (p≤0,05). In overgrown habitats they numbered 22,930 (± 20698,64) of total 30905 (± 25665,4) ind./m^3, while in the freshwater habitats – 14602,9 (± 24129,9) of 14297,8 (± 29842,4) ind./m^3. The only one exception was the station with freshwater habitat – Povstyn village (Fig.3), where cladocerans prevailed by the density- 120 of 200 ind./m^3. Dominant zooplankton species by the density were not noted.

Density prevalence of copepods within the most of experimental stations of the Uday river can be explained by the massive development of the larval stages of copepods, namely nauplius and metanauplius.

According to the standard classification [3] the overall parameters of the biomass of littoral zooplankton in overgrown habitat – common reed and yellow water-lily were "very low" for stations Leliaky village (0.06 g/m^3) and the island Masalsky (0.14 g/m^3), "low" – Keybalivka village (0.53 g/m^3), Povstyn village (0.58 g/m^3), Gurbentsi village (0.51 g/m^3) and "below average" – Sumskiy Bridge (1.27 g/m^3), Kroty village (4.3 g/m^3), Velyka Krucha village (2.35 g/m^3). In freshwater biotope parameters of biomass were "very low" – 0,005-0,13 g/m^3, except Kroty village – "below average" – 2.3 g/m^3 (Fig.3).

In the majority of the experimental aquatories of both habitats dominated copepods (p≤0,05). In overgrown habitats they amounted to 1,33 (± 1,11) of the total biomass of zooplankton groups 2,11 (± 1,61) g/m^3, while in the freshwater habitats – 0,3 (± 0,49) of 0,4 (± 0,64) g/m^3. Cladocerans prevailed in overgrown biotope of the Leliaky village – 0.05, Masalskiy island – 0.08, and in both habitats of the Povstyn village – 0.32; 0.005 g/m^3, and in the Gurbentsi village- 0.32; 0.02 g/m^3 (Fig.4). Cladocerans and copepods have great individual weight, therefore biomass indices were higher compared with other groups of zooplankton.

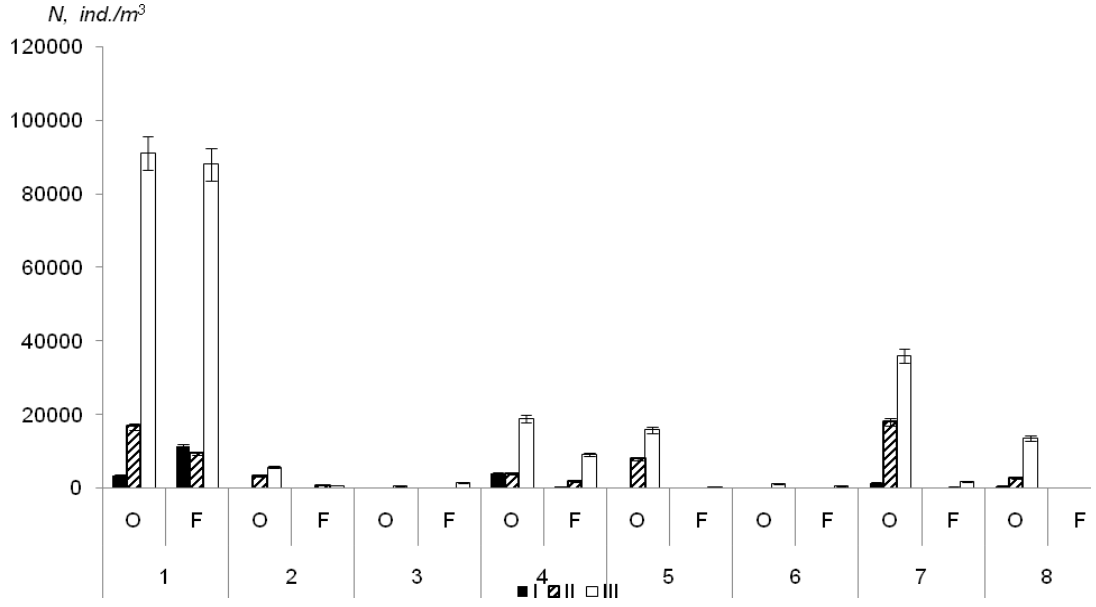

Figure 3. The ratio of the zooplankton densities of different groups in the two habitats of Uday river.
Marks: 1 – outskirts of the Kroty village; 2 – Gurbentsi village; 3 – Leliaky village; 4 – Keibalivka village; 5 – Sumskiy bridge, Pyriatyn city; 6 – Masalskiy island, Pyriatyn city; 7 – Velyka Krucha village; 8 – Povstyn village; I – rotifers, II – cladocerans, III – copepods; O – overgrown habitat, F – freshwater habitat

For overgrown habitat of the Lelyaki village the dominant by the biomass cladocerans species was distinctly identified – *Eurycercus lamellatus* – 66,6% (0,04 total of 0.06 g/m^3). Within overgrown habitat of the station Sumskiy bridge dominated representatives of copepods – *Macrocyclops albidus* – 33,1% (0,42 from 1,27 g/m^3). Also, monodominance of the copepoda *Eurycercus lamellatus* – 57,1% (0,08 from 0,14 g/m^3) was pronounced on the island Masalsky within the overgrown habitat.

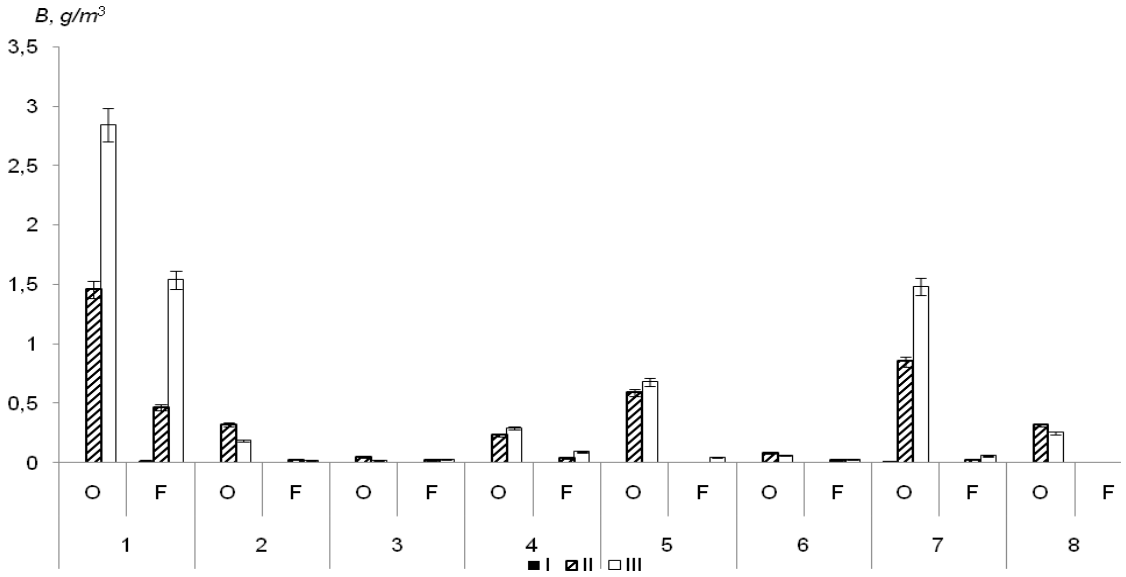

Figure 4. The ratio of the zooplankton biomass of different groups in the two habitats of Uday river.
Marks: 1 – outskirts of the Kroty village; 2 – Gurbentsi village; 3 – Leliaky village; 4 – Keibalivka village; 5 – Sumskiy bridge, Pyriatyn city; 6 – Masalskiy island, Pyriatyn city; 7 – Velyka Krucha village; 8 – Povstyn village; I – rotifers, II – cladocerans, III – copepods; O – overgrown habitat, F – freshwater habitat

In freshwater biotope of the station Velyka Krucha village dominated the copepoda – *Macrocyclops albidus* – 37,5% (0,03 from 0,08 g / m^3). In both habitats of the Povstyn village cladocerans dominated by the biomass, within the overgrown one – *Simocephalus vetulus* – 51,7% (0,3 of 0.58 g/m^3) and within the freshwater – *Daphnia cucullata* – 80% (0,004 of 0,005 g / m^3). In the Gurbyntsi village cladocera *Simocephalus vetulus* dominated in both overgrown biotope – 51% (0.26 from 0.51 g/m^3) and the freshwater – 50% (0.02 from 0.04 g/m^3). Within other investigated stations dominant complex was not determined because of the low biomass.

Ostracods had "very low" parameters [3] of the density – 20-2400 ind./m^3 and of the biomass – 0,002-0,24 g/m^3. Larvae of bivalves were not found at any station.

Conclusions

1. Within the Uday river in the Park "Pyriatynskiy" 58 species of zooplankton were recorded: rotifers – 23, cladocerans – 22, copepods – 13.

2. Zooplankton's species composition of different habitats had low similarity, as evidenced by Jaccard index (J = 4,7 – 44,5).

3. In the ecological spectrum the prevalence of the phytophilous zooplankton group was discovered – 26 species (45%) over the pelagic – 18 (31%) and bottom – 14 (24%).

4. By the type of feeding among representatives of zooplankton communities prevailed the peaceful groups – 44 (75.9%) of the 58 species.

5. By the method of feeding littoral zooplankton was assigned to 9 groups. The dominant groups were vertication – 15 species (25.9%) and secondary filtration – 12 (20.7%).

6. The parameters of density were "very low", "low" and "below average" for overgrown habitat – 820-111600 ind. / м3, and for freshwater – 200-109260 ind. /m3. Copepods dominated by the density within all research stations, except the freshwater habitat of the Povstyn village, where cladocerans dominated.

7. The biomass parameters were "very low", "low", "below average" in the overgrown biotope – 0,0596-4,30392 g/m3, and in the freshwater- 0,00538-2,02556 g/m3. The complex of cladocerans and copepods dominated.

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З. Бур'ян, асп., В. Трохимець, канд. біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

СТРУКТУРНО-ФАУНІСТИЧНА ОРГАНІЗАЦІЯ ЛІТОРАЛЬНОГО ЗООПЛАНКТОНУ Р. УДАЙ НПП "ПИРЯТИНСЬКИЙ"

Представлено результати дослідження структурно-функціональної організації угруповань літорального зоопланктону р. Удай в межах Національного природного парку "Пирятинський", проведені влітку 2015 року. В результаті досліджень визначено 58 видів зоопланктону та проаналізовано їх щільність, біомасу, екологічний спектр угрупування, тип і спосіб живлення.

Ключові слова: зоопланктон, р. Удай, НПП "Пирятинський", Україна.

З. Бурян, асп., В. Трохимец, канд. биол. наук
Киевский национальный университет имени Тараса Шевченка, Киев, Україна

СТРУКТУРНО-ФАУНИСТИЧЕСКАЯ ОРГАНИЗАЦИЯ ЛИТОРАЛЬНОГО ЗООПЛАНКТОНА Р. УДАЙ НПП "ПИРЯТИНСКИЙ"

Представлены результаты исследования структурно-функциональной организации сообществ литорального зоопланктона р. Удай в районе Национального природного парка "Пирятинский", проведенные летом 2015 года. В результате исследований определено 58 видов зоопланктона и проанализировано их плотность, биомассу, экологический спектр сообщества, тип и способ питания.

Ключевые слова: зоопланктон, р. Удай, НПП "Пирятинский", Украина.

УДК 581.52;631.811.98

Y. Kavulych, PhD stud, M. Kobyletska, PhD, O. Terek, DSc
Ivan Franko National University of Lviv, Ukraine

CHANGE IN THE CONTENT OF XANTHONES AND LIGNIN IN BUCKWHEAT AND WHEAT PLANTS UNDER SALICYLIC ACID AND CADMIUM IONS

Investigated the effect of cadmium and salicylic acid on phenols content (xanthones, lignin) in plants buckwheat (Fagopyrum esculentum Moench.) and wheat (Triticum aestivum L.). It is established that the action of cadmium ions increases the content of xanthone and produces lignin. To reduce the impact of stress factors it is expedient to use salicylic acid, which normalize the amount of xanthones and lignin in plants of buckwheat and wheat. With this stress regulator, phytohormons can significantly reduce the toxic effects of cadmium ions.

Key words: Fagopyrum esculentum Moench., Triticum aestivum L., cadmium chloride, salicylic acid, lignin, xanthones.

Introduction. Plants organism are extremely sensitive to the state of the environment and actively respond to change. The influence a different anthropogenic factors are seriously damaging for all plants. Plants resistance to stressful factors are controlled by the hormonal system. In recent years attention give hormonal compounds, especially for the induction of plants resistance to different stress factors. For this compounds include salicylic acid. Salicylic acid (SA) is considered as an endogenous plant growth regulator which has been found to generate a wide range of physiological and metabolic responses in plants [10]. The important role of plant protection under the action of stressors in necessary for life restored condition, belongs to the phenolic compounds. Polyphenols – secondary metabolites of the plant organism [6,12], which protect it from oxidative stress. The synthesis of polyphenols is enhanced under stressful conditions [8,13]. It is known that polyphenols counteract oxidative stress: neutralize active forms of oxygen, support the internal environment of cells and have a positive effect for activity of antioxidant enzymes [13].

In the present work, we made an attempt to explore whether external treatment of salicylic acid could mitigate the adverse effect of Cd toxicity on buckwheat and wheat plants and also investigate changes in the phenol contents under these conditions.

Matherials and methods. Seeds of wheat (*Triticum aestivum* L. cv. Podolianka) and buckwheat (*Fagopyrum esculentum* Moench. cv. Rubra) were sterilized and divided into two groups. First group of seeds were soaked in 0.05 μM SA respectively for 5 h, another group was soaked in distilled water (control). Then both groups were allowed to germinate on moist filter paper in the dark. Two-days-old seedlings were transported in pots filled with washed and inciderated sand artificially contaminated with Cd (25 mg/kg substrate). The concentration was chosen by comparing with the literature sources [5,7]. For our research were the plants are grown without Cd ions and SA (control), also plants, wich seed are soked SA, and plants are grown with Cd. The concentration of salicylic acid (0.05 μM) were chosen experimentally, basis of our previous studies. For investigation were used 14-days-old and 21-days-old plants.

Determination of xanthons was based on the determination the main and most common glucoside – mangiferin. For investigation were used spectrofotometric assays. The results of mangiferin was expressed in % of dry weight (DW) [2,3].

Determination of lignin in stem was based on color reaction with floroglucine [4]. For investigation were used optical microscope and program Image Tool. The size was obtained using the formula A=P/0,8, де A-size of aperture (micrometers), P- size of aperture (pixels), 0,8 – the conversion factor. The conversion factor was determined by photographing a ruler and then determined the number of pixels in 1 micrometers.

Results and discussion. Plants are frequently exposed to stress factors, that greatly influence growth, development, survival, crop productivity, and species distribution. Many plants can acquire tolerance in response to this factors. In this article all attention is for secondary metabolites of the plant organism – phenolic compounds, exactly xanthones. Many plants for stressful reaction including changes in physiological and biochemical processes [3]. Information about the change of xanthones contents under influence of heavy metals doesn't find. Known that xanthones as a phenolic compounds and secondary metabolites of plant organism has a protective role under stressful condition and oxidative stress, and their synthesis increase under this conditions [8,12,13]. The most common C-glucoside – mangiferin. Mangiferin was isolated from mango (*Mangifera indica* L.), but known that it is common

in other flower plants. This substance is known for their biological activiti.

Changes of xanthons content are represented in Figure 1. Analyzing the xanthons content of plants under influence of SA and Cd, we can see increase in 14-days-old wheat plants and decrease in shoots 21-days-old wheat plants in all variants, except non-stressed plants (control). (Fig. 1, A). Pre-treatment with 0.05 μM salicylic acid stimulated xanthons accumulation, this is due to the common synthesis of phenolic compounds. Salicylic acid is known as the compound which may play a protective role in stressful conditions. The difference in buckwheat plants in the content of investigated compounds was not detected, possibly due to the high total content of phenolic compounds and insignificant xanthon contents in these plants.We observed increase of this parameters in 21-days-old buckwheat plants with Cd treatment (Fig.1,B), because phenolic compounds are a natural stress metabolite of plants. Also, it can be a variental characteristic.

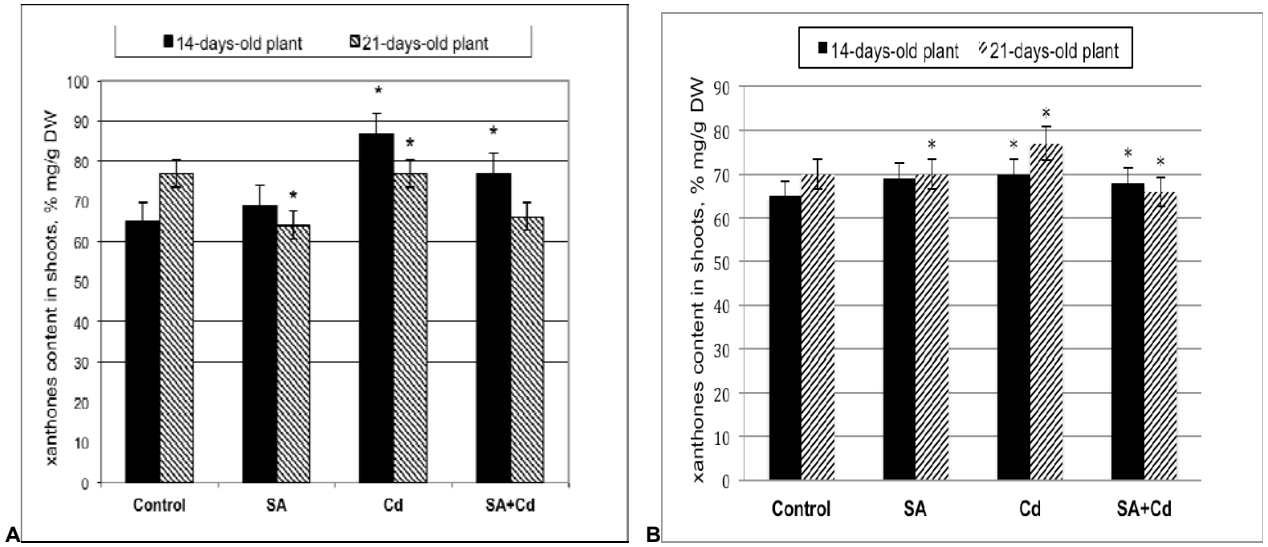

Fig.1. Effect of Cd and salicylic acid treatments on xanthone contents in wheat (A) and buckwheat (B) plants, %

Comments: * – significant at P≤0.05, (M±SD, n=3)

Except to many internal changes (the contents of important metabolites, activity of enzymes etc.), the plant organism can be resistand due to external changes. In recent years, research on the metabolism of lignin and plant resistance hasn't been plentiful.

