enhance the level of HIV resistance to these drugs). Mutations of resistance to PIs were detected in 11 samples, but mutations associated with high levels of resistance to PIs were detected in 8 of them (8/60, 13.33%). Among the major mutations of resistance to PIs nucleotide substitutions at position 46 (M46L, M46LI) were detected – in 7 of the investigated sequences, at position 82 (V82A, V82F) – in 5 samples. M46I/L contribute reduced susceptibility to few PIs (indinavir (IDV), nelfinavir (NFV), fosamprenavir (FPV), atazanavir (ATV), and lopinavir (LPV)) [10]. V82A decreases susceptibility to IDV and LPV and confers cross-resistance to ATV and NFV [12]. The mutation I54V and L76V were detected in 3 and 2 samples, respectively. Among the minor mutations substitutions at position L10I dominated, which were found in 13 of the samples.

Conclusions. Thus, HIV strains with mutations of high level resistance to ARVs were found in the majority (in 39 from 60) of blood samples obtained from children with virological inefficiency HAART. The frequency of detection of resistance mutations to NRTIs was 55.0 %, to NNRTIs – 48.33%, to PIs – 13.33 %. In total group 21 children (35.0 %) had treatment failure, probably related to their low adherence to therapy, abnormalities in the mode of taking the drugs. These results indicate the high relevance of problem of the formation and spread of resistant strains of HIV among HIV- positive children in Ukraine and the necessity for further study of that problem.

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Н. Бабій, канд. біол. наук, А. Щербінська, проф.
КНУ імені Тараса Шевченка, Київ

ПОШИРЕННІСТЬ ШТАМІВ ВІЛ-1, СТІЙКИХ ДО АНТИРЕТРОВІРУСНИХ ПРЕПАРАТІВ, СЕРЕД ДІТЕЙ, ЯКІ ОТРИМУЮТЬ НЕЕФЕКТИВНУ ВИСОКОАКТИВНУ АНТИРЕТРОВІРУСНУ ТЕРАПІЮ

Представлені результати аналізу поширеності резистентних до АРВ – препаратів штамів ВІЛ- 1 серед дітей з неефективною ВААРТ. Для проведення досліджень з виявлення резистентних до АРВ – препаратів штамів ВІЛ були відібрані зразки крові 60 ВІЛ-інфікованих дітей у віці до 15 років. Частота виявлення штамів ВІЛ з мутаціями, що забезпечують стійкість високого рівня хоча б до одного препарату, включеному в схему лікування, склала 65,0 %, 51,67 % дітей потребували корекції схеми терапії. Більшість проаналізованих послідовностей РНК ВІЛ (96,67%) належали до субтипу А ВІЛ-1.

Ключові слова: АРВ прапарати штамів ВІЛ-1, ВААРТ.

Н. Бабий, канд. биол. наук, А. Щербинская, проф.
КНУ имени Тараса Шевченко, Киев

РАСПРЕДЕЛЕНИЕ ШТАММОВ ВИЧ-1, УСТОЙЧИВЫХ К АНТИРЕТРОВИРУСНЫМ ПРЕПАРАТАМ, СРЕДИ ДЕТЕЙ, КОТОРЫЕ ПОЛУЧАЮТ НЕЭФФЕКТИВНУЮ ВЫСОКОАКТИВНУЮ АНТИРЕТРОВИРУСНУЮ ТЕРАПИЮ

Представлены результаты анализа распространенности резистентных к АРВ-препаратам штаммов ВИЧ-1 среди детей с неэффективной ВААРТ. Для проведения исследований по выявлению резистентных к АРВ-препаратам штаммов ВИЧ были отобраны образцы крови 60 ВИЧ-инфицированных детей в возрасте до 15 лет. Частота выявления штаммов ВИЧ с мутациями, обеспечивающими устойчивость высокого уровня хотя бы к одному препарату, включенному в схему лечения, составила 65,0%, 51,67% детей нуждались в коррекции схемы терапии. Большинство проанализированных последовательностей РНК ВИЧ (96,67%) принадлежали к субтипу А ВИЧ-1.

Ключевіе слова: АРВ препараты штаммов ВИЧ-1, ВААРТ.

