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A. Kyrychenko[1], I. Shcherbatenko[1], I. Antipov[2], K. Hrynchuk[2]

[1] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Acad. Zabolotny Str., Kyiv, MSP, D03680, Ukraine
[2] National University of Life and Environmental Sciences of Ukraine
15 Heroiv Oborony Str., Kyiv, 03041, Ukraine

TYPING OF PLUM POX VIRUS ISOLATES IN THE CENTRAL UKRAINE

Aim. *During the spring of 2016 in three locations of Central Ukraine – Cherkasy, Kyiv and Yahotyn, we found plum and pear trees displaying foliar symptoms similar to those causing by an important quarantine pathogen Plum pox virus (PPV), and cherry trees showing symptoms typical for Apple mosaic virus (ApMV) – one of the most widespread viruses which can infect over 65 plants species of Rosaceae family. The aim of this study was to perform detection, identification and molecular analysis of viruses causing diseases symptoms found by us on fruit trees in Central Ukraine.* ***Methods****. Total RNA extracted from leaves of infected and healthy plants was used to generate cDNA with known PCR primers as well as with own primers designed by «Primer3» software. The amplified DNAs of Cp gene fragments of ukrainian PPV isolates were sequenced by Sanger dideoxy sequencing method and were compared with those available in GeneBank. The comparison and molecular analysis of virus isolates were performed using BLAST, MultAline and MEGA 6 softwares, and a set of own a simple computer programs (utilities), tightly specialized for the solution of a narrow objectives of sequence analysis.* ***Results.*** *The primers have been developed for Plum pox virus detection proved to be effective for revealing and molecular diagnostic of PPV in three regions of the Central Ukraine. Using these primers three PPV-D isolates, named seq1, seq2, seq3, have been selected from pears in Cherkasy (seq1) and from plums in Yahotyn (seq2) and Kyiv (seq3). According to sequence comparison of 181-base Cp gene fragments, the plum isolates seq2 and seq3 turned out to be identical among themselves and have 95.6% sequence identity to pear isolate (seq1). Ukrainian isolates obtained have shown high identity (95 - 99.4%) with all of 33 PPV-D isolates from different countries and host plants tested in our study. The 181-base sequences of these isolates contain 30 nucleotide replacements concerning seq1 sequence. The vast majority of replacements (27 of 30) are synonymous and do not cause of amino acid substitutions in the viral coat proteins. PPV-D isolates form 6 groups of members with identical nucleotide replacements, which remind Vavilov's homologous series of heritable variation of morphological traits.* ***Conclusion.*** *The primers have been developed were successfully used for detection of PPV-D isolates from pear and plum trees in three regions of Central Ukraine. It is the first time the PPV has been reported in Cherkasy.Our studies provide information about spreading PPV isolates, that can be valuable in understanding the epidemiological role of this virus. Groups of identical nucleotide replacements, revealed by us in Cp gene sequence fragments of different PPV isolates, remind Vavilov's homologous series of heritable morphological traits, and are of interest for clarification of the molecular basis of hereditary variation and parallel mutations.*

*Keywords***:** *Plum pox virus, sharka, ukrainian isolates of Plum pox virus, groups of virus isolates with identical nucleotide replacements*

Sharka is the most damaging viral disease of stone fruit trees caused by Plum pox virus (PPV) – a member of the genus *Potyvirus* in *Potyviridae*

family. Sharka is widespread virus infecting mainly plums: (*Prunus domestica*), Damson plums (*Prunus insitia*), apricots (*Prunus armeniaca*), peaches (*Prunus persica*), and blackthorns (*Prunus spinosa*). This virus reduces fruit yield and quality. It also shortens the productive lifespan of orchards and can render stone fruit trees useless for fruit production. Even symptomless trees produce reduced fruit quantities. The economic impact of PPV to the peach, plum and apricot industry worldwide is estimated to be $600 million per year [1]. PPV is an EPPO (European and Mediterranean Plant Protection Organization) A2 quarantine pest. It is also considered to be a quarantine pest by IAPSC (International Association of Professional Security Consultants) and NAPPO (North American Plant Protection Organization).