Cellulose, hemicellulose, and lignin comprise the main composition of cell walls of plants and are important components of natural lignocellulosic materials. Lignin is one of the most abundant organic polymers in plants, just behind cellulose. In the cell wall lignification process, lignin penetrates into the cell walls and fills in their framework, which increases the hardness of the cell wall, enhancing the mechanical and the compressive strengths, promoting the formation of mechanical tissues, and consolidating the plant body and water conduction. Lignin, as a secondary metabolite in plant growth and development, has important biological functions in the growth and development and disease resistance of plants. Lignin metabolism in plants has physiological significance, which was mainly present as the close relationship between changes of its enzyme activity, the increase of intermediate and lignin contents and cell differentiation, the resistance to pathogen infection, and other physiological activities in plant development [4]. Many studies have shown that, when the plant is infected or resistance is induced, the activity of enzymes related to lignin synthesis and the content of lignin would both increase, thereby enhancing the resistance of plants. Lignin biosynthesis can be regulated by changing external factors, such as the action of drought [11]. Heavy metals also influence the formation of lignin. Different plant tissue would have a different lignin content, because lignin biosynthesis regulated by changing the activities of different enzymes [9].

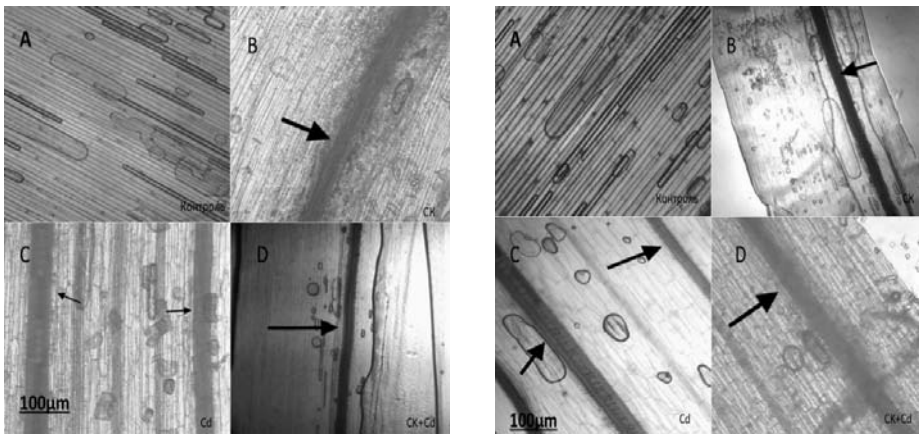

Fig.2. Effect of Cd and salicylic acid treatments on localization and the formation of lignin in shoots 14-days-old and 21-days-old wheat plants (A-Control, B-SA, C-Cd, D-SA+Cd)

In our case, for detection of lignin we chose different parts of plant (leaf, stem, node and internode). All research plants were on the second (14-days-old) or third (21-days-old) leaf. Presents results (photo) the part of stem, close to the node, where it was best seen accumulation of lignin (for 14 and 21 days-old) compared to other parts of the plant. Different parts of plants would have a different lignin content and composition. For example, the lignin content and structure are significantly different in the node and internode of reed (*Arundo donax*); the node has a higher density than the internode because of the high content of phenolic acids (p-coumaric acid and ferulic acid) [1,4,9]. The reasons for this difference are varied. For instance, methyl jasmonate can significantly improve the POD activity of seedlings and the lignin content. Both jasmonic acid and gaseous methyl jasmonic acid could induce the expression of chalcone synthase, thereby increasing the lignin content [9,13]. Our results show differences accumulation in plants, depending on the conditions of growth [13]. Discovered species differences, with a predominance of lignin in plants of buckwheat in comparison with wheat plants (Fig. 2,3). Also considered, that salicylic acid consumed increased formation of lignin, through phenolic composition (Fig. 3).

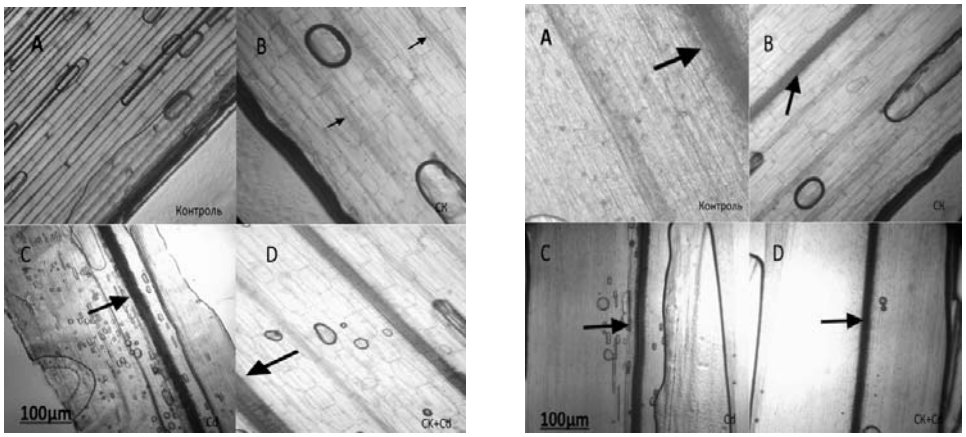

Fig.3. Effect of Cd and salicylic acid treatments on localization and the formation of lignin in shoots 14-days-old and 21-days-old buckwheat plants (A-Control, B-SA, C-Cd, D-SA+Cd)

Summary. Our results confirm stress protectiv role of phenolic compounds by the heavy metals action. We can talk about the involvement of different mechanisms of protection, as external (the process of lignification) and internal (changes in the content of xanthons) for the actions of cadmium chloride. Salicylic acid under these conditions reduced education (accumulation) of xanthons and stimulated lignification in buckwheat and wheat plants. Comparing data on different plants in species composition, more visible changes can be observed in the identification of lignin in plants of buckwheat, in our opinion is associated species and varietal characteristics, a high content of phenolic compounds in this culture. Also, through the high content of phenolic compounds was observed a low level xanthons. The influence of salicylate is the reason that it is a substance of phenolic origin, has a common way of synthesis with phenols, activated in stress conditions and salicylic acid is an active growth regulator in plants.

The use of external treatment salicylic acid in a concentration of 0.05 Mm can serve as an important regulator of the formation of phenolic compounds by the action of stressors, especially in the variant with the combined effect of two factors (SA+Cd), and indirect impact on plants growth and development.

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Я. Кавулич, асп., М. Кобилецька, канд. біол. наук, доц., О. Терек, д-р біол. наук, проф.
Львівський національний університет імені І. Франка, Львів, Україна

ЗМІНА ВМІСТУ КСАНТОНІВ ТА НАГРОМАДЖЕННЯ ЛІГНІНУ В РОСЛИНАХ ГРЕЧКИ ТА ПШЕНИЦІ ЗА ДІЇ САЛІЦИЛОВОЇ КИСЛОТИ ТА ІОНІВ КАДМІЮ

Досліджено сумісний вплив іонів кадмію та саліцилової кислоти на вміст поліфенолів у рослинах гречки (Fagopyrum esculentum Moench.) і пшениці (Triticum aestivum L.). Встановлено, що за дії іонів кадмію зростає вміст ксантонів та посилено утворюється лігнін. Для зниження впливу стресового чинника доцільно використовувати саліцилову кислоту, яка нормалізує нагромадження фенольних сполук – вміст ксантонів та лігніфікацію у рослин гречки та пшениці. За допомогою цього регулятора росту можна певною мірою зменшити токсичний вплив іонів кадмію.

Ключові слова: Fagopyrum esculentum Moench., Triticum aestivum L., кадмію хлорид, саліцилова кислота, лігнін, ксантони.

Я. Кавулич, асп., М. Кобылецкая, канд. биол. наук, О. Терек, д-р биол. наук
Львовский национальный университет имени Ивана Франко, Львов, Україна

ИЗМЕНЕНИЕ СОДЕРЖАНИЯ КСАНТОНОВ И НАКОПЛЕНИЕ ЛИГНИНА В РАСТЕНИЯХ ГРЕЧИХИ И ПШЕНИЦЫ ПРИ ДЕЙСТВИИ САЛИЦИЛОВОЙ КИСЛОТЫ И ИОНОВ КАДМИЯ

Исследовано совместное влияние ионов кадмия и салициловой кислоты на содержание фенолов (ксантонів, лигнина) в растениях гречихи (Fagopyrum esculentum Moench.) и пшеницы (Triticum aestivum L.). Установлено, что за действия ионов кадмия возрастает содержание ксантонов и усиленно образуется лигнин. Для снижения влияния стрессового фактора целесообразно использовать салициловую кислоту, которая нормализует содержание и снижает содержание ксантонов и лигнификацию в растениях гречихи и пшеницы. С помощью этого регулятора роста можно значительно уменьшить токсическое влияние ионов кадмия.

Ключевые слова: Fagopyrum esculentum Moench., Triticum aestivum L., кадмия хлорид, салициловая кислота, лигнин, ксантоны.

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A. Markova, PhD stud., V.Serebryakov, SDc.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

DIFFERENCES IN AGGRESSIVE BEHAVIOR OF RELATED SPECIES OF FLYCATCHERS (MUSCICAPIDAE) FAMILY

The article is devoted to the observation of differences in relation between aggressive behavior of related species of Flycatchers (Muscicapidae) family and behavior acts in naturally watering places. Observations have reviled the timing separation between presence and engagement of Flycatchers in morning hours and relation with acts of aggression. Each representative of Flycatchers family is using the watering place in different ways. The correlation between intraspecific and interspecific contacts with the predominance of interspecific and highly aggressive interactions has been analyzed. The rating of successfulness of the acts of aggression has been established for every particular group of Flycatchers. It demonstrates the energetic justification of aggressive behavior for spotter, red-breasted and pied flycatchers but energetic overspend and failure for collared flycatcher.

Keywords: behavior, aggression, Muscicapidae, watering place.

Introduction. Over the last decade, interest to the flycatcher's ecology significantly increased due to their synanthropic opportunities, habitat area expansion and population increasing in the southern Europe, as well as their relationships with the other species. For the social bird's interaction study, flycatchers are ideal species because some knowledge regarding the use of non-specific and cone-specific information in their choice of nesting place has already been partially disclosed. Besides these species are flexibly used an intraspecific and especially interspecific social information (for example, neighborhood with the great and the blue tits) [26].

Numerous publications of European authors, the question of the aggression's reasons and consequences among the animals are actively appeared. Especially essential attention is spared to behavior's differences investigation on different territories, its significance for biology, ecology and social relationships closed and competitive species. Much attention is paid to aggression study in intraspecific competition [14], but its significance and consequence in interspecific competition or other relationships of the closed species are the newer and not enough learnt issue so far [27, 36], but its mechanisms and consequences are still not clear.

Interspecific competition is an important factor which regulates niche overlapping in the resources use by the closed spices and relative density of the bird's population [38, 33]. Under natural conditions specimens of many spices are involved to this competition that certainly increases competition level and aggressive behavior as one of expression of competition [29].

Interspecific aggression has also an important consequence for ecological processes and provides with answers about the reasons of evolutional strategies behavior change. For today, there are still exist difficulties in understanding how exactly the behavior will influence on the structure, functions and stability of the ecosystem, interaction difficulties which exist between species and environment. Information exchange between specimens of the other species in relation to resources is extremely important and its mechanism may have impact to consistent patterns and consequences of species coexistence [21].

For forest and steppe zones of Ukraine breeding is spotted flycatcher (Muscicapa striata) and pied flycatcher (Ficedula hytoleuca) which are under protection of Bonn and Berne conventions and coralled flycatcher (Ficedula albicollis) and red-breasted flycatcher (Ficedula parva)

which have no environmental protection status but the number of which gradually decreased for the last years [11]. That's why flycatcher's ecological peculiarity and behavior study in Ukraine is extremely relevant which may help to keep them in the original habitats.

The purpose of this work is the investigation and interspecific (further InterS) ratios comparison and intraspecific (further IntraS) aggression for the four species of birds Muscicapidae family in the breading season at the natural watering place. The main objectives are:

- to detect the presence and significance of aggression with physiological needs which were determined according to the results of acts at the watering place and the presence and number of other species and their aggressiveness as a direct factor of aggression among flycatchers;
- to establish the justification of energy costs on aggressive behavior expression.

Methods and material. As materials served the data collected by the author in Kanev Nature Reserve (further KNR) of Cherkassy region in May and June 2010, 2012 and 2014 at the watering place in Mokry ravine in the household territory. Investigation territory has environmental protection status and it is characterized by the low anthropogenic influence. The total duration of observation in KNR is 324 hours. During this time about 1 324 interspecific and intraspecific contacts were fixed and 1 940 flycatchers' appearances were analyzed at the watering place.

Observations were conducted from 5.00 till 21.00 (hereinafter hours are corrected to daylight saving time). Light day period is conventionally divided into three periods: morning (from 5.00 till 12.00), day (from 12.00 till 17.00) and evening (from 17.00 till 21.00). Studying the dynamics of birds' activity at the watering places according to commonly applied ethological practices [1], possible variants of birds' behavior acts at the watering places were separated: water drinking, food consumption, bathing and cleaning feathers. Commonly applied ethological methods of "total observation" and "total record" [12] with author's modifications for birds observation in nature were applied to study interspecific behavior. Behavior act when two birds reduced the distance between them, obviously changing their behavior compared to previous period of time, demonstrating readiness to attack and fight is considered to be an act of aggressive behavior [9].

Among the data, related to aggressive reaction, following information was recorded:

- which of species/specimen was the first who arrived the study area and which was the second one
- which of species/specimen showed initiative to aggression
- which of species/specimen won, i.e. stayed at the place of observation.

We counted the cases of winning the fight for resources having reached the place as the first one and cases of winning after having reached the place as a second one. Based on these data the distribution of specie's success rating in interspecific aggressive contacts was obtained.

Only the data collected taking into account same weather conditions is used for analysis. Statistical processing of the data is made applying mathematical methods using software Microsoft Excel and STATISTICA 7.0. The data is checked for normality, and, accordingly, correlation ratios were defined using parametric and nonparametric methods.

Study was performed as stage of research topic of Department of Zoology, NSC "Institute of Biology," Taras Shevchenko National University of Kyiv "Saving of the biodiversity and comprehensive study of adaptation strategies of phyto-, zoo- and virobioty of Ukraine using bio informational technologies, topic number 11BF036-02.

Results and discussion.

Dynamics of birds' engagement in behavioral acts at watering places. According to the type of food flycatchers are insectivorous birds [2, 5, 6, 7] and therefore their need for drinking is not so significant as the need of crops eating birds. So the main purpose of staying at watering place is cleaning feathers and bathing, although drinking and search for food still is a large share of behavioral acts at watering place. We identified maximum need of flycatchers for replenishment water balance in the body, bathing and search for food in different areas (Table 1).

***Table* 1. Peaks of engagement in behavioral acts at watering place during the day**

Peaks	*Muscicapa striata*			*Ficedula albicollis*			*Ficedula hypoleuca*			*Ficedula parva*		
	M	D	E	M	D	E	M	D	E	M	D	E
presence	6	14	20	8	12	18	6-7	12, 14	19	8	12	-
bathing	5, 9	13, 15	17, 20	8	13	19	6	14-16	-	8	12	-
drinking	6	12	20	8	11	21	6	12	20	8	16	20
search for food	6	11	17	8	-	21	7, 9	-	18	6	16	20

Note: "M" – morning, "D" – day, "E" – evening, "-" – no visible peak

It was found out that biological needs of flycatchers at watering place are slightly separated in time. Especially important separation was noticed in morning hours. Maximum activity arrival of collared and red-breasted flycatchers was noticed at $8^{.00,}$ and of pied and spotted flycatcher – at 6.00. It should be noted that that the place of research was selected given the lack of other sources of water. So the results about the separation of close species in time indicate ecological behavioral adaptation to limited resources. Besides, it indicated the choice of individual behavioral adaptation type. Collared and pied flycatchers have very similar ration and linear dimensions, but behavioral strategy of collared flycatcher is similar to behavior of spotted flycatcher, the number of which is large enough in the national park. In return, the behavior of pied flycatcher is similar to the behavior of red-breasted flycatcher. However, this trend may be wrong because the number pied flycatcher in recent years has decreased dramatically, and the species is not considered to be nesting in the area anymore.

Aggression and behavioral acts. Through the analysis of quantitative acts of aggressive behavior, the indexes of different behavior acts at the watering place for each species were defined (Table 2). Generally, the correlation between the appearance of individuals of their own species and aggressive reaction was noticed for all flycatchers. For collared and red-breasted flycatcher this correlation is average, and for pied and spotted flycatcher this correlation is low. Only for spotted flycatcher low correlation of aggressive behavior and overall increase of birds number and average correlation to increasing aggression of all birds at watering place was noticed. Besides, the watering place has its strategic importance for each species. Ration od the species is very similar during the nesting period and if the resource available is sufficient the birds do not compete [5]. But the watering place is one of the areas of food searching for the collared flycatcher, so due to the limited water resource nearby its competitive importance significantly increases. Banding in the ravine facilitates, to some extent,

the search of flying insects because large specimens of butterflies and bees have often been meshed in the net for banding. Therefore, the correlation between the aggression and food searching at the watering place is high (r=0.842; p<0.01), and the correlation between the aggression and drinking is lower, but still considerable. The watering place has more important role as a place of cleaning feather for the spotted flycatcher and as a source of after for the red-breasted flycatcher. The data for the pied flycatcher is still insufficient for specific conclusions.

***Table* 2. Relation between the aggression, behavioral acts and other birds on watering places**

	Muscicapa striata	*Ficedula albicollis*	*Ficedula hypoleuca*	*Ficedula parva*
bathing	0.348*	-0.135	0.193	0.449*
drinking	-0.028	0.558*	0.229	0.436*
searching for food	0.115	0.842**	-0.111	-0.201
their kind	0.387*	0.489*	0.307*	0.544*
all birds	0.339*	-0.041	0.036	0.077
general aggression of birds	0.425*	-0.011	-0.032	0.217

Note: * p<0.05, ** p<0.01

Detailed research of the aggression of flycatchers with the presence of massive kinds of KNR on a watering place revealed a weak inverse correlation between the aggressiveness of the collared flycatcher and the number of the great tit, and the number and the aggressiveness of the blackcap. Instead, the connection between the aggressiveness of spotted flycatcher and the number and the aggressiveness of the great tit is direct and weak, but significant with the common chaffinch (with the quantity r=0.515; p<0.05 and the aggression r=0.504; p<0.05). The red-breasted flycatcher actively reacts on the aggressive behavior of the Eurasian blue tit (r=0.703; p<0.01), but weakly reacts on its number (r=0.413; p<0.05). An insignificant weak correlation is noticed between the pied flycatcher the aggressiveness of the robin, the great tit and the marsh tit.