UDK 578

A. Baidaliuk, stud., I. Budzanivska, Dr.Sci.
Taras Shevchenko National University of Kyiv, Kyiv

ADVANCED APPROACHES TO IDENTIFICATION OF VIRUSES INFECTING OF WILD HERBACEOUS PLANTS

Recently, interest in studying viruses in wild flora was gradually increasing. This is connected with necessity of better understanding plant virus evolution, ecology, virulence, and even to avoid economic losses due to crop-wild hybridization, followed by introgression of pathogen-resistant transgenes to wild populations. In this review brief information about last contributions in development of wild plant virology is given. Different approaches to the researches are present here.

Key words: viruses in wild flora, transgenes to wild populations.

Introduction. Viruses commonly infect wild plants. However, virus infection is easily overlooked in wild plant populations. Although infections can be visually unapparent, it is frequently assumed that absence of visual symptoms (such as leaf mottling or malformation) indicates lack of virus infection. Moreover, symptoms of virus infection are sometimes difficult to distinguish from environmental stresses. For these reasons, in part, virus ecology in natu-

ral plant populations is poorly studied [1]. Little is known about the prevalence or effects of virus infection in wild plant populations, much of our understanding of plant-virus interactions comes from economically important plants (e.g., crops, horticultural varieties, and pasture plants). In crops, virus infection can reduce plant growth by depressing photosynthesis, changing metabolism, and altering resource allocation. Virus infections can drastically reduce crop yield, resulting in economic losses. Moreover, many virus vectors are difficult to control, and for this reason, genetic resistance to virus infection is often the most practical means of controlling crop losses [2]. The use of transgenic crops with virus resistance offers promise for control of problematic viruses. The commercial release of virus-resistant transgenic crops has motivated research on plant-virus ecology in natural populations [1]. Knowledge from studies of viruses of cultivated plants may not be adequate for explaining the ecology and evolution of plant viruses in nature. Anthropogenic activities such as domestication of crops, travel and exploitation of natural habitats seem to influence plant virus spread and evolution in cultivated conditions.

Little is known about plant virus diversity, host specificity and evolution under natural conditions where human influence is limited. In wild plants, virus infection can affect plant growth, mortality, and seed production, but these effects vary among populations, species, and environments. Although these data indicate that viruses can affect community dynamics and have fitness impact on many wild plants, remarkably little is known about virus prevalence in wild populations [3]. Assuming recent information, which is connected with investigation of viruses in wild plants, several main reasons for further research on the given theme can be established. Regarding evolution, the phylogenetic relationships between the viruses in wild and domestic plants should be clearly assessed. In respect of virulence, several studies of the plant viruses in natural systems indicate no harmful effects or a mild influence on their host plant's fitness [4]. Moreover, new species of plant viruses can probably be found (e.g. Asclepias asymptomatic virus [5]). High rates of using transgenic crops may cause crop-wild hybridization, followed by introgression of pathogen-resistant transgenes to wild plant, which is an ecological risk of increasing wild population in size or becoming weedier [6]. Viruses are known to be presented in wild fungi and algae, woody perennials, and herbaceous plants. This review is focused on the recent investigations of the viruses found in wild herbaceous species.

Discovery of novel viruses from wild plants. During 21st century, the level of concern with viruses, which must exist in plants under wild conditions, was rising. Several researches directed to find new virus species in wild plants in Alaska have been accomplished. A new virus named Nootka lupine vein-clearing virus (NLVCV) was isolated from *Lupinus nootkatensis* plants that were confined to a relatively small area in the Talkeetna mountains of south-central Alaska. There were apparent leaf symptoms of pronounced vein clearing and mosaic on 3- to 4-week-old plants. The nucleotide sequence of RNA-dependent RNA polymerase (RDRP) did not match any known virus, but contained short regions of identity to several carmoviruses. Only species belonging to the *Fabaceae* were susceptible to NLVCV by mechanical inoculation. Based on precise studies of this virus and similarity of the RDRP gene to that of other carmoviruses, it is suggested that NLVCV is a member of the family *Tombusviridae*, and tentatively of the genus *Carmovirus* [12].

A novel potyvirus was discovered in wild celery and kneeling Angelica (family *Apiaceae*) in the Matanuska-Susitna Valley, Alaska, named after its natural plant hosts, angelica virus Y (AnVY) (Table 1) [13].

Recent studies describe a novel mastrevirus found in grass *Digitaria didactyla* in Africa. Analysis of the sequence shows the virus to be a typical mastrevirus, with four open reading frames, two in each orientation, separated by two noncoding intergenic regions. Although it showed the highest levels of sequence identity to CSMV (68.7%), their sequences are sufficiently diverse for the virus to be considered a member of a new species in the genus *Mastrevirus*, based on the present species demarcation criteria. It was proposed that the name first used during the 1980s be used for this species, Digitaria didactyla striate mosaic virus (DDSMV) [14].