Plum pox symptoms were first observed in Bulgaria between 1915 and 1918 and in the early 20th century Bulgarian researcher proof. D. Atanassov for a first described the reason of disease – PPV [2]. The virus spread across most of Europe by the 1970s. In the late 1990s, PPV had spread in many other countries in the eastern hemisphere. The disease has not been reported yet in California (USA), Australia, New Zealand and South Africa [3].

Nine strains of the virus are known: PPV-An, PPV-C, PPV-CR, PPV-D, PPV-EA, PPV-M, PPV-Rec, PPV-T and PPV-W. The strains differ by nucleotide sequence of genomic RNA, antigenic and epidemiological properties, geographical prevalence, host range, and pathogenicity for different species of stone fruit crops [4]. PPV strains are characterized by relatively low intra-strain diversity (reaching 1.1 - 3.9% at the nucleotide level for full-length genomes, except for PPV-W, where the divergence reaches 7.9%) and by comparatively high between-strains diversity (4.4 - 22.8 %) [5].

In Ukraine virus was first found in 1967 [6]. Currently, PPV is detected in 8 regions of Ukraine (Vinnitsa, Ivano-Frankivsk, Lviv, Mykolaiv, Odessa, Ternopil, Chernivtsi) and the Crimea [7]. By the Roy and Smith (1994) [8] Ukraine belongs to the central and eastern countries zone in which PPV spread relatively early and infection levels are generally high. During the last years it was shown the presence of PPV in stone fruit plantations in different regions of Ukraine and admitted a tendency of its fast distribution through the country and on the southern coast of Crimea [6, 7].

To date only D (Dideron) strain isolates have been found infecting trees in Ukraine [9] and there are no data on the presence of the virus in cherries.

The aim of this study was to conduct diagnostic, identification and molecular analysis of viruses causing PPV infection symptoms of fruit trees found by us in Central Ukraine.

Materials and metods. The leaves displayed typical viral infection symptoms were selected from the plum (*Prunus domestica*), cherry (*Prunus avium*) and pear (*Pyrus communis*) single grown trees in green plantations of the central Ukrainian region – Cherkasy, Kyiv and Yahotyn during the spring of 2016.

Total plant RNA was extracted from PPV infected or healthy leaves using the «RIBO-sorb kit» and «Reverta-L-100» (AmpliSens, Russia) was used to generate cDNA with PPV specific primers according to the manufacturer's instructions.

For the general detection of PPV we have developed PCR primers (PPV1 and PPV2), which amplifies a 181 bp fragment of 3'-end coat protein gene.

PPV strain determination of the Ukrainian isolates was performed by RT-PCR with PPV-W and PPV-D strain-specific primers we designed.

To search for nucleotide sequences of various PPV isolates the NCBI database was used (http://www.ncbi.nlm.nih.gov). Alignment of nucleotide sequences was performed using the software «MultAline» (Multiple sequence alignment) (http://multalin.toulouse.inra.fr/multalin/). The sets of primers were designed using software «Primer3» [10].

For the direct detection of Plum pox potyvirus-cherry subgroup we used specific oligonucleotide primers PPV-SoC [11]. «OligoAnalyzer 3.1» was used to evaluate physical properties of primers.

The reaction mixture for the PCR (a volume of 20 µl) contained: 1 × PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 - 50 ng of cDNA, 0,5U Taq polymerase. All primers were used at final concentration of 5 pmol per 20 µl in PCR reaction. The amplification was performed in DNA Thermocycler "Tertsyk" TP4-PCR-01.

Table 1.

Molecular characterization of primers for PPV identification

Primers annealing points	Name	Oligonucleotide primer sequences 5' - 3'	Annealing temperature	Amplicons size
9148 - 9167 (HG964686.1)	PPV1 (F)	tcaatggaatgtgggtgatg	55-60 °C	181 bp
9309 - 9328 (HG964686.1)	PPV2 (R)	cgctgaattccataccttgg		
9382 - 9401 (HG964686.1)	PPV-W1 (F)	cctcgacaacacctgttagg		195 bp
9556 - 9576 (HG964686.1)	PPV-W2 (R)	gagaccactacactcccctca		
9382 - 9401 (HG964686.1)	PPV-D1 (F)	gacttcaacgacacccgta		197 bp
9556 - 9576 (HG964686.1)	PPV-D2 (R)	gagaccactacactcccctca		
1508 - 1527 (HG328280.1)	ApMV1 (F)	aaaaggaccaaccgagaggt		109 bp
1599 - 1616 (HG328280.1)	ApMV2 (R)	gcggcgaaattcgtctta		

For all primer sets, the same cycling conditions were used: denaturation at 94°C for 5 min, 35 cycles of amplification (94°C for 30 s, 60°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 7 min. All PCR products were analyzed by nondenaturing electrophoresis in 1,5% (w/vol) agarose gel and ethidium bromide (0.5 mg/ml) staining. The «DNA Sorbo» (AmpliSens, Russia) was used to purify DNA fragments from agarose gels.