In general, in recent years to determine the interconnection between the most explored types of the behavior the strength of correlations of different possible combinations of features was estimated by meta-analysis. Thus, based on data from 81 scientific works, the researchers found that the correlations between the behavior in general are weak and quite varied because of the variation of the comparable characteristics. The presence of partial correlation between features indicates that certain connections do not depend on covariance with other features, while some connections (in particular aggression or exploration of new territory) successively decrease after the controlling of covariance. At the same time, the magnitude of effects (eg. correlation) is systematically higher when behavior is analyzed under the same experimental conditions. Differences in correlations arise not because of differences in recurrence, which are related to the measuring of different features, and most often assessed behavioral features do not necessarily form the same independent intervals (domains). Overall, between any behavioral acts there is a positive correlation of medium strength.

Data interpretation by such methods indicates that the recurrence research of certain behavioral acts is not statistically different, and their value influences more on correlations in the species-specific behavior than in the individual one [22]. Thus, the obtained data can be used to establish the behavioral plasticity of representatives of the Flycatchers.

Distribution of interspecific and intraspecific aggression. Interspecific relationships in a particular grouping of birds are closely related to intraspecific aggression of existing together species [3]. Usually, the number of interspecific contacts is considerably greater than intraspecific [4, 8, 10, 13]. We recorded species, to which flycatchers revealed the reaction of aggression for the whole time of observations (Table. 3). Thus, for the collared flycatcher among 19 species, that were encountered, there was an aggressive reaction to 9. The spotted flycatcher expressed an aggression to 7 out of 18 species, the pied flycatcher – to 2 out of 7, and the read-breasted flycatcher – to 1 out of 10. All flycatchers more frequently showed the reaction of aggression upon arrival at the watering place later than the object of attack.

***Table* 3. Species that are marked by the presence of aggressive behavior during interaction of species**

Species	Muscicapa striata	Ficedula albicollis	Ficedula hypoleuca	Ficedula parva
Dendrocopos medius	–			
Dendrocopos minor	–			–
Hippolais icterina		+	–	–
Sylvia atricapilla	+	–	–	–
Phylloscopus collybita	–	–		–
Phylloscopus sibilatrix		+		
Muscicapa striata	+	–		
Ficedula parva	–	–	–	–
Ficedula hypoleuca	–	+	–	–
Ficedula albicollis	–	+		
Erithacus rubecula	+	–		
Turdus merula	–	–		
Turdus philomelos	+	–		
Parus caeruleus	+	–		–
Parus palustris	–	+		
Parus major	+	+	+	–
Sitta europaea	–	–		
Certhia familiaris	–	+	–	
Fringilla coelebs	+	+	+	+
Chloris chloris				–
Carduelis carduelis		–		
Coccothrausres coccothraustes	–	+		–

Note**: "**+" – the reaction of aggression is present; "–" – the reaction of aggression is absent; " " – there was no encounter.

It is known that the amount of interspecific conflicts (aggressive contacts) in mixed populations is comparable to or greater than the frequency of intraspecific aggressive contacts [3]. These data are known for waterbirds, but this pattern was also confirmed for flycatchers (Table 4). It was established, that all four species of flycatchers pay a large share of attention to the interspecific aggressive interactions, but the percentage of intraspecific aggression of the collared flycatcher is still higher than of the spotted one. The total share of interspecific contacts is bigger than intraspecific, that is associated to the high competition. This is especially expressed for small flycatchers. In turn, this confirms recent researches on active and flexible use of interspecies information and social relations of close and competitive species by flycatchers [26].

***Table* 4. Percentage of behavioral reaction to all species' contacts**

	Muscicapa striata		*Ficedula albicollis*		*Ficedula hypoleuca*		*Ficedula parva*	
	InterS	IntraS	InterS	IntraS	InterS	IntraS	InterS	IntraS
Total number of contacts	84.29	15.71	75.39	24.61	69.39	30.61	91.67	8.33
Aggressive contacts	9.32	13.64	8.33	23.40	14.71	0	13.64	0
Non-aggressive contacts	90.68	86.36	91.67	76.60	85.29	100	86.36	100
Share or all aggressive contacts	78.57	21.43	52.17	47.83	100	0	100	0

Consequences of interspecific interaction, namely using the information, are asymmetric and they are used for interspecific coexistence modelling. Potential competitors are important component of efficient area use. Sometimes the presence of potential competitors even attracts birds [30, 31]. The more interspecific niches are overlapped, which is a case when the resource is limited, the better opportunities for using interspecific information appear, and strong competitors, for ensuring more accurate information [32, 35].

Importance of certain territory for territory species may depend not only on environment characteristics, but also on social structure of the area. Although interspecific competition can be asymmetric, as a rule it results in costs for all parties [19].

Protection of the territory is an energetically costly process [20], so keeping the information about territorial competitors and stable relationship is an additional advantage for the most part of birds. Birds often respond less aggressively to territorial specimen whom they often tolerate on their territory, than to distant neighbors or migratory birds [15, 16, 34, 37]. If the neighbors are less dangerous for secured recourses (food, individuals of their species, breeding), the respond is less aggressive in order to use as less energy as possible for accumulation of territorial competitiveness [19].

In our research the rating of pair aggressive contacts. It has no dimension but reflects the percentage of success (+) or loss (-) such interaction. Final calculation of results of flycatchers' pair collisions with each type separately indicates the competitive position of the flycatcher at some specific area (tab. 5). A sum of defense and attack indicates the overall justification of energy cost used for the competition and protecting the territory in a particular grouping of birds.

***Table* 5. Rating of aggressive contacts of Flycatchers**

	Muscicapa striata	**Ficedula albicollis**	**Ficedula hypoleuca**	**Ficedula parva**
Attack	-1.63	-3.22	0.50	1
Defense	2.63	-0.78	2.50	1
∑	1	-4	3	2

In the natural environment the collared and the spotted flycatchers typically have a low protection rating of an area, when they faced with a large number of other types of birds. This is due to the constant availability of the required resources. The same trend is noted for the collared flycatcher in case of an attack. Conversely, the spotted flycatcher is quite successful during attacks in pair interactions. However, for the pied and the red-breasted flycatchers, spending energy on aggression and attack protection is justified. As a result, there is rating of justification for energy costs of competitive aggression from the most depleted species of flycatchers – the collared flycatcher – to the most successful species – the red-breasted flycatcher. In summary, we note that flycatchers win the attack and lose protection, especially in contacts with the common chaffinch, the great tit and the blackbird.

This investigation reveals some possible scenarios of aggressive behavior on the ground watering to natural areas. Differences in flycatchers' participation in social interactions become more important in explaining adaptive individual differences in the behavior of animals and probably are part of the evolutionary process [17, 19]. Similar researches were performed in different areas and at different times on the example of European marmots. [25] Recent data regarding aggression among the greylag goose [39] also found that dominant behavior may depend not only on internal factors but also on the season and social environment. In addition, the best choice to achieve or maintain a high ranking domination can vary significantly between the stages of the life cycle. This highlights the importance of long-term research and multivariate approaches for understanding the complexity of the relations of domination for animals.

Overall, there is an individual right behavioral response of a group of individuals to external signs and the type of behavior of its species. For ethological studies such signs serve as a key to understanding behavioral ecology and quantitative genetics. Interaction between individuals (social conditions) is a major factor in changing behavioral variations at different levels of the hierarchy [18]. Social interactions lead to a restructuring of complex behaviors and tend to occur at the level of the group. This mechanism of behavioral change strategies has unknown evolutionary consequences, justifying its study.

Conclusions:

1. Detected distribution in time of occurrence flycatchers at the watering place during the day. A special feature is the morning dynamics of species: the collared and the red-breasted flycatchers actively visit the watering place at 8 am, and the pied and the spotted flycatchers – at 6 o'clock.

2. The spotted flycatcher's aggression correlate with the general level of birds' aggression and for the collared, the red-breasted and the pied flycatchers correlation is only available with the advent of their species in the natural environment and of the need for resources.

3. Have been revealed that in the ratio of interspecific and intraspecific contacts prevail aggressive interspecific

interactions, indicating the importance of establishing an interspecific hierarchy for flycatchers and active use of interspecific information.

4. Aggressive interactions for the collared flycatcher are the most debilitating and energy unjustified. Most energetically justified is the interaction of the red-breasted and the pied flycatchers in the reactions of protection and attack on the watering place. The spotted flycatcher occupies an intermediate position in the ranking of success at the natural watering places in Kanev Nature Reserve.

5. Obtained data in respect of the distribution of the flycatchers' aggressive behavior complement the already known knowledge and point to the diversity of behavioral strategies of birds of one family. Basic mechanisms and causes of differences have still needed further investigation.

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А. Маркова, асп., В. Серебряков, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ВІДМІННОСТІ У ПРОЯВІ АГРЕСІЇ БЛИЗЬКИМИ ВИДАМИ РОДИНИ МУХОЛОВОК (*MUSCICAPIDAE*)

Робота присвячена вивченню відмінностей зв'язку агресивної поведінки близьких видів родини Мухоловкові (Muscicapidae) із поведінковими актами на водопої у природі. Виявлено розподіл у часі присутності та зайнятості мухоловок у ранкові години та зв'язок із проявом агресії. Кожен із представників мухоловок використовує водопій по-різному. Розглянуто співвідношення міжвидових та внутрішньовидових контактів, серед яких переважають міжвидові, особливо агресивні, взаємодії. Встановлено рейтинг успішності прояву агресії мухоловками у конкретному угрупованні. Він вказує на енергетичну виправданість агресивної поведінки для сірої, малої та строкатої мухоловки, але максимальну затратність та програш для мухоловки білошиї.

Ключові слова: поведінка, агресія, Muscicapidae, водопій.

А. Маркова,асп., В.Серебряков, д-р. биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, украина

ОТЛИЧИЯ В ПРОЯВЛЕНИИ АГРЕССИИ БЛИЗКИМИ ВИДАМИ СЕМЕЙСТВА МУХОЛОВКОВЫЕ *(MUSCICAPIDAE)*

Работа посвящена изучению отличий между связью агрессивного поведения близких видов семейства Мухоловковых (Muscicapidae) и поведенческими актами на водопое в природе. Выявлено разделение во времени присутствия и занятости мухоловок в утренние часы и связь с проявлением агрессии. Каждый из представителей мухоловок использует водопой по-разному. Рассмотрены соотношения межвидовых и внутривидовых контактов, среди которых преобладают межвидовые, особенно агрессивные, взаимодействия. Установлен рейтинг успешности проявления агрессии мухоловками в конкретной среде обитания. Он указывает на энергетическую оправданность агрессивного поведения для серой, малой и пестрой мухоловки, но максимальную затратность и проигрыш для мухоловки-белошейки.

Ключевые слова: поведение, агрессия, Muscicapidae, водопой.

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P. Boltovets, PhD.
Institute of Semiconductor Physics NAS of Ukraine, Kyiv, Ukraine,
R. Radutny, ing.
Scientific and Technical Center of advanced technologies NAS of Ukraine, Kyiv, Ukraine,
V. Petlyovana, PhD., T. Shevchenko, PhD.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

INTERACTION BETWEEN PHAGES AND BACTERIA AS A TOOL FOR THE OBTAINING OF IMAGES

The obtaining of images by lytic action of bacteriophage T4 on the Escherichia coli bacterial lawn is considered. Methodical aspects of the approach are discussed, namely, use of different stencil types, total and partial staining of obtained image by different dyes. The perspectives of the practical use are proposed namely restriction of the action of microorganisms in out-of-the-way places etc.

Key words: bacteriophage T4, Escherichia coli, lytic action, lytic zone.

Introduction. Creation of images by controllable culturing of microorganisms in certain patterns (microbial art) now became a very special branch of skill at the interface between science and art [2]. In this case agar plates can be considered as a background, while pigmented or fluorescent bacteria or yeasts represent the paint. In the first case it can be performed by the application of the microorganisms with the intensively colored colonies. Another approach based on the using transgenic bacteria expressing fluorescent protein genes [3]. In this case images can be visible in ultraviolet light.

Images as well can be obtained by the growth of microscopic green alga on the nutrient medium [4]. In this case exposing different areas of the algal lawn to light over varying intervals of time changes their colors along the green spectrum.

In all these approaches the stiff nutrient medium (agar) is used as a background, where the image can be formed as the result of the growth of the bacterial colonies as well as by the change of the color of the medium or by the combination of these factors. In both cases bacteria appear as a tool.

However the authors did not found any scientific publication, where bacterial lawn itself was used as the as a background and the image was formed by the lytic action of the virus (bacteriophage). Whereas the mentioned approach could be used not only with artistic aim but for the practical use.

The aim of this work was to demonstrate a possibility to obtain the image on the bacterial lawn by the lytic action of the bacteriophage on the example of the bacteriophage T4 and *Escherichia coli*.

Materials and methods. For the obtaining of the bacterial lawn by the standard metod [1] the 1,5% agar with the nutrient medium was disposed to the Petri dishes. After the congelation of the solid medium its' surface was coated by the 0,7% agar containing *Escherichia coli* culture (concentration about 10^9 cells per ml).

For the introduction of the virus on the bacterial lawn the use of the micropipette and the application of the preliminarily autoclaved stencil from printing or filter paper were combined.

After the chilling of the substrat the stencils were applied and the preparation of the bacteriophage T4 (concentration 10^8 PFU (plaque-forming unit) per ml was introduced at the stencils by micropipette. Samples obtained by such a way were incubated during the twenty-four hours at +37°C.

The scheme of the experiment is shown on the Fig.1.

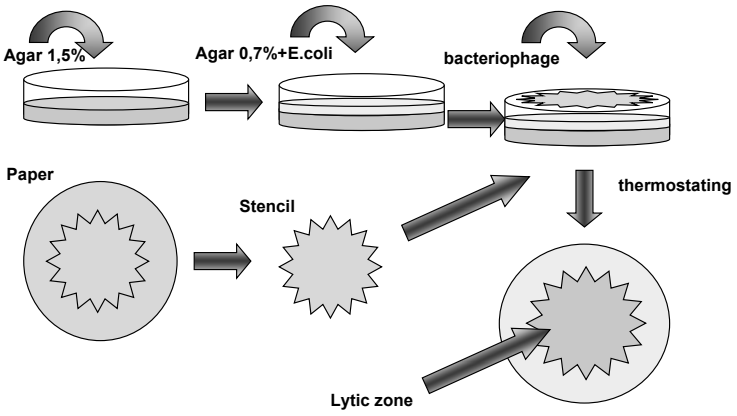

Fig.1.The scheme of the experiment

After the incubation the stencils were removed and the samples were stained by *Coomassie blue R-250 (Coomassie blue 100mg, methanol 50 ml, acetic acid 10 ml, H_2O 40ml or fuchsine (1 ml of concentrated solution – 9g of fuchsine in 100 ml of ethanol – diluted in 10 ml of H_2O) with further fixation by the 7% acetic acid.*

Results and discussion. For the obtaining of the image by the lytic action of the bacteriophage several approaches were applied. At the first series of the experiment stencils made from printing paper and filter paper were compared. It was demonstrated, that generally the use of filter paper stensil (Fig.2a) allows one to obtain more accurate and controllable image, than the use of the printing paper stensil (Fig.2b). This result can be explained by the porous structure of the filter paper which provides more regular and controllable penetration of the bacteriophage to the stencil. As the result more uniform distribution of the bacteriophage to the bacterial lawn can be achieved in

comparison with the print paper stencil. The last one can not absorb virus containing liquid effectively and therefore can not then distribute it regularly to the bacterial lawn causing the forming of wide area of lytic zones. Also It was shown, that the wetting of the whole stencil in the phage preparation instead of the using the micropipette for the application of the virus containing liquid leads to the total lysis of the bacterial lawn (data not shown).

a b

Fig.2. Unstained images, obtained by the lytic action of the bacteriophage T4 on the *Escherichia coli:*
a – using filter paper stencil, b – using printing paper stencil

In the second series of the experiment the possibility of the use of the reversed stencil (where the image is formed not by the lytic zone but by the zone of bacterial growth) was demonstrated (Fig.3) However this approach is more restricted because of the fact, that in this case the zone of lysis is more wide and thereof less controllable. Besides, this approach is more labor-consuming.

Fig.3. Image obtained by the use of the reversed stencil (stained by fuscine)

Also the possibility of the partial staining of the obtained image (namely, only zone of lysis) was explored (Fig.4). It gives an opportunity to obtain polychrome images using available colorants (for example, *Coomassie blue, fuchsine)*.

Fig.4. Partially stained by *Coomassie blue* image

Summarizing the above it should be noted, that it was the firs time when the graphical image was obtained by the lytic action of the virus on bacteria. This approach could be used not only for the artistic aims but as well for the practical use, for example, for the restriction of the action of microorganisms in out-of-the-way places.

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4. http://distractify.com/old-school/2014/08/08/lia-giraud-algaegraphs-1197777237

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П. Болтовець, канд. біол. наук
Інститут фізики та напівпровідників НАН України, Київ, Україна,
Р. Ратудний, інженер
Науково-технічний центр передових технологій НАН України, Київ, Україна,
В. Петльована, канд. біол. наук, Т. Шевченко, канд. біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ВЗАЄМОЗВ'ЯЗОК ФАГІВ ТА БАКТЕРІЙ ЯК ІНСТРУМЕНТ ОТРИМАННЯ ЗОБРАЖЕНЬ

Розглянуто отримання зображень шляхом літичної дії бактеріофага T4 на бактеріальний газон Escherichia coii. Обговорюються методичні аспекти підходу, зокрема, використання шаблонів різних типів, повне та часткове фарбування отриманого зображення різними барвниками. Запропоновано можливі перспективи використання, зокрема, обмеження дії мікроорганізмів у важкодоступних місцях.

Ключові слова: бактеріофаг, Escherichia coii, літична дія, літична зона.

П. Болтовец, канд. биол. наук
Институт физики та полупроводников НАН Украины, Киев, Украина,
Р. Ратудний, инженер
Научно-технический центр передовых технологий НАН Украины, Киев, Украина,
В. Петлевана, канд. биол. наук, Т. Шевченко, канд. биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

ВЗАИМОСВЯЗЬ ФАГОВ И БАКТЕРИЙ КАК ИНСТРУМЕНТ ПОЛУЧЕНИЯ ИЗОБРАЖЕНИЙ

Рассмотрено получение изображений путем литического действия бактериофага T4 на бактериальный газон Escherichia coii. Обсуждаются методические аспекты похода, в частности, использование шаблонов разных типов, полное и частичное окрашивание полученного изображения различными красителями. Предложены возможные перспективы использования, в частности, ограничение действия микроорганизмов в труднодоступних местах.