Yellow oat-grass (*Trisetum flavescens* L. Beauv) is a perennial grass native to Europe, West Asia and North Africa. Yellow oat-grass plants with mild mosaic and pronounced dwarfing symptoms were observed at different locations in the Czech Republic. Serological assays of infected plant extracts using antiserum specific to the closest species in the family *Potyviridae* were negative. Based on phylogenetic analyses of the coat protein cistron and flanking genomic regions, it was proposed to be a distinct viral species of the genus *Tritimovirus*, tentatively named Yellow oat-grass mosaic virus (YOgMV) [15].

Research in Australia revealed that a range of viruses, both indigenous and exotic, infect native orchids. A novel potyvirus was identified from a wild plant of *Diuris laxiflora* that did not exhibit symptoms. The name Donkey orchid virus A (DOVA), isolate SW3.1 was applied. Its predicted genome organization was typical of those of other potyviruses [16].

***Table* 1. Examples of viruses identified in wild herbaceous plants, presented in chronological order**

Name of virus	Family and/or Genus of virus	Name of wild host plant	Family of wild host plant	Symptoms	Author(s) and year of indication
Glycine mosaic virus (GMV)	*Comovirus*	*Glycine clandestina* and *G. tabacina*		mosaic symptoms and marginal deformation in leaves	Bowyer et al. 1980
Turnip yellow mosaic virus	*Tymoviridae Tymovirus*	*Cardamine lilacina*	*Brassicaceae*	mild	Guy and Gibbs 1985
Tobacco mild green mosaic virus (TMGMV)	*Virgaviridae, Tobamovirus*	*Nicotiana glauca*	*Solanaceae*	mild or unapparent	Rodríguez-Cerezo et al. 1991
Kennedya yellow mosaic virus	*Tymoviridae Tymovirus*	*Desmodium scorpiurus; Kennedya rubicunda*	*Fabaceae*	unapparent	Skotnicki et al. 1996
Beet western yellow virus (BWYV)	*Luteoviridae*	*Brassica oleracea*	*Brassicaceae*	yellowing of tissue	Raybould et al. 1999
Barley yellow dwarf virus (BYDV)	*Luteoviridae*	1) *Bromus hordeaceus* 2) *Setaria viridis* 1) *Setaria lutescens*	*Poaceae*	1) unupparent 2) reduction in panicle length 3) increase in panicle length	Remold 2002
Hardenbergia mosaic virus	*Potyviridae, Potivirus*	*Hardenbergia comptoniana*	*Fabaceae*	unapparent	Webster et al. 2007

Closing table 1

Name of virus	Family and/or Genus of virus	Name of wild host plant	Family of wild host plant	Symptoms	Author(s) and year of indication
Passion fruit woodiness virus	*Potyviridae, Potivirus*	*Passiflora aurantia*	*Passifloraceae*	unapparent	Webster et al. 2007
Angelica virus Y (AnVY)	*Potyviridae, Potivirus*	*Angelica lucida* L. and *A. genuflexa* Nutt.	*Apiaceae*	leaf mottling	Robertson 2007
Polygonum ringspot virus (PolRSV)	*Buniaviridae, Tospovirus*	*Polygonum convolvulus*	*Polygonaceae*	chlorotic or necrotic ringspots, mosaic and deformation	Ciuffo et al. 2008
Eragrostis curvula streak virus (ECSV)	*Geminivirigae*	*Eragrostis curvula*	*Poaceae*	mild streak	Varsani et al. 2009
Yellow oat-grass mosaic virus (YOgMV)	*Potyviridae Tritimovirus*	*Trisetum flavescens*	*Poaceae*	mild mosaic and dwarfing	Hassan 2009
Sweet potato feathery mottle virus (SPFMV)	*Potyviridae, Potivirus*	*Ipomoea* sp., *Hewittia* sp., and *Lepistemon* sp.	*Convolvulaceae*	unapparent	Arthur et al. 2010
Narcissus late season yellows virus (NLSYV)	*Potyviridae, Potivirus*	*Narcissus* sp.	*Amaryllidaceae*	leaf streaking and yellowing, leaf distortion and plant stunting	Wylie et al. 2010
Digitaria didactyla striate mosaic virus (DDSMV).	*Geminivirigae, Mastrevirus*	*Digitaria didactyla*	*Poaceae*	streak	Briddon et al. 2010
Asclepias asymptomatic virus (AsAV)	*Tymoviridae Tymovirus*	Asclepias viridis	*Apocynaceae*	unapparent	Melcher 2013
1)Bean yellow mosaic virus; Ornithogalum mosaic virus; Blue squill virus A 2) Turnip yellows virus	*1) Potyviridae, Potivirus 2)Luteoviridae, Polerovirus*	*Diuris* sp.	*Orchidaceae*	unapparent	Wylie et al. 2013