The amplified DNAs were sequenced by Sanger dideoxy sequencing method using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA). The samples were run on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, USA). Obtained sequences were compared with those available in GeneBank using the BLAST, MultAline and MEGA 6 softwares, and a set of own a simple computer programs (utilities), tightly specialized for the solution of a narrow objectives of sequence analysis [12].

For comparative analysis the 38 nucleotide sequences belonging to PVV-D strain were retrieved from GenBank (Tabl 2).

Results and discussion. In the spring of 2016 the disease incidences were recorded at the three locations of the Central Ukraine. The samples were obtained from homeowner-grown gardens located in Cherkasy, Kyiv and Yahotyn. It should be noted that in these locations the PPV infection had not been previously reported. The leaves of plum, pear and sweet cherry showing PPV-like symptoms — chlorotic spots, bands or rings, vein clearing, leaf deformation (Fig. 1) were sampled from six different branches of the tree and analyzed for PPV infection. Also for the analysis the leaf samples of the outwardly healthy trees were collected. The orchards under the study were young – 4 - 5 years old.

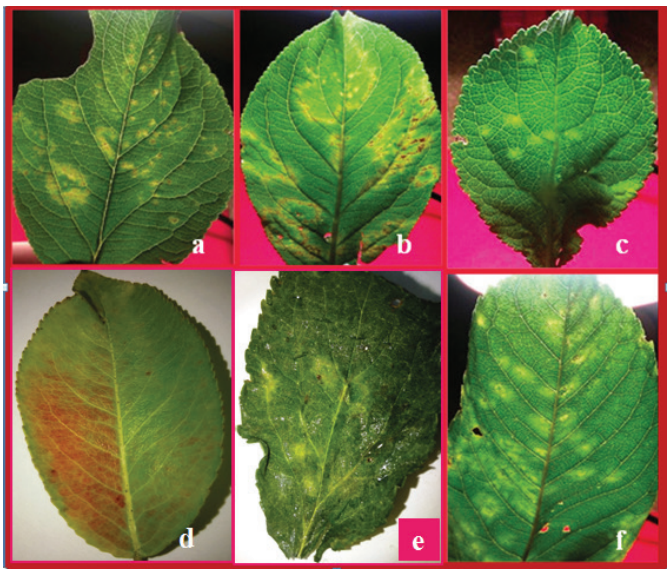

Fig. 1. Symptoms on leaves of plum (a - c), pear (d, e) and sweet cherry (f) plants

Using RT-PCR methods with general primers the virus was detected in analyzed samples of plum and pear leaves with typical sharka symptoms. After separation of amplification products on agarose gels PCR fragments of the expected size (181 bp) were obtained (Fig. 2).

Virus diseases of sweet cherry were observed in private cherry orchards of Yahotyn (Kyivsky region). The plants showed severe symptoms, including mosaic, yellow to creamy line pattern, diffused chlorotic rings and spots. Being that the symptoms on the leaves were very similar to the symptoms caused by Apple mosaic virus (ApMV), a RT-PCR with specific primers has been applied to the detection of this ilarviruses. The results of the analysis show that isolate from cherry tree failed amplification neither with Plum pox mosaic virus (PPV1, PPV2) specific primers nor Apple mosaic virus (SoC-2) ones. Both viruses were not detected in the samples. Even though our tests were limited of two cherry viruses, the results obtained suggested that further investigation on Ukrainian PPV isolates and viruses infecting sweet cherry is necessary and should be carried out more thoroughly.