Ключевые слова: бактериофаг, Escherichia coii, литическое действие, литическая зона.

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A. Kosyan, PhD, O. Smirnov, PhD, N.Taran, DSc.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,
V. Burdyga, PhD
State Agrarian and Engineering University in Podilya, Kamianets-Podilsky, Ukraine

TEST SYSTEM BASED ON ROOT EXUDATES FOR HIGH-YIELDING COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH.) FORM SCREENING

A new effective non-invasive method of screening of highly productive forms of buckwheat sowing (Fagopyrum esculentum Moench.) based on rapid testing of buckwheat seedling intensity exudation of organic acids root system in the laboratory is offered. Buckwheat seeds were germinated on agar gel layer which contains in its composition acid-base indicator followed by visual assessment of the indicator color changes around primary root and plants with the largest area of color change were selected. The effectiveness of the method was confirmed in the field conditions by phenotyping of plants and significant differences in determining the structure and yield performance of selected plants were found. Statistical analysis of indicators grain number and grain weight showed that these indicators in selected plants were over 6 times higher than in the control variant with the degree of reliability of 99%.

Keywords: screening, buckwheat, phenotyping, seedlings, root exudates.

Introduction. Buckwheat is a well known valuable agricultural cereal culture used traditionally in the food industry of Ukraine. However, due to the peculiarities of the secondary metabolism this culture can be widely used in the pharmaceutical and medical industries as a source of a significant amount of bioactive substances [1]. But buckwheat may not have the performance as cereal crops because of their biological characteristics. At the same time the potential of buckwheat are not always used sufficiently. Development of new methods of selection can greatly affect the disclosure of genetic potential of the crop and expand its use. Taking into account the substantial value of buckwheat in the food industry the search for effective and rapid methods for the selection of high-performance forms of culture do not stop. Development of new methods requires complex analysis and improvement and modification of previous methods of selection.

Most known methods of selection of high-performance forms of buckwheat is selection at the stage of budding or flowering plants [2, 3, 4]. They are held on the last phenological phases of plant development and aimed at creating productive large inflorescences. A method of selecting plants for buckwheat complex features [5] is that plants are selected for buckwheat sign of breeding and structure characteristics at the crop ripening phase. Plants selected are propagated *via* meristem culture *in vitro*, encouraging re-blooming. Seeds from regenerative plants are got by directed re-pollination with known genotypes. The essence

of the method is that buckwheat plants selected visually at the phase of ripening. From the plants selected isolate axillaries buds that are sterilized in aseptic conditions laminar box, separate axillaries meristems, inoculate in the culture medium, induce morphogenesis of shoots and the formation of roots and get plants rooted *in vitro*. The resulting plants are planted in containers with unsterile soil in greenhouses and after acclimatization get plants that are genetically identical to the selected ones; after flowering plants pollinate with known genotypes to produce seeds that are subcultured for breeding in subsequent generations. The disadvantage of this method is the having of multiple stages and using tissue culture method, which in turn makes the method complicated in execution and rather expensive.

The above mentioned methods of selection forms highly focused on functional signs of aerial parts, which are fixed at the later phases of phenological development. But root system of plants that performs multiple functions is neglected thereat, although we know that it plays an important role in the life of plants and formation of productive capacity. The surface of the root system is in 50-150 times greater than the surface of the aerial part. In addition to the mechanical functions the roots serve important physiological processes: transport (providing transportation of substances in above ground organs), absorption of water and mineral elements from the soil) and the excretion of various chemical nature and the biological value substances to the environment. Their role in the formation of production capacity is investigated not enough. This is especially true for such unique culture as buckwheat characterized by specific peculiarities of the root system.

The prototype developed method of selection of high-performance forms are selection based on visual assessment of buckwheat seedling root system at the early stages of development [6]. According whose separate sprouted seeds planted in a test tube with nutrient medium and grown until reaching the length of the primary root of majority of seedlings over 10 cm. Specimens of the plants with a maximum length of root, taken into account the intensity of lateral roots are selected. The disadvantage of this method is the need to ensure each plant separate tubes with nutrient medium, which in turn makes the method quite cumbersome in performing. In addition, despite the fact that the selection is made in the early stages of plant roots to reach a certain length required for some time that something slow down the selection process.

The purpose of our work was to develop a new non-invasive method of screening for high-performance forms buckwheat functional characteristics of the root system. The main criteria were: the search of express-marker for selection; reducing significantly the time of selection; the possibility of increasing the samples to handle an increasing number of plants for breeding practices.

Material and methods. Buckwheat seeds of Rubra variety within 3 hours were soaked in distilled water, then transferred to a Petri dish on wet filter paper and placed in a thermostat with a temperature of 27°C for germination. For visual control of root secretions acid-base indicator color with the transition in the pH range of 3 to 6.8 was used. Sprouted seeds planted at the bottom of a flat cell on a layer of 1% agar-agar gel thickness ~ 3 mm, containing 5 mg/100 ml of bromocresol green (variant 1) or bromocresol purple (Variant 2) so that the root was immersed in the thick gel and kept in an incubator at 27°C without light. After a visual assessment of root exudate intensity two versions with 10 plants in each were collected: low intensity root secretions – control; high intensity of root exudate – experiment. The selected plants were planted in the field and grown at conventional farming methods of cultivation of buckwheat [7]. Phenotyping of plants in the field was performed according to the recommendations of V.O.Yeshchenko [8]. Statistical analysis of data was performed by analysis of variance according to Fisher [9].

Results and discussion.

The root system of buckwheat has highly synthetic and excretory activity – root exudates buckwheat, namely organic acids, dissolve remote forms of mineral nutrients, resulting in a relatively small mass of roots characterized by a high intensity of nutrient absorption. So the average absorption of minerals by buckwheat is 38.8 mg/g of root, while millet – 22 mg/g, spring wheat – 14.5 mg/g barley – 7 mg/g and winter wheat – 4,9 mg/g [10]. It is clear that buckwheat plants that have a high level of root secretions can provide themselves more fully with mineral nutrients and consequently have more favorable conditions for the disclosure of its productive capacity.

Our preliminary research the quality of the root exudates showed extensive excretion of oxalic acid by buckwheat plant root system [11]. To visualize the intensity of root secretions model system was developed based on the ability of chemical dyes change color depending on the pH. In a culture *in vitro* on the medium with the addition of acid-base indicators sprouted buckwheat seeds begin to form the primary root, which provides oxalic acid in the root environment changing gel color (Fig. 1).

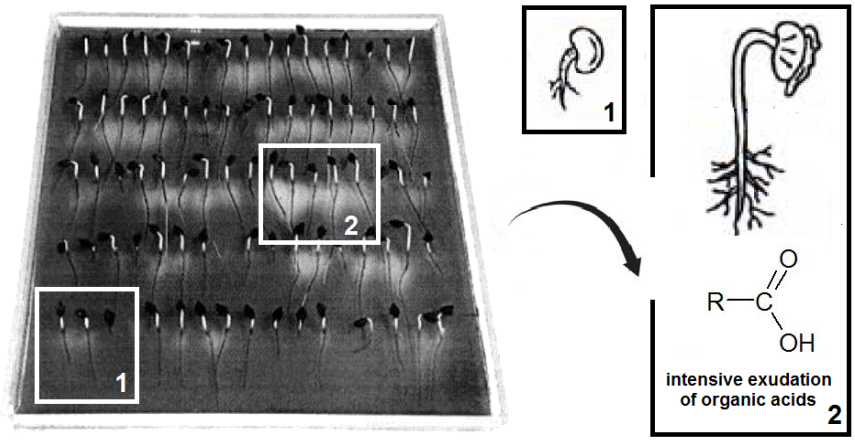

Fig. 1. Intensity of root exudates of buckwheat sowing plants (*Fagopyrum esculentum* Moench.):
1 – low intensity excretion root exudates; 2 – high intensity excretion root exudates

The intensity of color change was assessed excretion activity of organic acids not evaluating them biochemically (quantitative), that reduces significantly the selection term. In the first phase seedlings were divided into two groups according to the color change intensity for further field studies: with low intensity root secretions – the control group, and with high intensity-experiment.

In the second phase, during field research observations of the plants was observed that plants grown from seeds of buckwheat with a high level of root secretions outstripped significantly in the development of plants with low root secretion level (Fig. 2).

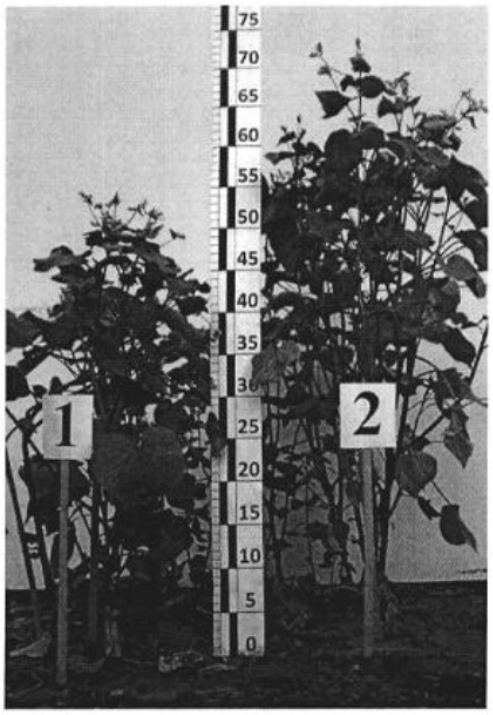

Fig. 2. The phenotype of buckwheat sowing plants (*Fagopyrum esculentum* Moench.) with different secretion intensity of root exudates:
1 – plants with low intensity root secretions; 2 – plants with high intensity root secretions

At the final stage of experiment plant phenotyping was performed in order to determine the peculiarities of crop formation structure. The potential productivity of buckwheat plants is largely determined by the number of vegetative and generative organs. We revealed a direct correlation between the performance of plants and their grain number, determined by the number of inflorescences per plant and the number of grains in them [12]. In the experimental variant plants with height 125.7 cm were formed being higher by 27% than control (Table. 1).

***Table* 1. Crop structure dependence of plants sowing buckwheat (*Fagopyrum esculentum* Moench.) from the intensity of root exudate excretion**

Group of investigated plants	Total plant height, cm	Number, pieces				Average weight of grain from plant, g
		Nodes	Branches	Inflorescences	Grains from plant	
Variant 1 (bromocresol green)						
Control	99,7	11,7	4,0	35,2	33,4	0,97
Experiment	122,6	14,0	4,5	51,2	222,8	6,91
Difference	22,9	2,3	0,5	16,0	189,4	5,94
Variant 2 (bromocresol purple)						
Control	97,1	11,8	4,1	33,6	37,8	1,10
Experiment	128,7	14,1	4,6	53,8	231,8	7,19
Difference	31,6	2,3	0,5	20,2	194,0	6,09
The average						
Control	98,4	11,8	4,1	34,4	35,6	1,04
Experiment	125,7	14,1	4,6	52,5	227,3	7,05
Difference	20,3	2,3	0,5	18,1	191,7	6,02
LSD_{01}	20,7	1,8	1,0	15,8	104,2	3,21

Another important morphological feature is the number of nodes on the stem, which varies depending of genotype and growing conditions. Increase of the nodes in the zone of fruiting contributes to greater stem productivity and therefore plants in general. The node number in the branching area is a parameter that defines the precocity of buckwheat and its growth opportunities [13]. Analyzing the number of nodes on the stem showed also a significant difference between the study options. Their number was 14.1 pc in experiment, while 11.8 pc in control.

Concerning plant branching one could see fairly significant differences between the variants. Counting the num-

ber of inflorescences and seeds showed the true advantage of these indicators in experiment version. Number of inflorescences and seeds in the control variant was 34.4 and 35.6 pc., while 52.5 and 227.3 pieces in experiment version. It is worth to note that some plants that never formed grain in the control variant were observed.

Plant productivity is complex trait resulting from the interaction of the aggregate morphological characteristics and properties that determine the peculiarities of plant growth and development. The value of each individual complex features from a total is different. The result of all components and features are grain number and weight of grain from the plant, which exceed in experiment by 6.8 times the control variant with the degree of reliability of 99%.

Conclusions. Thus, it was proved the efficacy of high-yielding common buckwheat early screening method of root exudates using acid-base indicators. Both proposed indicators proved effective. Our results yield structures in the field indicate that developed a new non-invasive screening method is highly effective and achieve the claimed technical result and can serve as a reliable tool for creating original material in breeding buckwheat sowing.

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А. Косян, канд. біол. наук, О. Смірнов, канд. біол. наук, Н. Таран, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна,
В. Бурдига, канд. с.-г. наук
Подільський державний аграрно-технічний університет, Кам'янець-Подільський, Україна

ТЕСТ-СИСТЕМА НА ОСНОВІ КОРЕНЕВИХ ЕКСУДАТІВ ДЛЯ СКРИНІНГУ ВИСОКОПРОДУКТИВНИХ ФОРМ ГРЕЧКИ ПОСІВНОЇ (*Fagopyrum esculentum* Moench.)

Запропоновано новий ефективний неінвазивний метод скринінгу (відбору) високопродуктивних форм гречки посівної (Fagopyrum esculentum Moench.), який базується на експрес-тестуванні проростків гречки в лабораторних умовах на інтенсивність ексудації органічних кислот кореневою системою. Насіння гречки пророщували на шарі агарового гелю, який містить в своєму складі кислотно-основний індикатор з наступною візуальною оцінкою зміни забарвлення індикатора навколо первинного кореня, і відбирали рослини з найбільшою зоною зміни кольору індикатора. В польових умовах підтверджена ефективність методу шляхом фенотипування рослин та виявлені значні відмінності у формуванні структури врожаю та продуктивності відібраних рослин. Статистичний аналіз показників озерненості та ваги зерна показав, що у відібраних рослини ці показники у понад 6 разів перевищували контрольні варіанти, зі ступенем достовірності 99%.

Ключові слова: метод відбору, фенотипування, проростки, кореневі ексудати.

А. Косян, канд. биол. наук, А. Смирнов, канд. биол. наук, Н. Таран, д-р биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина,
В. Бурдыга, канд. с-х. наук
Подольский государственный аграрно-технический университет, Камянец-Подольский, Украина

ТЕСТ-СИСТЕМА НА ОСНОВЕ КОРНЕВЫХ ЭКССУДАТОВ ДЛЯ СКРИНИНГА ВЫСОКОПРОДУКТИВНЫХ ФОРМ ГРЕЧИХИ ПОСЕВНОЙ (*Fagopyrum esculentum* Moench.)

Предложен новый эффективный неинвазивный метод скрининга (отбора) высокопродуктивных форм гречихи посевной (Fagopyrum esculentum Moench.), который основан на експресс-тестировании проростков гречихи в лабораторных условиях на интен-

сивность экссудации органических кислот корневой системой. Семена гречихи проращивали на слое агарового геля, который содержит в своем составе кислотно-основный индикатор с последующей визуальной оценкой изменения окраски индикатора вокруг первичного корня, и отбирали растения с наибольшей зоной изменения цвета индикатора. В полевых условиях подтверждена эффективность метода путем фенотипирования растений и выявлены значительные отличия в формировании структуры урожая и продуктивности отобранных растений. Статистический анализ показателей озерненности и массы зерна показал, что у отобранных растений эти показатели более чем в 6 раз превышали контрольные варианты, со степенью достоверности 99%.

Ключевые слова: метод отбора, фенотипирование, проростки, корневые экссудаты.

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V. Trokhymets, PhD, D. Lukashov, DSc
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

SPATIAL-TEMPORAL DYNAMICS OF LITTORAL ZOOPLANKTON COMMUNITY OF THE OLEKSANDRIVKA RESERVOIR

The analysis results of spatio-temporal dynamics of zooplankton communities from littoral of the Oleksandrivka reservoir are presented. The features of the seasonal changes in species composition, faunal and ecological spectrums, quantitative indicators (density and biomass) and the dominant species complexes of littoral zooplankton was revealed. The analysis of seasonal dynamics of qualitative and quantitative development of zooplankton in the littoral zone within the upper, middle and lower parts of the Oleksandrivka reservoir was conducted.

Keywords: Ecology, the Oleksandrivka reservoir, littoral, zooplankton community.

Introduction. At present, the anthropogenic factors have ecological importance for aquatic ecosystems [1-2]. Examples of human activities involve the restructuring of individual components of ecosystems (including groups of animals) [3], their structural and functional organization [4], and transforming rivers to reservoirs with different hydrological regime [5]. Many reservoirs were created over the past 50-60 years [6] and today they are the main type of water in Ukraine [7]. Special interests have littoral hydrobiocenosis that differ significantly from the pelagic and play an important role in the functioning of aquatic ecosystems [8]. They are characterized by high rates of biodiversity [9] and biological productivity [10] and the complex structural and functional organization [11]. This littoral zone occupies a large area of water, such as in the Kiev reservoir it is 38% [9]. Particular attention is drawn to the reservoir of the South-Ukrainian energy complex, which is an important part of the Oleksandrivka reservoir.

Zooplankton is an important component of aquatic ecosystems, which plays an important role in the circulation of matter and the energy transformation [2]. Most of the zooplankton belongs to the primary and secondary consumers [12]. Zooplankton is the foundational supply base for the young and planktonophagous fish at higher trophic levels [13].

Purpose – analysis of seasonal dynamics of structured littoral zooplankton communities in the Oleksandrivka reservoir.

Materials and methods. The object of our research were species of same groups of zooplankton: rotifers (class Eurotatoria), cladocerans (class Branchiopoda, order Cladocera), copepods (class Copepoda) and ostracods (class Ostracoda). Monogononth rotifers, copepods and cladocerans were determined to the species, bdelloid rotifers and ostracods – to subclass and class. The dynamics of the same seasonal changes was analyzed on the basis of data obtained from the Oleksandrivka reservoir during the different seasons (spring, summer and autumn) in 2009–2010.

The material sampled by filtering 50 liters of water through a conical plankton net [14] from four standardized sampling stations [15]: I – the left bank of the upper part, N $47^0$51.429’ E $31^0$07.721’; II – the right bank of the middle part, N $47^0$42.802’ E $31^0$11.267’; III – the left bank of the middle part, N $47^0$44.110’ E $31^0$44.681’; IV – the right bank of the lower part, N $47^0$42.042’ E $31^0$13.704’. Within each station, the samples were taken at various points (habitats): in the thickets of higher aquatic plants (overgrown) and clean areas of littoral macrophytes pond (not overgrown). At the station of the upper part of the Oleksandrivka reservoir dominated the formations of the common reed (*Phragmites australis* (Cav.) Trin. Ex Steud.). At the station of the middle and lower parts of the reservoir dominated the formations the claspingleaf pondweed (*Potamogeton perfoliatus* L.). The projective cover of higher aquatic plants increases from the top of the reservoir to its middle reaches – from 30 to 50%.