Transgenic crops and viruses. Because not so much is known about the prevalence or effects of virus infection in wild plant populations, most of our understanding of plant-virus interactions comes from economically important plants. In crops, virus infection can reduce plant growth by depressing photosynthesis, changing metabolism, and altering resource allocation. Controlling the virus vectors is mostly difficult; hence using transgenic crops is one of the most novel and popular methods to avoid losses of yield. For example, in the United States, 27 crop species with virus-resistance transgenes have been issued permits for field trials, and a handful of crops have been deregulated for commercial production (i.e., squash, papaya, and potato; Information Systems for Biotechnology, 2012). Dealing with transgenic plants we must be aware of crop–wild hybridization followed by the introgression of transgenes into wild relatives, which located in proximity to crops. This process may have certain negative consequence: the size or dynamics of the wild plant population, which is limited by pathogen attack, may increase, or wild population may become weedier due to ecological advantages obtained from virus-resistant transgenes. A research has been conducted to contribute in our awareness of such very problem. It surveyed wild *Cucurbita pepo* populations in the south-central United States over 4 years for five virus species. These populations were examined for the presence of virus-resistance transgenes. Although results of the survey constitute that the virus-resistance transgene was not present in any of the 1256 leaf samples of wild *C. pepo* collected from 21 sites over 4 years in south-central United States [2], we cannot still certainly exclude the possibility of the transgene introgression, for instance, into other wild plant in the other countries. In addition, prevalence of viruses in wild *C. pepo* was examined. Cucumber mosaic virus (CMV), Squash mosaic virus (SqMV), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV) were detected. It was also confirmed that RT-PCR is more sensitive than ELISA, and results suggest that neither method is 100% accurate. At least one of these viruses surveyed was present in 17 of 21 wild *C. pepo* populations and prevalence ranged from 4–74%, and the average prevalence for all viruses was 23%. Another interesting data is that, in field survey, 80% of infections were visually unapparent [2].

Ecogenomics: advantages and disadvantages. Several recent metagenomic studies have analyzed prokaryotic viruses in a variety of unexpected environments [7]. Metagenomics has been very valuable in directing the rethinking of the global 'virome', i.e. there are orders of magnitude more viruses in nature than previously anticipated, but it has not been able to link any viruses found in environmental samples to their hosts. Ecogenomics can fill this gap in our understanding. In addition, almost all metagenomics studies of viruses have characterized bacterial viruses, while the methods described here give us a way to analyze eukaryotic hosts and their viruses. However, the sample processing for this type of study is much more labour intensive than what is used in metagenomics, and hence ecogenomics can simply give a different perspective on the global virome [8] and, particularly, on investigation of viruses of wild plants. Clear example of this kind of method should be noticed. That was remarkable survey of viruses in wild flora of the Tall Grass Prairie Preserve in northeastern Oklahoma, and the Area de Conservacio´n Guanacaste in northwestern Costa Rica. These areas have low and high level of biodiversity respectively. dsRNA were used, as a form of nucleic acids that is generally unique to viruses, to assess RNA virus infection in plants, by converting it to cDNA through a process specific for dsRNA. The resulting cDNA then was amplified with tagged primers that could cross reference each sample to the sequences obtained by pyrosequencing in pools of 24 to 96 uniquely tagged samples [8]. As a result, there were discovering thousands of plant viruses that are generally unique, and only distantly related to known viruses. The term 'Ecogenomics' to distinguish this study from the metagenomic studies from environmental samples since given sequences are directly linked to the original plant hosts. Another essential survey on the same territory (Tall Grass Prairie Preserve) was accomplished. One considerable result was discovering novel *Tymovirus* – Asclepias as-