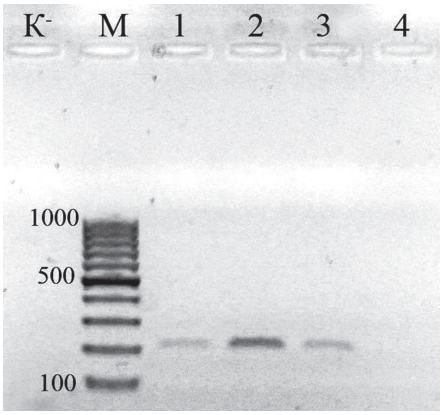

Fig. 2. Agarose gel electrophoretic analysis of RT-PCR amplification products from leaf samples infected with PPV isolates. Lane M – O'GeneRuler™ DNALadder, #SM 0241 (Fermentas); lane 1 – isolate from pear (Cherkasy); lane 2 – isolate from plum (Yahotyn); lane 3 – isolate from plum (Kyiv); lane 4 – isolate from cherry tree (Yahotyn); lane K – negative control. The PPV-specific primers PVP1/PVP2 were used.

In order to differentiate PPV strains RT-PCR with subgroup specific primers enabling direct discrimination of PPV-D and PPV-W isolates was done. PPV-D strain specific primers produce amplicons with expected size 197 bp (Fig. 3). Thus, the molecular analyses have confirmed the presence of the PPV-D strain in all prunus and pear hosts. No Amplification was observed with primers, specific to PVP-W virus strains. Thus, PCR-based identification and strain typing confirmed that PPV-D strains were present in different prunus and pear hosts.

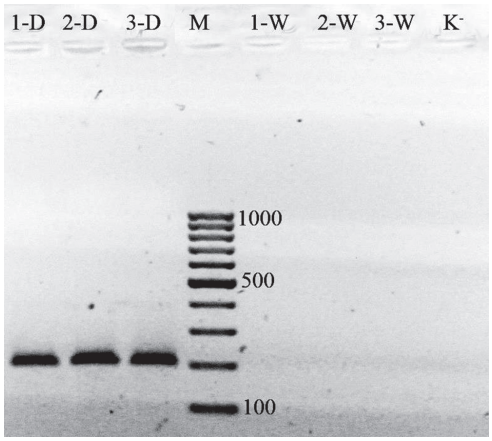

Fig. 3. Agarose gel electrophoretic analysis of RT-PCR amplification products from leaf samples infected with PPV isolates. Lane M – O'GeneRuler™ DNALadder, #SM 0241 (Fermentas); lane 1D, 1W – isolate from pear (Cherkasy); lane 2D, 2W – isolate from plum (Yahotyn); lane 3D, 3W – isolate from plum (Kyiv); lane K – negative control. The PPV-W1/W2, PPV-D1/D2 primers were used

In the present work, the partial genome sequence covering 181-base fragment of CP coding regions of three PPV isolates were aligned with the respective sequences of PPV-D strains available in the GenBank database. Sequence comparisons for a PCR fragments of pear isolate from Cherkasy (seq1) and plum isolates from Yahotyn and Kyiv (seq2, seq3) showed a very high nucleotide identity between themselves and with those of available in databases. All studied isolates was separated into one clade and clustered

together with each other strongly reducing efficiency of the phylogenetic analysis using the popular MEGA 6 program.

For this reason in our work also was used a set of own a simple computer programs (utilities) tightly specialized for the solution of specific objectives of comparative sequence analysis such as definition of types, positions and number of nucleotide replacements etc [12].

It was established that the selected 181-base PCR fragments seq1, seq2 and seq3 contain the TAG terminal codon (the end of coat protein gene) and the first 8 nucleotides of the 3' untranslating region. Plum isolates from Yahotyn and Kyiv (seq2 and seq3) turned out to be identical among themselves and have shown 95.6% of identity to pear isolate from Cherkasy (seq1). The seq1 have the most high level of identity (99.4%) with another ukrainian isolate KJ914573.2, which have replacement of G by A in 26 position (Table 2, number 2). A high level of seq1 identity is observed also: with turkey peach (KX423945) and plum (KX423949) isolates (96.7%; 4, 1, and 1 nucleotide substitutions within three sequence fragments; Table 2, numbers 6 and 7); with ukrainian isolate KR028389 (96.1%; 3, 1, and 3 nucleotide substitutions, Table 2, number 3); with our seq2 and seq3 isolates, germanian isolate Ku508427 and 26 PPV isolates from different countries and host plants (95.6%; 4, 1 and 3 substitutions; Table 2, numbers 4, 5 and 8). The seq1 identity with japanese AB560080 and france X56258 isolates is equal to 95.0% (5, 1, 3 and 3, 2, 4 nucleotide substitutions, respectively; Table 2, numbers 9 and 10).