For general qualitative analysis 34 samples were collected using the conical net. Further processing of samples and analysis were performed on the basis of generally accepted methods. The species composition of zooplankton was identified in the laboratory due to identification guide [14, 16-19].

The results and their discussion. In the spring within all research stations was marked only the sites free of vegetation, and diversity of zooplankton was presented with 29 species: rotifers – 13 species, cladocerans – 85, copepods – 8. During the daily studies in the summer in common reed sites and in the sites without water plants 73 species of littoral zooplankton were registered: rotifers – 33 species, cladocerans– 26, copepods crustaceans – 14. In autumn 54 species of zooplankton were found in sites without aquatic plants and with reeds: rotifers – 16 species, cladocerans – 26, copepods – 12.

If we consider the representation of the species, during different seasons the serious reconstruction occurred. Species diversity of the littoral zooplankton increased in summer in 2,5 times in comparison with spring, and decreased in 1,4 times in autumn. Seasonal changes in species diversity of littoral zooplankton can be explained by the same reasons [5]. In summer comparing with the spring, the projective coverage and the overgrowness level of the habitat significantly increased, creating more favorable conditions for the development of aquatic littoral. Thus, in the spring samples were registered cryophilic species of rotifers – *Brachionus angularis*, *Br. nilsoni*, *Notholca acuminata*, *N. squamula*, which were absent during the summer and autumn research. Instead, a number of thermophilic representatives were met in the summer: rotifers of the genus *Lecane*, *Leptadella* and *Trichocerca*, *Tripleuchlanis plicata* et al.

The similarity of the species composition lists in different seasons was characterized by the Jaccard index as the very low: between spring and summer – J = 30,4; between spring and autumn – J = 27,2; between summer and autumn – J = 39,3. Especially low was the similarity between

species composition lists of rotifers, while crustaceans, especially copepods, were characterized by more stable composition during different seasons (Table 1). Thus 17 species of littoral zooplankton encountered during all three seasons: rotifers *Brachionus calyciflorus*, *Br. quadridentatus*, *Euchlanis deflexa*, *E. Dilatata* and *Keratella quadrata*; cladocerans *Alona rectangula*, *Bosmina longirostris*, *Chydorus piger*, *Ch. sphaericus*, *Daphnia cucullata* and *Graptoleberis testudinaria*; copepods *Acanthocyclops americanus*, *Cyclops vicinus*, *Eucyclops serrulatus*, *Eurytemora velox*, *Mesocyclops leuckarti* and *Thermocyclops crassus*.

Table 1. **The seasonal dynamics of species composition (J) of different groups of littoral zooplankton in the Oleksandrivka reservoir**

Seasons / Groups	Spring and summer	Spring and autumn	Summer and autumn
Eurotatoria	20,4	24,3	27,6
Cladocera	25,7	24,3	45,0
Coprepoda	48,1	36,7	50,0

The faunal spectrum of littoral zooplankton during different seasons was characterized by the prevalence of rotifers complex in spring, in summer – the prevalence of rotator and rotifers-cladocerans complexes and in autumn – the prevalence of cladocerans complex (Fig. 1). This was due to the formation during different seasons favorable conditions for filter feeders in the Oleksandrivka reservoir, and the prevailing were the rotifers and cladocerans. So, in the original research, the rotifers and cladocerans crustaceans had the highest species richness in different seasons.

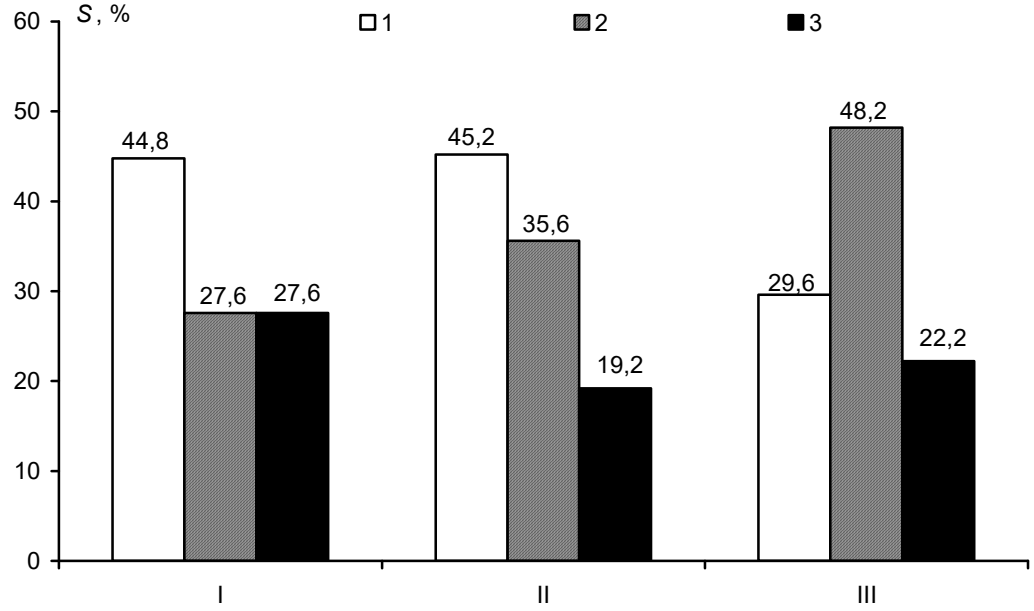

Fig. 1. The seasonal dynamics of faunal spectrum of littoral zooplankton in the Oleksandrivka reservoir.
Notes: *S* – number of species; 1 – rotifers, 2 – cladocerans crustaceans, 3 – copepods crustaceans; I – spring, II – summer, III – autumn

The ecological spectrum of different littoral zooplankton groups of the Oleksandrivka reservoir in the spring was characterized by a predominance of thepelagic group: pelagic – 16 species (55,2% of the total), littoral-phytophilous – 9 (31,0%), demersal-phytophilous – 4 (13,8%). Summer pelagic species continued to dominate in the ecological group (28 species – 38,3% of the total), exceeding slightly the proportion of littoral-phytophilous (24 species – 32,9%) and demersal-phytophilous (21 species – 28,8%) groups. In autumn littoral zooplankton did not have the dominant group: pelagic – 18 species (33,3% of the total), littoral-phytophilous – 17 species (31,5%) and demersal-phytophilous – 19 species (35,2%) groups. The dominance during different seasons of the pelagic ecological groups can be explained by the persistence of river conditions within the littoral zone of the Oleksandrivka reservoir, like in the Kyiv reservoir [5, 20]. Growth in summer particles of demersal-phytophilousand littoral-phytophilous groups can be explained by the formation of overgrown habitat [21]. The summarized value of the ecological zooplankton groups considering its faunal spectrum in different seasons is presented in Fig. 2.

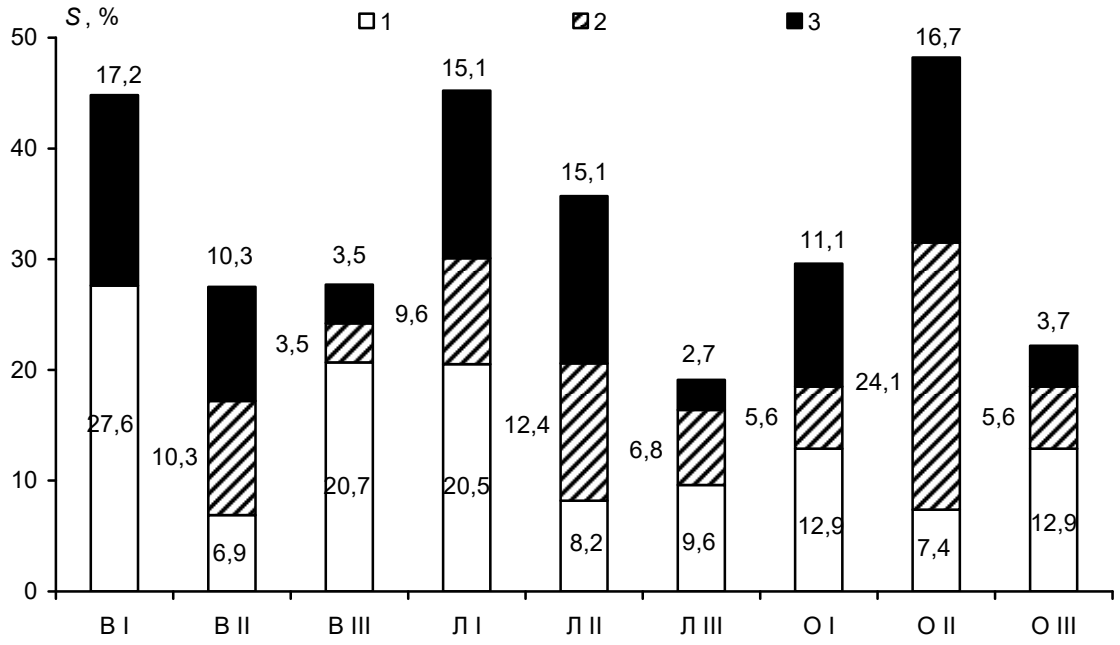

Fig. 2. The ecological spectrum changes of the Oleksandrivka reservoir littoral zooplankton in different seasons.
Notes: *S* – size of species; 1 – pelagic group, 2 – demersal-phytophilous,
3 – littoral-phytophilous; I – rotifers, II – cladocerans, III – copepods

Spring littoral zooplankton in quantitative terms in afternoon time was characterized by "very low" or "low" development. Its density varied within 2,8-19,9 thousand ind./m^3, and biomass – 0,02-0,21 g/m^3. Among quantitative indicators the species of rotifers complex and larvae of copepods crustaceans mainly nauplia stages of development dominated (Table 2).This trend is normal for communities of spring littoral zooplankton in reservoirs [5, 22].

Table 2. **The seasonal dynamics of density (thousand ind./m^3) and biomass (g/m^3) of different groups of littoral zooplankton in the Oleksandrivka reservoir (M±m)**

Seasons / Groups	Spring n = 8	Summer n = 16	Autumn n = 10
Eurotatoria	3,3±0,9/ 0,02±<0,01	13,6±4,8/ 0,03±0,01	0,6±0,2/ <0,01±<0,01
Cladocera	0,7±0,2/ 0,01±<0,01	17,8±5,2/ 0,63±0,21	1,5±0,3/ 0,10±0,03
Copepoda	0,4±0,1/ 0,02±<0,01	3,9±1,7/ 0,21±0,10	1,6±0,7/ 0,09±0,03
Copepoda larvae	4,8±1,1/ 0,04±0,01	21,7±6,5/ 0,17±0,06	2,8±1,0/ 0,03±0,01
Ostracoda	–	0,3±0,1/ 0,03±0,01	0,1±<0,1/ 0,01±<0,01
Bivalvia larvae	–	1,0±0,5/ <0,01±<0,01	<0,1±<0,1/ <0,01±<0,01
In general	9,2±2,0/ 0,09±0,02	58,3±18,6/ 1,08±0,39	6,7±1,8/ 0,23±0,07

Notes: the numerator – the density, the denominator – the biomass

In summer daytime the littoral zooplankton development was "very low", "low", "below average" and "average" (Table 2). Its density varied within 0,2-282,4 thousand ind./m^3, and biomass – <0,01-5,35 g/m^3. Rotifers and copepods' larvae dominated for quantitative indicators, with the predominance of the copepods' stages of development. The rotifers prevailed in the upper part of the reservoir, in other parts of reservoir – cladocerans did. The domination of cladocerans-copepods complex is customary for the development of summer zooplankton communities in a reservoir, that associated with a more complex biotopical variety, with the ecological and trophic spectrum of zooplankton and the complexity of the living conditions for small filter feeders. Although representatives of the rotifers complex had significant density within the upper part of reservoir, which is associated with the high flow in Oleksandrivka reservoir and the influence of the complex of geophilic species from the river Pivdennyi Bug.

The littoral zooplankton was characterized by "very low" and "low" development in daily time of autumn (Table 2). Its density varied within 0,4-21,3 thousand ind./m^3, and biomass – 0,01-0,73 g/m^3. Among the quantitative indicators larvae of copepods dominated, mainly copepodite stages of development. Prevalence of rotifers complex associated with a longer period of the vegetation of higher aquatic plants, remains of which have formed overgrown habitat and create conditions for the development of various ecological groups of zooplankton [5, 22].

Significant seasonal changes occurred in the dominant groups of zooplankton communities. Rotifers *Brachionus calyciflorus* and *Keratella quadratain*, and the larval stage of copepods were the dominant in the spring in different

parts of the reservoir. Rotifers *Brachionus quadridentatus*, *Euchlanis dilatata* and *Trichocerca bidens*, cladocerans – *Bosmina longirostris*, *Diaphanosoma brachyurum* and *Disparalona rostrata*, copepods – *Mesocyclops leuckarti* form the summer dominant complex of species. In autumn dominant complex was formed of rotifers *Euchlanis dilatata*, cladocerans – *Daphnia cucullata*, *D. rostrata* and *Sida crystallina*, and of copepods – *Acanthocyclops americanus* and *Eurytemora velox*.

We used Jaccard dominant index for the comparison of the dominant species complexes, and very low number of similarities in different seasons was recorded (Table 3). Meantime none species was not presented in dominant complexes for three seasons. The significant restructuring of dominating complex of littoral zooplankton is linked with temperature conditions, significant fluctuations in the concentration of organic matter in the water [5, 21].

Table 3. **The seasonal dynamics of dominante specias complex ($J_{dom.}$) of littoral zooplankton in the Oleksandrivka reservoir**

Seasons	Spring	Summer	Autumn
Spring	X	0,0	0,0
Summer	0,0	X	18,2
Autumn	0,0	18,2	X

The seasonal distribution of littoral zooplankton in different parts of the Oleksandrivka reservoir is characterized by the middle degree of similarity in the species composition in spring, but much lower in summer and autumn (Table 4). In spring the biotopical diversity was absent, and littoral was characterized by the presence of sites free of vegetation. The middle size of the reservoir facilitates mixing littoral and pelagic zooplankton. In the summer and autumn, the reed formation developed actively, which entered in association with other types of higher aquatic plants. Zooplankton species composition of the upper and lower part of the reservoir in the summer had the lower similarity (J = 8,4 and 36,2), in middle part – higher (J = 53,3). In the autumn habitats were mild and macrophytes began to fall off the bottom.

Table 4. **The seasonal dynamics of species composition (J) of littoral zooplankton in different parts of the Oleksandrivka reservoir**

Parts of reservoir	The upper part	The middle part	The lower part
Spring			
The upper part	X	50,4	55,6
The middle part	50,4	X	40,4
The lower part	55,6	40,4	X
Summer			
The upper part	X	24,5	16,7
The middle part	24,5	X	31,8
The lower part	16,7	31,8	X
Autumn			
The upper part	X	26,1	10,5
The middle part	26,1	X	33,4
The lower part	10,5	33,4	X

In spring littoral zooplankton was characterized by "very low" and "low" density and biomass in hydrobiocenoses without macrophytes (Table 5). The quantitative indicators increased from the upper to the lower parts of the reservoir. The latest trend is clearly seen in summer when density and biomass of littoral zooplankton were "very low" and "low" in the upper part of the reservoir. Density and biomass of littoral zooplankton were "very low", "low" and "below average" in the middle part of the reservoir. Density and biomass of littoral zooplankton were "very low", "low" and "below average" in the middle part of the reservoir. Density and biomass of littoral zooplankton were "low", "below average" and "average" in the lower part of the reservoir. In autumn littoral zooplankton was characterized by "very low" and "low" density and biomass. The complex of the dominant species within the intertidal zone of the reservoir in a season had changed and most of them have a similar structure (J dom. = 50–100).

Table 5. **The seasonal dynamics of density (thousand ind./m^3) and biomass (g/m^3) of littoral zooplankton in different parts of the Oleksandrivka reservoir (M±m (lim))**

Seasons / Reservoir	Spring n = 8	Summer n = 16	Autumn n = 10
The upper part	3,8±1,1 (2,8–4,9)/ 0,04±0,02 (0,02–0,06)	1,2±0,4 (0,2–1,9)/ 0,03±0,01 (0,01–0,04)	4,2±1,5 (1,5–6,8)/ 0,11±0,04 (0,05–0,19)
The middle part	10,6±3,2 (5,4–19,9)/ 0,10±0,04 (0,05–0,21)	46,4±14,0 (7,8–110,3)/ 0,85±0,35 (0,12–2,76)	5,2±1,5 (0,4–9,6)/ 0,22±0,08 (0,01–0,53)
The lower part	11,5±2,8 (8,8–14,2)/ 0,08±0,03 (0,06–0,11)	140,0±41,0 (42,4–282,4)/ 2,59±0,91 (0,59–5,35)	14,0±6,3 (6,7–21,3)/ 0,42± 0,21 (0,11–0,73)

Notes: the numerator – the density, the denominator – the biomass; n – size of sumples

Conclusions. 1.In spring littoral zooplankton of the Oleksandrivka reservoir comprises 29 species (13 species of rotifers, crustaceans – 8, copepods – 8), in summer – 73 species (33 species of rotifers, cladocerans – 26, copepods – 14), in autumn – 54 species (16 species of rotifers, cladocerans – 26, copepods – 12). 2. The similarity of the species lists, obtained in different seasons, was characterized by the Jaccard index as the low levels: between spring and summer – J = 30,4, between spring and autumn – J = 27,2, between summer and autumn – J = 39,3. Especially low was the similarity between the species composition of rotifers (J = 20,4–27,6), while crustaceans were

characterized by greater stability during different seasons (J = 24,3-50,0). 3. In spring in the faunal spectrum of the species composition of littoral zooplankton dominated the rotifers complex (44,0% of all species of zooplankton), in summer – rotifers (45,6%) and rotifers-cladocerans complexes (80,8%), in autumn – cladocerans complex (48,2%). 4. The ecological spectrum of different groups of littoral zooplankton was characterized by a predominance of pelagic group in spring and summer (55,2 and 38,2 %) In autumn littoral zooplankton didn't have the dominate group: pelagic – 18 species (33,3% of the total), littoral-phytophilous – 17 species (31,5%) and demersal-phytophilous – 19 species (35,2%) groups. 5. In spring in afternoon time littoral zooplankton in quantitative terms was characterized by "very low" or "low" development. Its density varied within 2,8-19,9 thousand ind./m^3, and biomass – 0,02-0,21 g/m^3. In summer in daytime littoral zooplankton development was "very low", "low", "below average" and "average". Its density varied within 0,2-282,4 thousand ind./m^3, and biomass – <0,01-5,35 g/m^3. The littoral zooplankton was characterized by "very low" and "low" development in daily time of autumn. Its density varied within 0,4-21,3 thousand ind./m^3, and biomass – 0,01-0,73 g/m^3. 6. None species was presented in the dominant complexes for three seasons. Dominant species complexes were very low for similarities in different seasons ($J_{дом.}$ = 0–18,2). 7. The seasonal distribution of littoral zooplankton in different parts of the Oleksandrivka reservoir is characterized by middle degree of similarity in species composition in spring (J = 40,4–55,6), but much lower in summer (J = 16,7–31,8) and autumn (J = 10,5–33,4). The seasonal dynamics of density and biomass of littoral zooplankton were similar in different parts of the reservoir.