ymptomatic virus (Table 1) in *Asclepias viridis* [5]. Plant samples were screened for virus-like sequences in double-stranded RNA and in nucleic acids associated with particulate fractions of plant homogenates. Furthermore, among the plant specimens analyzed for amplifiable VLP-VNA, only 2.3% were noted at collection as having any symptoms of disease. In each year of harvest the proportion of samples that were PCR positive was the same among plants with and without symptoms. Thus, presence or absence of amplification was not an indicator of disease, manifested as symptoms [9]. Therefore this massive sequencing cannot allow evaluating of virulence, and gives information to understand evolutionary and ecological relationships among plant viruses in wild flora. Similar approach to identification plant viruses in wild plants is described by another research. A remarkable recent advance in plant virus discovery has been the utilization of massively parallel pyrosequencing (next-generation sequencing, 'deep' sequencing), which is capable of yielding megabases to gigabases of sequence information, coupled with bioinformatics [10]. It was described the use of a massively parallel sequencing approach whereby polyadenylated plant RNA from multiple plants was pooled and sequenced together before the output was analyzed for the presence of viral genomes. This research represents part of a project to describe the ecological roles viruses play in the indigenous flora of the south-west Australian floristic region. After analysis, complete or partial genome sequences representing 20 virus isolates of 16 polyadenylated RNA species were identified. In three cases, 2-3 distinct isolates of a virus species co-infected the same plant. Twelve of the viruses identified were described previously and belonged to the genera *Potyvirus*, *Nepovirus*, *Allexivirus*, and *Carlavirus*. Four were unknown and are proposed as members of the genera *Potyvirus*, *Sadwavirus*, and *Trichovirus* [11].

Nowadays, studying viruses persisting in non-cultivated plants become more popular. The knowledge allows us to understand general plant virus ecology better, because, previously, only viruses of economically essential plants were discovered, and an ability to make whole picture of ecological processes of plant viruses was restricted by lack of information about viruses in wild plants. Obviously, wild plants are natural reservoir of plant viruses. Thus, the plenty of surveys in order to indicate viruses of crops in wild populations have been done. As a result, it became clear that visual symptoms in wild plants are mostly unapparent. It could be suggested that milder symptoms in wild plants are connected with long-term co-evolution with certain virus, which, obviously, is not present in the population of cultivated plants. Moreover, studying viruses in wild populations seems to be useful for biotechnology: to assess a risk of virus-resistant transgene introgression into wild plant. Development of new methods of sequencing, coupled with bioinformatics, caused metagenomics, while metagenomics was followed by ecogenomics – beneficial to massive simultaneous discovery of viruses in wild flora, linked to their hosts.

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А. Байдалюк, студ., І.Будзанівська, д-р біол. наук
КНУ імені Тараса Шевченка, Київ

НОВІ ПІДХОДИ ДО ІДЕНТИФІКАЦІЇ ВІРУСІВ, ЯКІ УРАЖАЮТЬ ДИКОРОСЛІ ТРАВ'ЯНИСТІ РОСЛИНИ

Останнім часом зацікавленість у вивченні вірусів дикорослої флори поступово зростає. Це пов'язано з необхідністю кращого розуміння еволюції, екології, вірулентності вірусів рослин, і також задля уникнення економічних втрат через взаємну гібридизацію трансгенних технічних культур та диких рослин та наступної інтрогресії вірус-стійких трансгенів в дикі популяції. В даному огляді надана коротка інформація про останні внески в розвиток вивчення вірусів диких рослин. Представлені різні підходи до досліджень.

Ключові слова: віруси дикорослої флори, транс гени в дикій популяції.

А. Байдалюк, студ., И. Будзанивска, д-р биол. наук
КНУ имени Тараса Шевченко, Киев

НОВЫЕ ПОДХОДЫ К ИДЕНТИФИКАЦИИ ВИРУСОВ, КОТОРЫЕ ПОРАЖАЮТ ДИКОРАСТУЩИЕ ТРАВЯНИСТЫЕ РАСТЕНИЯ

За последнее время интерес к изучению вирусов дикорастущей флоры постепенно возрастает. Это связано с необходимостью лучшего понимания эволюции, экологии, вирулентности вирусов растений, и также во избежание экономических потерь из-за взаимной гибридизации трансгенных технических культур с дикими растениями, и, впоследствии, интрогрессии вирус-устойчивых трансгенов в дикие популяции. В данном обзоре дана короткая информация о последних вкладах в изучение вирусов диких растений. Здесь представлены разные подходы к исследованиям.

Ключевые слова: вирусы дикорастущей флоры, трансгены в дикой популяции.