Table 2.

Nucleotide substitution in the Plum pox virus Cp gene fragments

Length [1]	Sequence [2]	Number [3]
1 - 60	TGCGTGCACGTGAAGCTCATATACAGATGAAGGCAGCAGCATTGAGAAATGTTCAGAATC	1
	.....a....	2
	a..g.....c.....	3
	a..g.....c.....a....	4,5,6,7,8
	.a..g.....c.....c.....a....	9
	.....g.....cc.....	10
61 - 120	GTTTATTTGGCTTGGATGGAAACGTCGGAACACAAGAAGAGGACACAGAGAGGCACACCG	1
	.....	2
	.....a.....	3,4,5,6,7,8,9
	.....t..t.....	10
121-181	CTGGTGACGTGAATCGCAACATGCACAACCTCCTCGGTGTGAGGGGAGTGTAGTGGTCTCA	1
	.....	2
	.....t.....	6,7
	.....t..t.....g	3,4,5,9
	.....t..t.....a.....	8
	.....t..t.....t.....a.....	10

[1] 60-nucleotide regions of coat protein gene 3'-end sequences. [2] Sequence regions of tested viruses with identical nucleotides (points), base substitutions (small letters) and the absence of eleven terminal nucleotides in the KJ914573.2 sequence (11 spaces). [3] Numbers of viral sequences with their accessions: **1** – pear isolate from Cherkasy (seq1); **2** – KJ914573.2; **3** KR028389; **4**– plum isolates from Yahotyn (seq2) and Kyiv (seq3); **5** – 26 sequences: AB576049, AB576071, AB576075, AM260934, AY591253, AY750961, AY953261, AY953266, DQ299538, DQ883816, HG452356, HG452357, JN637991, KC417483, KJ849228, KJ914573.2, KP198598, KP998124, KR028387, KT595211, KT595213, KU948432, KX423948, KX423952, X16415, X81079; **6** – KX423945; **7** – KX423949; **8** – KU508427; **9** – AB576080; **10**– X56258

All 32 PPV-D isolates containing a coat protein gene fragments which correspond to seq1, seq2 and seq3 sequences turned out to be highly identical (95.0 – 99.4%). The vast majority of their nucleotide replacements (27 of 30) are synonymous which localized in the third codon position and does not cause of amino acid substitutions in the viral coat proteins. Two nonsynonymous replacements of G by A in the first codon positions (Table 2, numbers 8 and 10) cause substitution of alanine (GCA) by threonine (ACA), and T by C replacements in second codon position leading to valine (GT*) by alanine (GC*) substitution (Table 2, number 10).

The PVV isolates analyzed form 10 groups, which contain from 2 to 26 members with identical nucleotide replacements. This phenomenon reminds Vavilov's homologous series of heritable morphological traits. [13]. Elucidations of molecular basis for Vavilov's homologous series are, currently, restricted to only few studies on variation of meiosis [14], rare amino acid replacements [15] and analysis of important plant traits on the gene level [16]. These experiments were conducted on eukaryotic organisms. The groups of virus isolates containing identical nucleotide replacements and reminding Vavilov's homologous series are presented in this report.

The reason of homologous series, according to our assumption, may be the existence of several genetic codes within each genome [17, 18]. So a mutation possibility is strongly limited to some gene regions because of participation of many gene nucleotides in conservative (functionally important) sites of different genetic codes coexisting within genomes.

Conclusion. The primers for Plum pox virus detection and identification by RT-PCR assay have been developed and their affectivity was proved in our investigation. Using these primers three isolates (seq1, seq2 and seq3) of Plum pox virus originating from *Prunus domestica* L., *Prunus avium* L. and *Pyrus communis* L. trees have been revealed in the Central Ukraine. It is the first time the PPV has been reported in Cherkasy region. The presence of PPV was not detected in the plum and pear trees surrounding the diseased ones and in the cherry trees showing diseases symptoms.