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В. Трохимець, канд. біол. наук, Д. Лукашов, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ПРОСТОРОВО-ЧАСОВА ДИНАМІКА УГРУПОВАНЬ ЛІТОРАЛЬНОГО ЗООПЛАНКТОНУ ОЛЕКСАНДРІВСЬКОГО ВОДОСХОВИЩА

Представлено результати аналізу просторово-часової динаміки угруповань зоопланктону літоралі Олександрівського водосховища. Виявлено особливості сезонних змін видового складу, фауністичного та екологічного спектрів, кількісних показників (щільності та біомаси) та домінуючих комплексів видів літорального зоопланктону. Здійснено аналіз сезонної динаміки якісних і кількісних показників розвитку зоопланктону в межах літоралі верхньої, середньої та нижньої частин Олександрівського водосховища.

Ключові слова: екологія, Олександрівське водосховище, літораль, угруповання зоопланктону.

В. Трохимец, канд. биол. наук, Д. Лукашов, д-р биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

ПРОСТРАНСТВЕННО-ВРЕМЕННАЯ ДИНАМИКА СООБЩЕСТВ ЛИТОРАЛЬНОГО ЗООПЛАНКТОНА АЛЕКСАНДРОВСКОГО ВОДОХРАНИЛИЩА

Представлено результаты анализа пространственно-временной динамики сообществ зоопланктона литорали Александровского водохранилища. Выявлено особенности сезонных изменений видового состава, фаунистического и экологического спектров, количественных показателей (плотности и биомассы) и доминирующих комплексов видов литорального зоопланктона. Осуществлено анализ сезонной динамики качественных и количественных показателей развития зоопланктона в пределах литорали верхней, средней и нижней частей Александровского водохранилища.

Ключевые слова: экология, Александровское водохранилище, литораль, сообщества зоопланктона.

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G. Shayakhmetova, PhD
SI "Institute of Pharmacology and Toxicology NAMS of Ukraine" Kiev, Ukraine

COMPARATIVE INVESTIGATION OF ANTI-TUBERCULOSIS DRUGS EFFECTS ON TESTICULAR CYP2E1 EXPRESSION AND MALE REPRODUCTIVE PARAMETERS UNDER SEPARATE AND COMBINED ADMINISTRATION IN MALE RATS

Comparative study of anti-tuberculosis drugs anti-androgenic effects and effects on testicular CYP2E1 has been performed. Testicular CYP2E1 mRNA and protein expression, serum total testosterone level, fertility and spermatogenesis parameters in male rats under simultaneous and separate administration of ethambutol, isoniazid, rifampin and pyrazinamide have been investigated. Analysis of the obtained data has proved the prominent role of ethambutol and isoniazid in gonadal toxicity of anti-tuberculosis drugs combination. Activation of CYP2E1-dependent metabolizing systems in testicular steroidogenic cells could stipulate at least a part of ethambutol, isoniazid and anti-tuberculosis drugs combination negative effects on testosterone level and spermatogenesis processes. Mechanisms of spermatogenesis alteration by rifampin and pyrazinamide need to be explored more extensively, but in the light of our observations they do not depend from testicular CYP2E1.

Key words: anti-androgenic effects, anti-tuberculosis drugs, protein expression, ethambutol, isoniazid.

Introduction. The epidemiological situation of tuberculosis in the world keeps worsening [1]. In general, all patients from countries with a known high incidence of resistant *M. tuberculosis* strains, all patients who had been treated previously, and all patients with life-threatening tuberculosis, receive as initial anti-tuberculosis therapy the same combination of isoniazid (INH), rifampin (RMP) and pyrazinamide (PZA), together with at least one additional medicine (ethambutol (EMB) and/or streptomycin) [2].

In such situation investigation of these compounds adverse effects becomes of vitally importance. We have previously shown the antifertility effects of anti-tuberculosis medicines combination in male rats with simultaneous increase in cytochrome P-450 2E1 (CYP2E1) mRNA in their testes [3, 4]. It is important to note that series of **studies clearly demonstrated inducibility of** CYP2E1 in testis, suggesting its possible role in chemicals bioactivation to their toxic metabolites directly in male gonads [4, 5, 6, 7]. Among this, it is well known that both toxic intermediates (which are able to interact with vitally important cells structures) and reactive oxygen species (ROS) overproduction (with the further development of oxidative stress) take place during CYP2E1-mediated xenobiotics metabolism [8].

It remains unclear which one of the four co-administered (ATD) plays a crucial role in testicular CYP2E1 expression modulation and the development of antifertility effects. Thus, in terms of *o*ur above mentioned results [3, 4] analysis of potential effects on male gonads of each component of the combination are urgently required. Such data could substantially contribute to our general understanding of causes of the man subfertility. To get the answer on this question we have decided to compare testicular CYP2E1 mRNA and protein expression, serum total testosterone (TS) level, fertility and spermatogenesis parameters in male rats under combined and separate administration of EMB, INH, RMP and PZA.

Materials and methods. Substances of EMB, INH, RMP and PZA were supplied by the SIC "Borzhagovsky Chemical-Pharmaceutical Plant" CJSC, Ukraine.

Wistar albino male with initial body weight (b.w.) 150–170 g (8-9 weeks old) and female rats 150-170 b.w. (9-10 weeks old), were purchased from Biomodel Service (Kyiv, Ukraine). They were kept under a controlled temperature (from 22 °C to 24 °C), relative humidity of 40 % to 70 %, lighting (12 h light-dark cycle), and on a standard pellet feed diet ("Phoenix" Ltd., Ukraine).

The male rats were divided randomly into 6 groups: 1-control (n=12); 2 – EMB administration (n=12); 3 – INH administration (n=12); 4 – RMP administration (n=12); 5 – PZA administration (n=12); 6 – simultaneous ATD administration (n=12). All ATD were suspended in 1% starch gel and was administered intragastrically by gavage in doses used in clinic [9], which for rats (with the coefficient for conversion of human doses to animal equivalent doses based on body surface area) were following: EMB – 155 mg/kg b.w./day, RMP – 74.4 mg/kg b.w./day, INH – 62 mg/kg b.w./day, PZA – 217 mg/kg b.w./day [10]. ATD were administered during entire spermatogenesis cycle, which (with

time of germ cell maturation in epididymis) is 60 days for rats. The control group received only starch gel in corresponding volumes (5 ml/kg b.w.).

After 46 days of repeated administrations, the males from both groups were mated with intact females at the ratio 1 male: 2 females during 14 days (3 oestrous cycles). During this period administrations of ATD to male rats were continued.

According to generally accepted guidelines for the fertility study in laboratory rats [11] the first day of pregnancy was established by vaginal cytology (the first day of sperm detection in vagina). Most males were mated within the first 5 days of cohabitation (i.e. at the females first available oestrus), but part of them demonstrated infertility. This fact was taken into account for evaluation of effects on male fertilizing capacity, which was determined by the index:

$$\frac{number\ of\ pregnant\ females}{number\ of\ females\ mated\ with\ males} \times 10$$

The pregnancy was confirmed by necropsy. The females were sacrificed under mild ether anaesthesia via cervical dislocation on day 20 of pregnancy.

Males were sacrificed on the morning under mild diethyl ether anaesthesia by decapitation after 60 days of experiment. The study was carried out according to the national and international guidelines and the law on animal protection was observed. All animal studies were performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee.

The expression of CYP2E1 mRNA in testes was determined by a reversed transcriptase polymerase chain reaction (RT-PCR). After collection of the testes samples (25 mg), they were quickly frozen in liquid nitrogen, and stored at -80 °C before RNA extraction. The isolation of total mRNA was carried out with a TRI-Reagent (Sigma-Aldrich, Inc., USA). The integrity and concentration of RNA was analysed in a 2 % agarose gel. First-strand complementary DNA (cDNA) was synthesized using a First-Strand cDNA Synthesis Kit (Fermentas, Germany) according to the manufacturer's protocol. The reaction mixture contents for PCR, amplification protocol, and specific primers for the CYP2E1 gene were chosen according to Lankford et al. (2000). The primer sequences were: sense, 5'-CTTCGGGCCAGTGTTCAC-3' and anti-sense, 5'-CCCATATCTCAGAGTTGTGC-3'. RT-PCR with primers of β – actin sense, 5' –GCTCGTCGTCGACAACGGCTC – 3' and antisense 5' – CAAACATGAT CTGGGTCATCTTCT –3') was carried out for internal control. All of the primers were synthesized by "Metabion" (Germany). The MyCycler termocycler (BioRaD, USA) was used for amplification. PCR products (CYP2E1-744 bp and β-actin-353 bp) were separated in a 2 % agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator (BIORAD, USA). Data analysis was carried out with Quantity One Software (USA) and presented in relative units as CYP2E1 mRNA contents / β-actin mRNA ratio.

Immunohistochemical staining for testicular CYP2E1 was performed using 4 μm thick sections of BS-fixed, paraffin-embedded sections. Briefly, tissue sections were dewaxed in xylene and were placed in water through graded alcohols. Antigen retrieval has been performed by microwaving slides in 10 mM citrate buffer (pH 6.2) for 30 min at high power, according to the manufacturer's instructions. Human polyclonal antibody against CYP2E1 (Thermo scientific, USA) were used as primary antibodies. To remove the endogenous peroxidase activity, the sections have been treated with freshly prepared 1.0 % hydrogen peroxide in the dark for 30 min at 37 °C temperature. Non-specific antibody binding was blocked by means of blocking serum. The sections were incubated for 30 min, at 37 °C temperature, with the primary antibodies against CYP2E1 diluted 1:100 in phosphate buffered saline (PBS) pH 7.2 then a triple washing with PBS follows. Anti-(rabbit IgG)–horseradish peroxidase conjugate (1:40000 dilution) has been fulfilled for the detection of the CYP2E1 primary antibodies, then the sections were incubated for 20 min, at 37 °C temperature. The reaction products were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB). Immunohistochemical study of testicular CYP2E1 was performed in EMB-treated and vehicle-treated rats, respectively. Any intensity of reactivity for CYP2E1 in testes was considered positive. The proportion of testes staining positive was scored semiquantitatively as positive, focal/weakly positive, or negative.

The sperm count in epididymal suspensions was estimated as described Chitra et al. [12] using Goryaev's counting chamber and light microscope (200 x).

The determination of the spermatogenic index in testicles was carried out according to four points system. It was based on the estimation of number of cell layers, types of cells, and the presence of late spermatids in the seminiferous tubules. The criteria were as follows: 1 – only spermatogonia present; 2 – spermatogonia and spermatocytes present; 3 – spermatogonia, spermatocytes and round (early) spermatids present with < 5 late spermatids per tubule; 4 – spermatogonia, spermatocytes, and round spermatids present with up to 25 late spermatids per tubule [11]. Spermatogenic index was calculated as a ratio of stages of spermatogenesis total to number of examined tubules. Two hundred seminiferous tubules per testis of each animal were observed by microscopy.

Blood samples from femoral vein were collected. Serum samples were separated and kept frozen at −70°C. Serum total testosterone levels were measured using DRC testosterone ELISA kit (Germany) according to manufacturer's instruction.

The obtained data were calculated by one-way analysis of variance (ANOVA) and compared using the Tukey test. Differences were considered statistically significant at $p<0.05$. Numerical data are represented as means ± SEM.

Results and discussion. We have shown that combined administration of ATD caused significant decrease in serum TS (Fig.1). At this group it was lower 2.8 folds as compared with control. Separate administration of EMB and INH also significantly influenced TS level, lowering it in average 1.6 folds as compared with control (Fig.1). At the same time RMP and PZA did not alter this parameter (Fig.1).

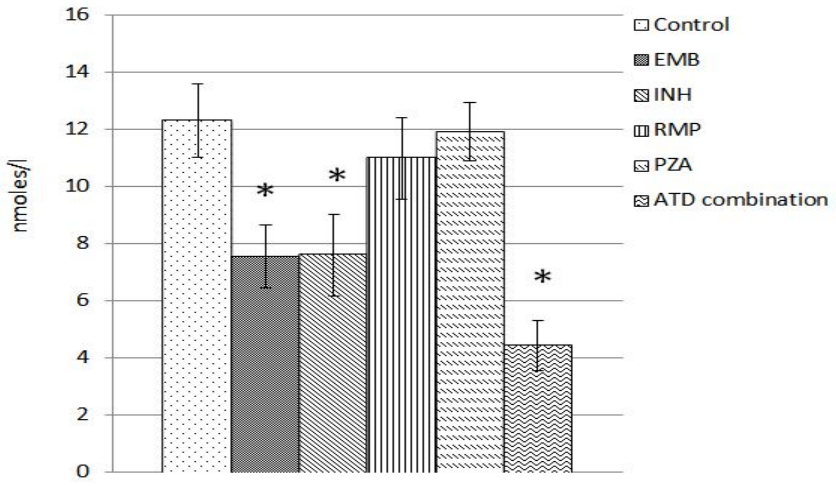

Fig. 1. Total testosterone level in blood serum of male rats following separate and combined ATD treatment

* – P<0.05 in comparison with control

Thus, our results indicate considerable TS level impairment in EMB, INH and ATD-combination-treated male rats, which, in turn, caused lowering of sperm count in these groups (Fig.2).

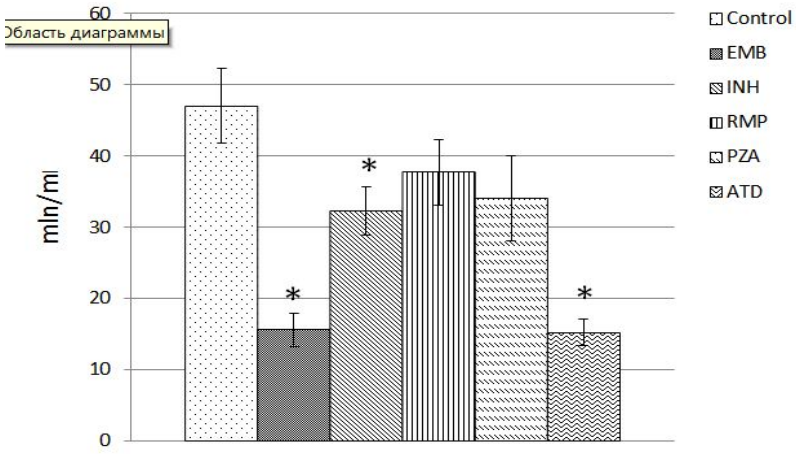

Fig. 2. Epididymal suspension sperm count following separate and combined ATD treatment

* – P<0.05 in comparison with control

Interestingly that administration (separate and combined) of all ATD caused a development of destructive changes in spermatogenic epithelium. It is seen from the data summarized in Table 1 that spermatogenic index at all experimental groups was *significantly* decreased in comparison with control group. Primary spermatogenesis cell population also was affected, namely mitotic activity was inhibited and number of spermatogonia in testes tubules sections was decreased. In addition, the number of cells at XII stage of spermatogenesis (characterizing primary spermatocytes meiotic division processes) in EMB and ATD combination-groups was lower than in control. This parameter was not significantly changed by RMP, INH, and PZA, but it demonstrated the tendency to decreasing. At ATD combination-treated group we detected epithelium exfoliation into the lumen of seminiferous tubules in substantial quantity too.

***Table* 1. Parameters of spermatogenic epithelium in testes following separate and combined ATD treatment**

Groups of males	Spermatogenic index (stages of spermatogenesis total / number of examined tubules)	Number of spermatogonia (per tubular cross section)	Cells at XII stage of spermatogenesis, %	Exfoliation of epithelium, %
Control	3.615±0.011	69.393±0.742	3.563±0.365	0.313±0.120
EMB	3.484±0.008*	57.840±0.465*	2.000±0.316*	1.000±0.316
RMP	3.530±0.012*	59.540±0.901*	2.200±0.510	0.600±0.400
INH	3.494±0.007*	62.110±0.936*	2.200±0.663	0.800±0.374
PZA	3.552±0.007*	61.060±1.016*	2.400±0.245	1.000±0.316
ATD combination	3.535±0.014*	59.573±0.861*	2.412±0.508	1.882±0.363*

* – P<0.05 in comparison with control

Our data on fertilizing capacity of experimental males was in accordance with TS level and sperm count results (table 2).

***Table* 2. Male rats' fertility index following separate and combined ATD treatment**

Groups of males	Number of mated females	Number of pregnant females	Fertility index, %
Control	12	11	91.66
EMB	12	4	33.33
RMP	12	10	83.33
INH	12	8	66.67
PZA	12	10	83.33
ATD combination	12	1	8.33

Counting of the number of pregnant intact females which were mated with experimental males demonstrated a significant reduction in the fertility of males receiving ATD combination. Of the 12 mated females only one became pregnant, while in the control fertility index was 92%. Evaluation of fertility index in animals separately treated with ATD found that all four drugs reduced this parameter to some extent, but in case of INH (index – 66.67%) and EMB (index – 33.33%) – most definitely.

In addition, we have recorded fatal decrease of the number of live fetuses in EMB (6.3 folds) or ATD combination-treated (36.6 folds) groups (table 3).

***Table* 3. Number of live fetuses in offspring of males following separate and combined ATD treatment**

Groups of males	Parameter	
	total number of live fetuses, abs.	number of live fetuses per one female, abs.
Control	110	9.16±1.14
EMB	16	1.33±0.62 *
RMP	86	7.17±1.55 #
INH	111	9.25±1.30 #
PZA	112	9.33±1.30 #
ATD combination	3	0.25±0.25 *

* – P<0.05 in comparison with control
# – P<0.05 in comparison with ATD combination-treated animals

RT-PCR was performed to evaluate the effect of separate and combined ATD administration on CYP2E1 mRNA expression in testes.

We have not found statistically significant increase in testicular CYP2E1 mRNA expression following administration of RMP and PZA (Fig. 3). At the same time, there was a significant rise in CYP2E1 mRNA expression after administration of INH (28 folds), EMB (8.7 folds), and ATD combinations (19 folds). Slightly lower CYP2E1 gene expression level in ATD-treated probably could be due to the competitive antagonistic relationship of EMB and INH.

The evaluation of the testicular CYP2E1 protein level was performed using immunohistochemical analysis. The immunoexpression of CYP2E1 in testes was confirmed by the presence of brown staining. Figure 4 illustrates broad expression of CYP2E1 in EMB, INH and ATD-combination treated rats' testes that were significantly higher than in PZA, RMP, and control groups (Fig.6).

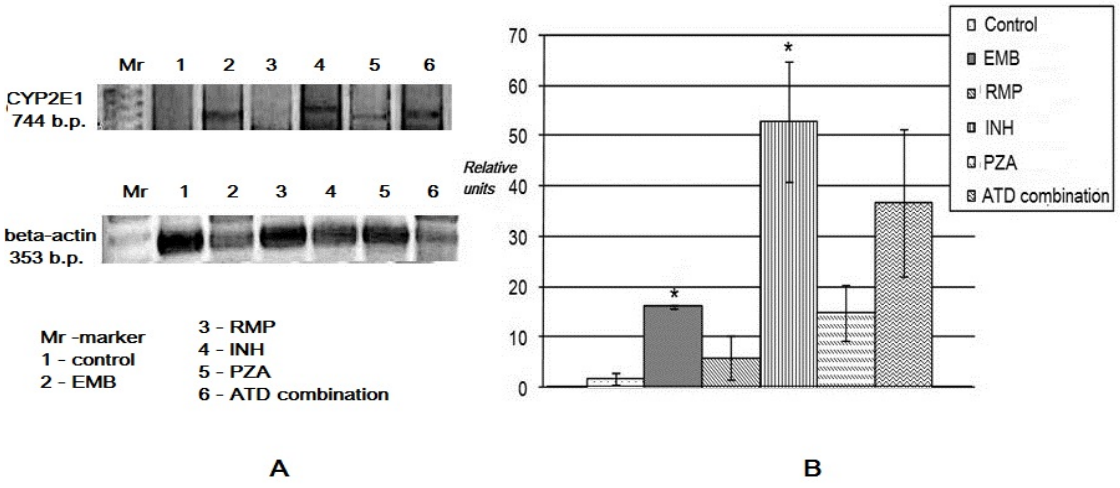

Fig 3. Representative electrophoregrams of CYP2E1 (744 b.p) and reference-gene β-actin (353 b.p.) RT-PCR (panel A); average rate of CYP2E1 mRNA expression (panel B) in rats testes following separate and combined ATD treatment

*- P<0.05 in comparison with control.

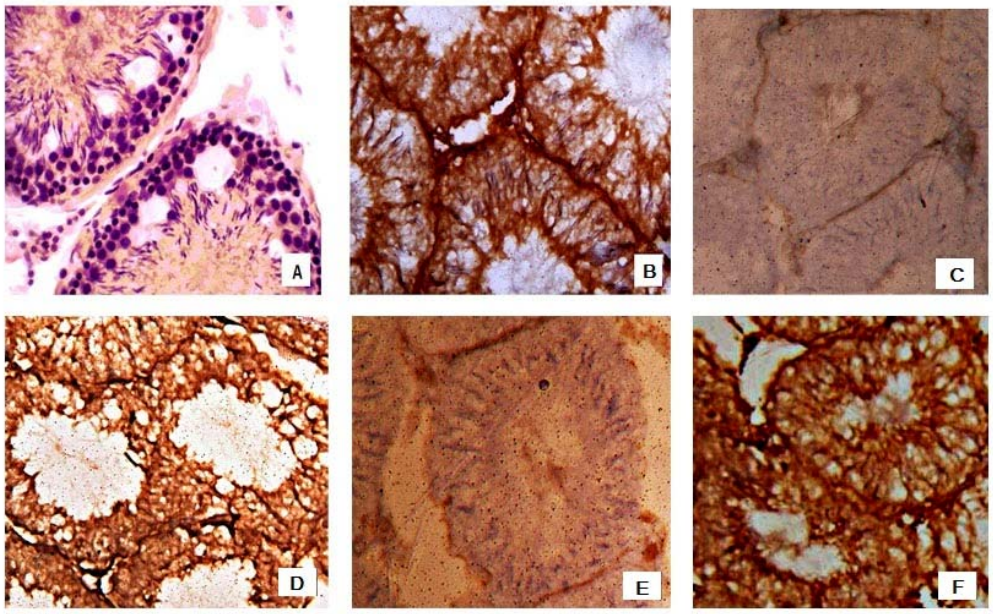

Fig. 4. Immunohistochemistry analysis of CYP2E1:
A – negative reaction in testis of control rat; B, D, and F – strongly positive reaction in testis of EMB, INH, and ATD combination-treated rats; C and E – weakly positive reaction in testis of RMP and PZA-treated rats

It should be noted that adult spermatogenesis consists of three phases: proliferation of spermatogonia, meiosis of spermatocytes, and differentiation of spermatids or spermiogenesis. These events are precisely controlled at each stage to ensure the formation of genetically balanced gametes [13]. Affecting of any step could dysregulate the process of spermatogenesis and the spermatozoa may become defective [14]. After analyzing our results, firstly, it can be seen the definite evidences of the spermatogenesis impairment in separate and combined ATD-treated male rats. For instance, the decrease of spermatogenic index and spermatogonia number confirms depression of spermatogenic cells activity. Moreover we have detected intensification of germ cells exfoliation into the lumen of the seminiferous tubules, as evidence of loss of their adhesion with Sertoli cells or shearing of Sertoli cells cytoplasm [15].

We also demonstrated that following separate EMB and INH, as well as combined ATD administration alterations of spermatogenesis along with low TS level, have led to a reduction in spermatozoids production. As a result the fertility index and number of live fetuses in offspring at these groups was three folds lower than in control. Also at these groups we have demonstrated the significant increase of testicular CYP2E1 mRNA and protein levels. This phenomenon could be an indication of this iso-enzyme induction.

Our results on testicular CYP2E1 induction following EMB, INH and ATD combination administration is of importance because this process occure in Leydig cells [6, 7], which provide the synthesis of androgens necessary for the maintenance of spermatogenesis and extra-gonadal androgen actions in mammals [16]. Consequently, these structures, and their microenvironment damage by free radicals as a result of ATD-mediated CYP2E1 induction could be one of the reasons of steroidogenesis enzymes inhibition and spermatogenesis disruption. It is known that CYP2E1 is an effective generator of hydrogen peroxide [17], and it acts directly on Leydig cells to diminish TS production by inhibiting cytochrome P450 side chain cleavage enzyme (P450scc) activity and steroidogenic acute regulatory (StAR) protein expression [18]. Moreover, recently it has been reported, that ROS signaling-mediated c-Jun upregulation suppresses the expression of steroidogenic enzyme genes by inhibiting Nur77 transactivation (one of the major transcription factors that regulate the expression of steroidogenic enzyme genes), resulting in the reduction of testicular steroidogenesis [19]. The fact that lowered level of TS can affect Sertoli cells function and also negatively influence spermatogenesis is of importance too [20].

Conclusion. Our findings prove the prominent role of EMB and INH in male reproductive toxicity of ATD combination. In our opinion, activation of CYP2E1-dependent metabolizing systems in steroidogenic cells could stipulate at least a part of EMB, INH and ATD-combination negative effects on testosterone level and spermatogenesis processes. Mechanisms of spermatogenesis alteration by RMP and PZN need to be explored more extensively, but in the light of our observations they do not depend from testicular CYP2E1. It seems warranted to conclude that the critical examination of both laboratory animals' and epidemiological data is required.

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Г. Шаяхметова, канд. біол. наук
ДУ "Інститут фармакології та токсикології НАМН України", Київ, Україна

ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ЕФЕКТІВ НАРІЗНОГО І КОМБІНОВАНОГО ВВЕДЕННЯ ПРОТИТУБЕРКУЛЬОЗНИХ ЛІКАРСЬКИХ ЗАСОБІВ НА ТЕСТИКУЛЯРНУ ЕКСПРЕСІЮ CYP2E1 І РЕПРОДУКТИВНІ ПОКАЗНИКИ САМЦІВ ЩУРІВ

Було проведено порівняльне вивчення анти-андрогенних ефектів протитуберкульозних препаратів, а також їх впливу на тестикулярний CYP2E1. Досліджували експресію мРНК і білка CYP2E1 в сім'яниках, рівень загального тестостерону, фертильність і показники сперматогенезу у самців щурів при окремому і сумісному введенні етамбутолу, ізоніазиду, рифампіцину і піразинаміду. Аналіз отриманих даних доводить важливу роль етамбутолу і ізоніазиду в гонадотоксичної дії комбінації протитуберкульозних препаратів. Активація CYP2E1-залежних метаболізуючих систем в тестикулярних стероїдогенних клітинах може обумовлювати, щонайменше, частину негативних ефектів етамбутолу, ізоніазиду і комбінації протитуберкульозних препаратів на рівень тестостерону і процеси сперматогенезу. Механізми порушень сперматогенезу, викликані рифампіцином і піразинамідом, вимагають більш детального дослідження, але, виходячи з наших спостережень, вони не залежать від тестикулярного CYP2E1.

Ключові слова: анти-андрогенний ефект, протитуберкульозні препарати, експресія мРНК, етамбутол, ізоніазид.

А. Шаяхметова, канд. биол. наук
ГУ "Институт фармакологии и токсикологии НАМН Украины", Киев, Україна

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ЭФФЕКТОВ РАЗДЕЛЬНОГО И КОМБИНИРОВАННОГО ВВЕДЕНИЯ ПРОТИВОТУБЕРКУЛЕЗНЫХ ЛЕКАРСТВЕННЫХ СРЕДСТВ НА ТЕСТИКУЛЯРНУЮ ЭКСПРЕССИЮ CYP2E1 И РЕПРОДУКТИВНЫЕ ПОКАЗАТЕЛИ САМЦОВ КРЫС

Было проведено сравнительное изучение анти-андрогенных эффектов противотуберкулезных препаратов, а также их воздействия на тестикулярный CYP2E1. Исследовали экспрессию мРНК и белка CYP2E1 в семенниках, уровень общего тестостерона, фертильность и показатели сперматогенеза у самцов крыс при раздельном и совместном введении этамбутола, изониазида, рифампицина и пиразинамида. Анализ полученных данных доказывает важную роль этамбутола и изониазида в гонадотоксичного действия комбинации противотуберкулезных препаратов. Активация CYP2E1-зависимых метаболизирующих систем в тестикулярных стероидогенных клетках может обуславливать, по меньшей мере, часть отрицательных эффектов этамбутола, изониазида и комбинации противотуберкулёзных препаратов на уровень тестостерона и процессы сперматогенеза. Механизмы нарушений сперматогенеза, вызванные рифампицином и пиразинамидом, требуют более детального исследования, но, исходя из наших наблюдений, они не зависят от тестикулярного CYP2E1.

Ключевые слова: анти-андрогенные эффекты, противотуберкулезные препараты, экспрессия мРНК, этамбутол, изониазид.

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A.Yurchenko, PhD-student, N. Raksha, PhD, O.Savchuk, DSc
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

THE INFLUENCE OF KIDNEY BEANS (*PHASEOLUS VULGARIS*) PODS EXTRACT ON OBESITY DEVELOPMENT

The influence of kidney beans pods extract on obesity development was investigated. It was found that administration of P. vulgaris pods extract led to decrease of body weight and body mass index of the animals which were on high-calorie diet. Found changes could be result of decrease of food intake by rats treated with extract in compare with rats in high-calorie diet group.

Key words: obesity, kidney beans pods extract, high-calorie diet.

Introduction. In our modern world with increasingly cheap, high calorie food, prepared foods that are high in things like salt, sugars or fat, combined with our increasingly sedentary lifestyles, increasing urbanization and changing modes of transportation, it is no wonder that obesity has rapidly increased in the last few decades, around the world. Obesity can be the basis for the development of related diseases and complications that are often the cause of early death and disablement, including diabetes, hypertension, coronary heart disease, cancer, etc. [1]. That is why the search for new treatment approaches of this disease remains an urgent medical problem, because of number of patients continues to grow steadily.

In recent years, worldwide scientific interest focuses on the study of the properties of plant extracts due to the multifactorial nature of their therapeutic effects on the obesity and its concomitant diseases. Plant extracts unlike synthetic drugs practically non-available toxic effects [2]. Available raw for the drugs development is kidney beans (*Phaseolus vulgaris*). Kidney bean pods are believed to be helpful in obesity and weight loss programs, as well as obesity-related diseases, such as diabetes mellitus type 2 and heart disease [4]. Bean pods may lower blood sugar level. Kidney bean pods extract naturally blocks the absorption and expulsion of the carbohydrates eaten as part of a daily diet. Bean pods have been proposed as an effective agent in the fight against weight gain and obesity [5].

For today, the complex investigation about effects of *P. vulgaris* extract on obesity development is absent, so the aim of this study was to investigate the influence of kidney bean pods on the development of obesity in rats which were on high-calorie diet.

Materials and Methods. Experiments were carried out on white nonlinear male rats with initial weighing of 135-160 g. All animals were at room temperature 19-24 ºC, humidity of less than 50%, natural light mode "day-night" in plastic cages during the experiment.

During first seven days, all rats received standard food "Purina rodent chow" and water ad libitum. On the 8th day the animals were randomly divided into 3 groups. Animal of the first group ("Control") have been fed with a standard food and water during the experiment. Animals of the second group ("HCD") were on a high-calorie diet which consisted of a standard meal (60%), pork fat (10%), eggs (10%), sugar (9%), peanuts (5%), dry milk (5%), and sunflower oil (1%) [6], and drank water ad libitum. Animal of the third group ("HCD+Ex") were also on high-calorie diet and water ad libitum. After 4 weeks of experiment they started to receive the extract of *P. vulgaris* (200 mg / kg). One day all rats of this group have received the extract and another day they drank water.

Body weights were recorded once a week and feed intake was recorded daily in all animal groups. Body mass index (BMI) (body weight (g) /nose-to-anus length^2 (cm^2)) and Lee index (cube root of body weight (g) /nose-to-anus length (cm)) were calculate at the end of experiment [7].

Statistical analysis was performed using statistical analysis applications of Microsoft® Excel. To assess intergroup differences the parametric Student test was used. The difference between the parameters was considered statistically significant at $p<0,05$.

Results and Discussion. As the result of our experiment it was shown a significant increase in body weight of rats which received a high-calorie food compared with a control group of animals that were on standard diet. Also, it was found that the dynamic of body weight increase of animals that received the *P. vulgaris* pods extract simultaneously with high-calorie food was not very different from that of the control rats (Fig.1).

After 10 weeks of experiment body weight of rats in the control group increased by 110% from an initial value. It was shown that the body weight of rats in the "HCD" group was increased by 161% that was significantly higher compared with the "Control". Our results revealed that body weight in the "HCD+Ex" group was not different from the control and increased by 113% from the initial value.

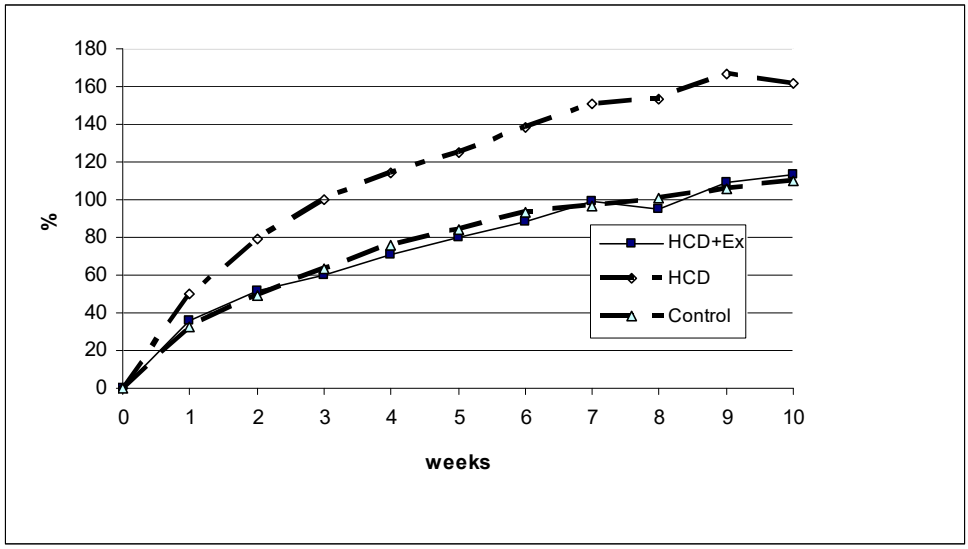

Fig.1. The dynamics of body weight increase of rats in the "Control", "HCD" and "HCD+Ex" groups (M ± m; n = 10)

During 10 weeks of study daily food consumption by rats of the control group was unchanged and was 28±1,4 g per day (Fig. 2). The rats that were on high-calorie diet ate an average of 30,6±1,5 g of high-calorie food per one animal. But the quantity of daily food that consumed rats of the "HCD+Ex" group was even lower compared with the control group of animals and composed 24,6±1,2 g per one rat.

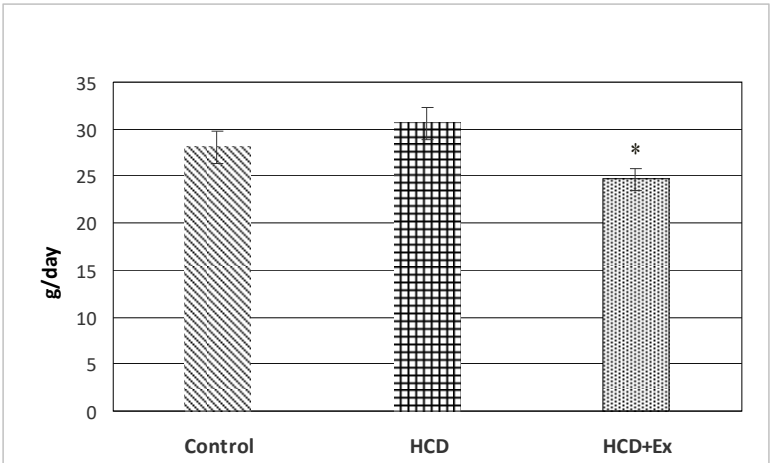

Fig.2. Food consumption by rats in "Control", "HCD" and "HCD+Ex" groups (M ± m; n = 10)
Note: * – p <0.05 differences credible with respect to the control

Also, our studies showed the higher liquid consumption in group of the control rats compared with rats that were on high-calorie diet and rats that received the *P. vulgaris* pods extract (Fig. 3). Liquid consumption was in average 38±1,9 ml, 32,5±1,6 ml and 28,7±1,4 ml for the control animals, "HCD" and "HCD+Ex" groups, respectively.