Our studies provide information about spreading PPV isolates in the Central Ukraine that can be valuable in understanding the epidemiology of this virus. Plum isolates from Yahotyn and Kyiv (seq2 and seq3) turned out to be identical among themselves and have shown 95.6% of identity to pear isolate seq1 from Cherkasy. All ukrainian isolates turned out to be highly identical (95.0 – 99.4%) to PPV-D isolates from different countries and host plants containing a coat protein gene fragments, which correspond to seq1, seq2 and seq3 sequences. The vast majority of their nucleotide replacements (27 of 30) are synonymous, localized in the third codon position and does not cause of amino acid substitutions in the viral coat proteins.

Nucleotide replacements of the PVV isolates analyzed remind Vavilov's homologous series of hereditary variations. The reason of this phenomenon, according to our assumption, may be a rigorous restriction of a mutation possibility to some gene regions because of participation of many nucleotides in conservative (functionally important) sites of many genetic codes coexisting within genomes [17, 18].

Кириченко А.М. [1]***, Щербатенко І.С.*** [1]***, Антіпов І.О.*** [2]***, Гринчук К.В.*** [2]

[1] *Інститут мікробіології і вірусології ім. Д.К.Заболотного НАН України, вул. Академіка Заболотного, 154, Київ ДСП, Д03680, Україна;*
[2] *Національний університет біоресурсів і природокористування України, вул. Героїв Оборони, 15, Київ, 03041, Україна*

ТИПУВАННЯ ІЗОЛЯТІВ ВІРУСУ ШАРКИ СЛИВ, ВИЯВЛЕНИХ В ЦЕНТРАЛЬНІЙ УКРАЇНІ

Резюме

Мета. Навесні 2016 р. в трьох регіонах Центральної України – Черкасах, Києві та Яготині, нами виявлені фруктові дерева сливи та груші з симптомами ураження, характерними для карантинного патогена – вірусу шарки сливи (Plum pox virus, PPV), а також дерева вишні з симптомами ураження вірусом мозаїки яблуні (Apple mosaic virus, ApMV), патогенним для більше, ніж 65 видів рослин родини Розоцвітих. Мета цієї роботи – провести діагностику, ідентифікацію та молекулярний аналіз вірусів, що викликають виявлені нами симптоми ураження фруктових дерев в центральній частині України. **Методи.** Тотальну РНК, екстраговану з листків інфікованих і здорових рослин, використовували для отримання кДНК за допомогою відомих ПЛР праймерів, а також власних праймерів, розроблених за програмою «Primer3». Ампліфіковані фрагменти ДНК гена Ср українських ізолятів вірусу секвенували методом Sanger і порівнювали з аналогічними сиквенсами з генетичної бази GeneBank. Для порівняльного та молекулярного аналізу ізолятів вірусів використовували комп'ютерні програми BLAST, MultAline та MEGA 6, а також низку власних коротких програм (утиліт), вузько спеціалізованих для конкретних задач аналізу сиквенсів. **Результати**. Праймери, розроблені для типування PPV, виявились ефективними для виявлення і молекулярної діагностики цього вірусу в трьох регіонах Центральної України. За допомогою цих праймерів три ізоляти PPV-D (seq1, seq2, seq3) були виділені з груш у Черкасах (seq1), а також із слив в Яготині (seq2) та Києві (seq3). За порівнянням сиквенсів 181-нуклеотидних фрагментів гена Ср ізоляти seq2 і seq3 виявились ідентичними між собою і близько спорідненими й з ізолятом seq1. Виділені українські ізоляти мають високу ідентичність (95 – 99,4%) з усіма 33-ма ізолятами PPV-D з різних країн світу і різних рослин-хазяїв, протестованими нами. 181-нуклеотидні сиквенси 33-х ізолятів містять 30 замін нуклеотидів відносно сиквенсу seq1. Переважна більшість замін (27 з 30) є синонімічними і не викликають заміщень амінокислот у вірусних білках. Ізоляти PPV-D утворюють 6 груп представників з однаковими замінами нуклеотидів, що нагадує гомологічні серії спадкової мінливості морфологічних ознак, відкриті Н.І. Вавиловим. **Висновки**. Праймери, розроблені нами, виявились ефективними для детекції ізолятів PPV-D з рослин груш і слив у трьох регіонах Центральної України. Наявність PPV в Черкаській області вперше виявлено нами. Отримані дані є важливими для розуміння епідеміологічної ролі цього вірусу. Виявлені нами групи ізолятів PPV з однаковими замінами нуклеотидів нагадують серії однакових морфологічних ознак, відкриті Н.І. Вавиловим, і являють інтерес для з'ясування молекулярних основ детермінації гомологічних серій спадкової мінливості та паралельних мутацій.