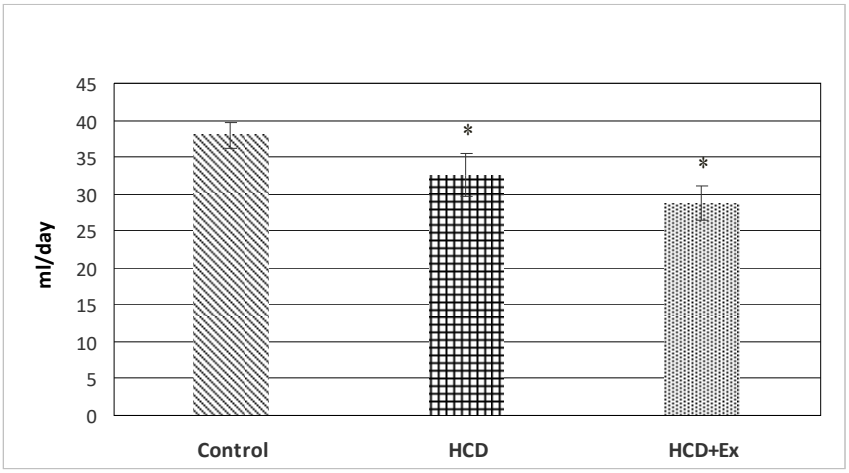

Fig.3. Liquid consumption by rats in "Control", "HCD" and "HCD+Ex" groups (M ± m; n = 10)
Note: * – p <0.05 differences credible with respect to the control

Table 1 represented data that summarized the daily food intake, liquid intake and changes in body weight of animals in the "Control", "HCD" and "HCD+Ex" groups. Also, Table 1 showed the body mass index (BMI) as a body weight (g) /nose-to-anus length^2 (cm^2) and Lee index as cube root of body weight (g) /nose-to-anus length (cm) of the control," HCD" and "HCD+Ex" groups. It was found an increase of BMI in "HCD" group on almost 0,07 points and decrease of BMI in "HCD+Ex" group on 0,05 points compared to the control after 10 weeks of experiment. The Lee index in "HCD" and "HCD+Ex" groups of animals was increased on 0,07 and 0,05 points, respectively, compared with "Control" group.

***Table 1*. General characteristics of the rats that were on a high-calorie diet ("HCD") and animals that received the *P. vulgaris* pods extract simultaneously with high-calorie food ("HCD+Ex")**

	Food intake (g/day)	Liquid intake (ml/day)	Initial body weight (g)	Final body weight (g)	BMI (g/cm^2)	Lee index
Control	28,1±1,4	38±1,9	183±9,1	393±19,6	0,71	2,58
HCD	30,6±1,5	32,5±1,6*	171±8,5	443±22,1	0,78	2,65
HCD+Ex	24,6±1,2*	28,7±1,4*	186±9,3	396±19,8	0,66	2,63

*p<0.05 significantly different from the control group

The main result of current study was the detection the fact that animals that received the *P. vulgaris* pods extract and were on high-calorie diet not gaining weight compared with rats that consumed only a high-calorie diet. Such result partly could be explained from the position of kidney beans pods extract influence on digestion of carbohydrates in the gastrointestinal tract. Also, our results confirmed the development of obesity in group of animals that were on high-calorie diet. Excessive body weight of these rats was probably associated with the accumulation of adipose tissue. This accumulation may be the result of an imbalance between the amount of energy consumed by rats and the amount of energy spent, because alongside with the increasing amount of consumed feed by the animals. The amount of energy received was increased either due to high caloric content of food. It may be due to high caloric content of food which in turn may be the result of accumulation of adipose tissue because of an imbalance between the amount of energy consumed by rats and the amount of energy spent.

Conclusion. Thus, our results demonstrated the ability of the *P. vulgaris* pods extract to influence the development of obesity, in particular, to reduce the amount of consumed food, which were accompanied by decrease of body mass index and body weight in compare with those for animals, which were only on high-calorie diet. Noted effects suggested that this extract may be used as functional ingredient in addition to regular therapy of obesity and its related complications.

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А. Юрченко, асп., Н. Ракша, канд. біол. наук, О. Савчук, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ВПЛИВ ЕКСТРАКТУ ЛУШПИННЯ КВАСОЛІ ЗВИЧАЙНОЇ (PHASEOLUS VULGARIS) НА РОЗВИТОК ОЖИРІННЯ

Досліджено вплив екстракту лушпиння квасолі звичайної на розвиток ожиріння. Встановлено, що введеня екстракту стручків P. Vulgaris призводить до зниження маси тіла та індексу маси тіла тварин, які споживали висококалорійну їжу. Виявлені зміни можуть бути результатом зниження споживання їжі щурами, які отримували екстракт в порівнянні з щурами, що перебували на калорійній дієті.

Ключові слова: ожиріння, екстракт лушпиння квасолі звичайної, висококалорійна дієта.

А. Юрченко, асп., Н. Ракша, канд. биол. наук, О. Савчук, д-р биол. наук
Киевський национальний университет имени Тараса Шевченка, Киев, Украина

ВЛИЯНИЕ ЭКСТРАКТА СТВОРОК ФАСОЛИ ОБЫКНОВЕННОЙ (PHASEOLUS VULGARIS) НА РАЗВИТИЕ ОЖИРЕНИЯ

Исследовано влияние экстракта шелухи фасоли обыкновенной на развитие ожирения. Установлено, что введение экстракта стручков P. Vulgaris приводит к снижению массы тела и индекса массы тела животных, которые потребляли высококалорийную пищу. Данные изменения могут быть результатом снижения потребления пищи крысами, которые получали экстракт по сравнению с крысами, которые находились на калорийной диете.

Ключевые слова: ожирение, экстракт створок фасоли обыкновенной, высококалорийная дзета.

УДК 578

A. Kharina, PhD, N. Kornienko, MD student, A. Stavniychuk, PhD student,
T. Kot, senior engineer, M. Liptuga, student
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

ISOLATION OF BACTERIOPHAGES, CAPABLE TO LYSE SERRATIA MARCESCENS AND EVALUATION OF THEIR ACTIVITY ON ONION AND GERANIUM

In this study, phages active against S. marcescens, causative agent of onion decay, were isolated from plant material. One virus isolate was shown to accumulate in high titers and was denoted as phage S. This bacteriophage exhibited a hexagonal head and tail and was attributed to Myoviridae family. It was shown the ability of bacteriophage S to suppress the development of bacteriosis on geranium plants. Investigated virus isolate also inhibited rooting of onion scales. This work focused on a biological control approach to use bacteriophages for reducing bacterial pathogen populations and disease severity on plants.

Key words: bacteriophages, plant bacteria, phytopathology, phage therapy.

Introduction. Plant pathogenic bacteria cause many serious plant diseases throughout the world stimulating intensive research of their ecology, pathology and epidemiology. Bacterial rot (*Erwinia carotovora*) and vascular bacteriosis (*Xanthomonas campestris*) cause epidemics and lead to large harvest destruction, resulting in requirement of the import of cabbages, tomatoes and pepper [1]. Bacteria of genuses *Salmonella, Serratia, Enterobacter* and *Enterococcus,* more frequently happen in the cases of nocosomial infections, e.g. food poisonings and sepsises [2-5]. The last researches, however, prove that these human pathogenic bacterial species also have ability to colonize a wide spectrum of plants and cause the disease development. For the instance *Serratia marcescens*, common soil bacteria, was described as causative agent of soft rot of onion [6]. However, it should be noted that the majority of these experiments were performed under laboratory conditions, while the development of crop diseases, caused by these pathogens in the environment, is still poorly understood.

Bacteriophages, bacterial viruses, are widely present in the environment, wherever the host bacterium is expected to be found. Some bacteriophages are potentially useful agents in the control of plant pathogens. There is a wide array of potential candidate phage isolates; hence, investigations are needed to determine which isolates will likely be the most effective for application in bacterial control strategies. Our purpose was to search bacteriophages, specific to *S. marcescens* and to verify their activity against this bacterium on the plants of onion and pelargonium.

Materials and methods.

Bacterial strain. Studies were carried out on bacterial culture *Serratia marcescens* IMBG291 [5], generously provided by colleagues from the laboratory of microbial ecology, Institute of Molecular Biology and Genetics (National Academy of Sciences of Ukraine). Working with phages we used an overnight culture of bacteria, in which bacteria was in exponential phase of growth. The concentration of bacteria cell culture was 10^8-10^9 c.f.u./ml. Bacteria was cultivated on plate count agar or in PC-broth. Incubation temperature was 25 ^{O}C.

Bacteriophage isolation. In order to isolate bacteriophages the samples of plant displaying the symptoms of bacteriosis were used. To amplify putative bacteriophages in samples enrichment method was applied. Investigated samples were centrifuged and handled by chloroform. The objects of research became phage, isolated from samples of tomatoes with symptoms of rot processes.

Spot-titer assay. The samples or serial dilutions of samples were applied dropwise on the plates with seeded bacterial culture. Following 20 minutes they were kept at room temperature in order to samples diffused into agar medium. Then plates were overturned and incubated in a thermostat at 37^{0}C for 12 hours. After that the plates were analyzed for the presence of phages. Results were recorded as the reciprocal of the highest dilution at which clearing the lawn was evident.

Double agar layer method. 0.2 ml of overnight bacterial culture (10^8 c.f.u./ml) was put together with 2.5 ml of 0.7% agar (the temperature of agar was 46-49^{0}C). Then 1 ml of the studied sample was added. The resulting mixture was accumulated on the bottom layer of 1,4% agar [1]. According to the results of spot-test, the concentration of phage particles in a sample of carrot was very high, so we diluted the phage lyzate to the 10^{th} degree in order to get separated plaques. After exposure within 15 minutes at the room temperature, plates were inverted and incubated at 37^{0}C for 12 hours. After incubation all resulting plaques were counted. Separate phage plaques were then picked. Isolated bacteriophages were purified by serial propagation of single plaques and amplified.

Electron microscopy. Morphology of virions was investigated using the electron microscope. Formvar films placed on 400-mesh copper grids were dipped into sample for 2 min and contrasted in 2% uranyl acetate. The preparations were dried and viewed under the electron microscope at an instrumental magnification of 20,000.

Investigation of bacteriophages influence on the expression of pathogenic properties of the bacteria.

In laboratory conditions the infectious process was modeled with the onion inoculation with investigated bacteria *Serratia marcescens*. Onion scales were obtained from sterilized onion bulbs and incubated in plates on paper filter discs. The onion scales were scratched with sterile scalpel. Then 10mkl of phage mixture (titer 10^7) and bacterial suspension (concentration of cells 10^8 c.f.u./ml) were applied on the scales. For controlling the initiation of pathogenic process with bacteria a drop of bacterial suspension was applied on the scales of onion, placed in other plate. Onion scales with deposited drop of physiological solution served as a control of the experiment. All plates were placed in the incubator at 25^{o}C. For statistical significance of data obtained each experiment was conducted in three repetitions.

In order to research the potential of isolated bacteriophages as therapeutic agents another model system was used. The ability of bacteriophages to suppress the development of bacteriosis was investigated on geranium plants. For this purpose geranium leaves were infected with bacteria in two ways: injection into veins with subsequent incubation in water or adding bacterial suspension to water. We utilized different ways for the treatment of plant leaves with bacteriophage preparations. In the first group the leaves was inoculated in a vein with the suspension of bacterium and phage, in the second group plant leaves were inoculated with a bacterium into a vein and incubated in water supplemented with phages.

Results and discussion. Initial studies on the application of bacteriophage for control of pathogens require the identification and isolation of an appropriate phage from the multitude of phages that exist in the environment. Phages

specific for pathogenic *Serratia marcrscens* were isolated after plating the samples of rotten tomatoes. We have observed the formation of different morphology plagues on bacterial lawn. Investigated isolates resulted in formation of large and small negative colonies (fig. 1). Large colonies had round, smooth shape (d=5-6 mm), while small colonies were 1-2 mm in diameter and characterized with irregular shape.

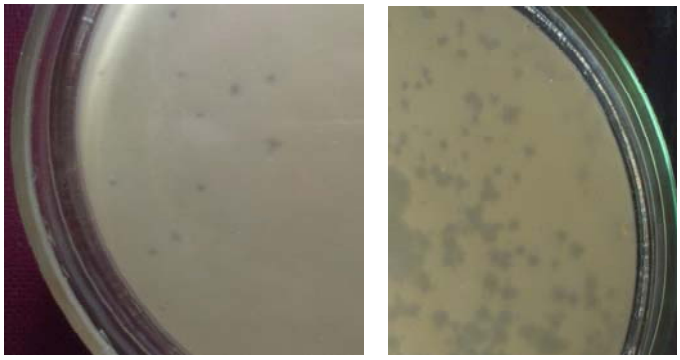

Fig.1. Bacteriophage plagues on *S.marcescens* lawn: isolate S (left), isolate L (right)

For subsequent research, we have chosen phages with small colonies because of their capability to accumulate in high titers compare to phages with large colonies. These phages were denoted as "S" in the experiment. To accumulate this isolate separate phage plagues were picked and transferred to sterile saline. Isolated bacteriophages were purified by serial propagation of single plaques. According to the results of spot-tests the titer of viruses after three passages was 10^{-7}. High titer lysates were routinely prepared from confluent lysis plates. Then we studied the morphological features of the selected bacteriophages using electron microscopy. The isolate S belongs to family of *Myoviridae* of order *Caudovirales* (morphotype A2, fig. 2).

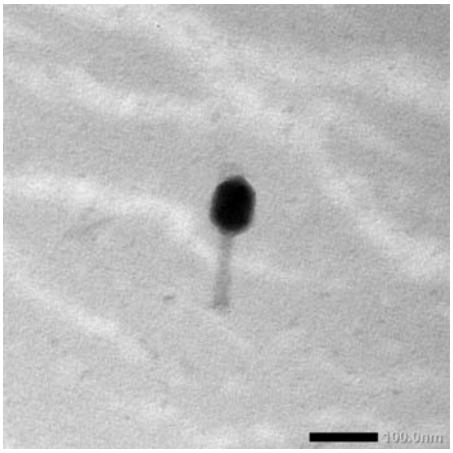

Fig.2. Transmission electron microscopy images of *S. marcescens* phage (S)

The next stage was to verify if the selected bacteriophage isolate S was active against *S. marcescens* in test-system on onion scales. In two days after inoculation we observed the development of soft rot on onion scales threated with bacterium and the absence of bacterial growth in the plate where phage was added.

Thus, our phage isolate was effective against *S. marcescens* in selected test system.

Fig. 3. Onion scales: inoculated with bacteria (left), inoculated with bacteria and phage (right)

In order to research the potential of isolated bacteriophages as therapeutic agents another model system was used. The ability of bacteriophages to suppress the development of bacteriosis was investigated on geranium plants. As a result, bacteria cased redness of leaves in five days after inoculation. The symptoms were the same in both variants of bacterial infection. Turning red was observed along the veins while the whole leaf plate remained green. In the site of bacteriophage inoculation we observed clean area. This suggested that bacteriophage repressed bacterial growth. However according to data obtained isolated phage was limited in the ability to spread through the plant tissue.

Conclusion. Consequently, the probed bacteriophage repressed the development of phytobacteriosis caused by *S. marcescens* on onion scales and geranium leaves. The use of bacteriophages to combat bacterial infections may help to solve the current problem of antibiotic resistance. For successful application of bacteriophages fundamental issues arising from the ecological dynamic of host, bacterium and phage should be investigated in detail.

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А. Харіна, канд. біол. наук, Н. Корнієнко, студ., А. Ставнійчук, асп.,
Т. Кот, пров. Інж., М. Ліптуга, студ.
Київський національний університет імені Тараса Шевченка, Київ, Україна

ВИДІЛЕННЯ БАКТЕРІОФАГІВ, ЗДАТНИХ ЛІЗУВАТИ SERRATIA MARCESCENS ТА ПЕРЕВІРКА ЇХ АКТИВНОСТІ НА ЦИБУЛІ ТА ПЕЛАРГОНІЇ

Бактеріофаги, активні проти S. marcescens, збудника гнилі цибулі, були виділені з рослинного матеріалу. Один із виділених ізолятів вірусів накопичувався у високих титрах і був позначений як бактеріофаг S. За морфологічними характеристиками (наявність голівки і хвостового відростку) даний вірус був віднесений до родини Myoviridae. Було показано здатність бактеріофагу S пригнічувати розвиток бактеріозу на рослинах пеларгонії. Досліджуваний ізолят також інгібував гнилісні процеси на лусочках цибулі. В даній роботі розглядається спосіб біологічного контролю фітопатогенів із застосуванням бактеріофагів для зниження чисельності бактеріальних популяцій і суворості прояву симптомів.

Ключові слова: бактеріофаги, фітопатогенні бактерії, фітопатологія, фаготерапія.

А.Харина, канд. биол. наук. Н. Корниенко, студ., А. Ставнийчук, асп.,
Т. Кот, вед. инж., М. Липтуга, студ.
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

ВЫДЕЛЕНИЕ БАКТЕРИОФАГОВ, СПОСОБНЫХ ЛИЗИРОВАТЬ SERRATIA MARCESCENS И ПРОВЕРКА ИХ АКТИВНОСТИ НА РАСТЕНИЯХ ЛУКА И ПЕЛАРГОНИИ

Бактериофаги, активные против S. marcescens, возбудителя гнили лука, были выделенны из растительного материала. Один из изолятов накапливался у высоких титрах и был обозначен как бактериофаг S. За морфологическими характеристиками (наличие головки и хвостового отростка) выделеный бактериофаг классифицирован как представитель семейства Myoviridae. Было показано способность бактериофага S подавлять развитие бактериоза на растениях пеларгонии. Исследуемый изолят вируса ингибировал также гнилостные процессы на чешуйках лука. В данной работе рассматривается способ биологического контроля фитопатогенов с применением бактериофагов для снижения чисельности бактериальных популяций и суровости проявления симптомов.

Ключевые слова: бактериофаги, фитопатогенные бактерии, фитопатология, фаготерапия.

Наукове видання

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ВПЦ "Київський університет"
01601, Київ, б-р Т. Шевченка, 14, кімн. 43
☎ (38044) 239 32 22; (38044) 239 31 72; тел./факс (38044) 239 31 28
e-mail: vpc@univ.kiev.ua
http: vpc.univ.kiev.ua
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