Ключові слова: Plum pox virus, sharka, вірус шарки сливи, українскі ізоляти вірусу шарки, групи ізолятів вірусу з ідентичними замінами нуклеотидів.

Кириченко А.Н. [1]***, Щербатенко И.С.*** [1]***, Антипов И.А.,*** [2] ***Гринчук*** [2] ***К.В.***

[1] *Институт микробиологии и вирусологии им. Д.К.Заболотного НАН Украины, ул. Академика Заболотного, 154, Киев ДСП, Д03680, Украина;*
[2] *Национальный университет биоресурсов и природопользования Украины, ул. Героев Обороны, 15, Киев, 03041, Украина*

ТИПИРОВАНИЕ ИЗОЛЯТОВ ВИРУСА ШАРКИ СЛИВ, ОБНАРУЖЕННЫХ В ЦЕНТРАЛЬНОЙ УКРАИНЕ

Резюме

Цель. Весной 2016 г. в трех регионах Центральной Украины – Черкассах, Киеве и Яготине, нами выявлены фруктовые деревья сливы и груши с симптомами поражения, характерными для карантинного патогена – вируса шарки сливы (Plum pox virus, PPV), и деревья вишни с симптомами поражения вирусом мозаики яблони (Apple mosaic virus, ApMV), патогенным для более 65 видов растений семейства Розоцветных. Цель данной работы – провести диагностику, идентификацию и молекулярный анализ вирусов, вызывающих выявленные симптомы поражения. **Методы**. Тотальную РНК, экстрагированную из листьев инфицированных и здоровых растений, использовали для получения кДНК с помощью известных ПЦР праймеров, а также собственных праймеров, разработанных посредством программы «Primer3». Амплифицированные фрагменты ДНК гена Cp украинских изолятов вируса секвенировали методом Sanger и сравнивали с аналогичными сиквенсами из генетической базы GeneBank. Для сравнительного молекулярного анализа изолятов вирусов использовали компьютерные программы BLAST, MultAline и MEGA 6, а также набор собственных коротких программ (утилит), узко специализированных для решения конкретных задач анализа сиквенсов. **Результаты**. Праймеры, разработанные для типирования PPV, оказались эффективными для выявления и молекулярной диагностики данного вируса в трех регионах Центральной Украины. С помощью этих праймеров три изолята PPV-D (seq1, seq2, seq3) были выделены из груш в Черкассах (seq1), а также из слив в Яготине (seq2) и Киеве (seq3). При сравнении сиквенсов 181-нуклеотидных фрагментов гена Cp изоляты seq2 и seq3 оказались идентичными между собой и близко родственными изоляту seq1. Выделенные украинские изоляты имеют высокую идентичность (95 – 99,4%) со всеми 33-мя изолятами PPV-D из разных стран мира и разных растений-хозяев, протестированными нами. 181-нуклеотидные сиквенсы 33-х изолятов содержат 30 замен нуклеотидов относительно seq1. Подавляющее большинство замен (27 из 30) являются синонимичными и не вызывают замещений аминокислот в вирусных белках. Изоляты PPV-D образуют 6 групп, представители которых имеют одинаковые замены нуклеотидов, что напоминает гомологические серии наследственной изменчивости морфологических признаков, открытые Н.И. Вавиловым. **Выводы**. Праймеры, разработанные нами, оказались эффективными для детекции изолятов PPV-D из растений груш и слив в трех регионах Центральной Украины. Наличие PPV в Черкасской области впервые выявлено нами. Полученные данные являются важными для понимания эпидемической роли данного вируса. Выявленные нами группы изолятов PPV с одинаковыми заменами нуклеотидов представляют интерес для выяснения молекулярных основ детерминации гомологических серий наследственной изменчивости и параллельных мутаций.

Ключевые слова: Plum pox virus, sharka, вирус шарки сливы, украинские изоляты вируса шарки, группы изолятов вируса с идентичными заменами нуклеотидов.

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