

**Mishchenko L.T.¹, Dunich A.A.¹, Shevchenko T.P.¹, Budzanivska I.G.¹,
Polischuk V.P.¹, Andriychuk O.M.¹, Molchanets O.V.¹, Antipov I.O.²**

¹ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv,
Volodymyrska str., 64/13, Kyiv, 01601, Ukraine

²National University of life and environmental sciences of Ukraine,
Heroyiv Oborony str., 15, Kyiv, 03041, Ukraine

DETECTION OF SOYBEAN MOSAIC VIRUS IN SOME LEFT-BANK FOREST-STEPPE REGIONS OF UKRAINE

Soybean (Glycine max L.) is strategic legumes world crop of XXI century, which is located in the center of attention of world agricultural science and production. Soybean mosaic virus (SMV) is the most prevalent virus and is recognized as the most serious, long-standing problem in many soybean producing areas in the world. Infection by SMV usually results in severe yield losses, seed quality, seedling viability reduction of soybean. Study of soybean viruses in Ukraine were conducted exclusively on the Right Bank of its parts. However, studies of possible spreading and circulation of these viruses in a left-bank forest-steppe has not yet been carried out. The aim of the study was to diagnose of viruses that infect soybeans in the territory of left-bank Ukraine, and investigate some of their properties. Methods: visual diagnostics, transmission electron microscopy, ELISA, RNA extraction from plant material, PCR, sequencing, phylogenetic analysis. Results. It was studied 29 samples of soybean plants in the territory of left-bank forest-steppe of Ukraine (Poltava, Sumy and Kharkiv region) on the presence of a viral infection. ELISA revealed that 48% of the samples (13 samples from the Poltava region and 1 - from Sumy) affected with Soybean mosaic virus. This is the first report about infecting soybean grown in a left-bank of Ukraine with SMV. On the basis of previously submitted data on circulation in the territory of the right bank of the country mixed infection of this virus with Bean yellow mosaic virus (BYMV) and Alfalfa mosaic virus (AMV), the test samples were checked on the presence of these viruses. Results of the analysis showed the absence of BYMV and AMV antigens. It was found that the studied SMV isolates differ in virions size from the previously identified Ukrainian SMV isolates. Phylogenetic analysis of the nucleotide sequence of the capsid protein gene of Soybean mosaic virus showed a 100% level of phylogenetic relatedness between the Ukrainian representative isolate and Chinese, Iranian isolates, American isolate 452, and Polish isolate M, which testifies to their common origin.

Keywords: Glycine max, Soybean mosaic virus, Bean yellow mosaic virus, Alfalfa mosaic virus, ELISA, PCR, sequencing, viruses morphology, phylogenetic analysis.

Soybean mosaic disease was first documented in the USA in 1915 [1] and its viral etiology in the detail was described by Gardner and Kendrick [2]. Since then, the virus has been found in China, Japan, South Korea, Canada, Brazil, Australia and other countries where soybean is grown, and in Ukraine [3-5].

Now it is known that soybean mosaic disease is caused by *Soybean mosaic virus (Potyvirus, Potyviridae)*, and that it is the most widespread and is recognized as the most serious, long-standing problem in many soybean producing areas in the world [6]. Infection by SMV usually results in severe yield losses from 8% to 50% [7] under natural field conditions and reach up to 100% in

severe outbreaks [8]. SMV-infection can cause change chemical composition of the seed, reduce seedling viability [9] and cause seed coat mottling.

Since SMV is a seed-borne viral pathogen and aphids efficiently spread it from plant to plant while they feed, it is difficult to control the virus and produce SMV-free seeds. Furthermore, SMV often infects soybeans with other viruses: *Bean pod mottle virus*, *Alfalfa mosaic virus*, *Tobacco ringspot virus* [6, 10] and *Bean yellow mosaic virus* [4]. The most effective and efficient measures to protect plants against viral diseases that interrupt the circulation of the virus in soybeans and significantly reduce their harmfulness is timely diagnostics of diseases, creation and cultivation of resistant soybean varieties and the use of virus-free seed.

First report about SMV in Ukraine was in 1938. Later this virus was detected in soybean fields in eastern and southern regions [3]. A more detailed study of the properties and origin of the Ukrainian SMV isolates were conducted in 2011-2012 only on the right bank part of Ukraine. Thus, a mixed infection of SMV with *Bean yellow mosaic virus* [4] and *Alfalfa mosaic virus* [10] in soybeans in Kyiv, Cherkassy and Vinnitsa regions was detected. However, a detailed study of viruses' circulation in soybeans under natural conditions of left-bank forest-steppe has not yet been carried out.

In view of the above, the aim of the study was to conduct the diagnostics and identification of viruses that infect soybeans in the territory of left-bank Ukraine, and investigate some of their properties.

Materials and methods. Plant samples with disease symptoms of a viral etiology were selected for soybean crops in Poltava, Kharkiv and Sumy regions [11].

Biological tests carried out by mechanical inoculation of indicator plants in phase two true leaves with sap of infected soybeans.

Morphology of virus particles was determined by transmission electron microscopy method. Contrasting has been made with 2% solution of phosphorotungstic acid [12]. Virions are investigated using electron microscope JEM 1230, 1400 (JEOL, Japan).

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with anti-SMV-IgG, anti-BYMV-IgG and anti-AMV-IgG («Loewe», Germany) were used for viruses identification. The results of reaction registered on the reader (Termo Labsystems Opsi, USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 nm. All samples showing values three times higher than the negative control are assumed as virus positive [13].

Total RNA was extracted from virus-infected soybean leaves using the Genomic DNA purification kit (Thermo Scientific, USA). The results were checked by RNA electrophoresis in 1.5% agarose gel used as a dye ethidium bromide solution at the concentration of 0.5 mg / ml.

RT-PCR was carried out using Qiagen one Step RT-PCR kit+Q-solution (Qiagen, USA) and thermo cycler (Genetic research instrumentation LTD, UK).

A pair of the specific oligonucleotide primers: SMV1 (5' tgcagcagaagcttaccattga 3'), SMV2 (5' tgtaactcccagagagctg 3') were used for amplification of the part (194 bp) of the SMV capsid protein gene. Places of primers annealing

have noted the example of the nucleotide sequence of isolate which is included in the GenBank under accession number GU015011.1.

Thermal cycling conditions were: 95°C for 15 min, 95°C for 30 s, 56°C for 30 s, 72°C for 1 min, 72°C for 5 min. Analysis of PCR amplification products was performed by electrophoresis in 1.5 % agarose gel using a DNA marker Gene Ruler Plus 100 bp (Fermentas, USA) [14].

Amplification products (cDNA) were purified using a set of reagents High Pure PCR Product Purification Kit (Roche Applied Science, Germany). Sequencing of purified amplified fragments was performed on the Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 (Applied Biosystems, USA).

Identification and comparison of the sequence with sequences of SMV isolates from GenBank were performed using BLAST analysis ([http:// www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The phylogenetic analysis was performed using MEGA 7 software. Phylogenetic tree was constructed by maximum likelihood algorithm (ML). Bootstrap test with 1000 bootstrap replications was used to check the reliability of the constructed trees.

Statistical analysis of experimental data was performed by parametric criteria of normal distribution option, the standard deviation of the mean values – with the conventional method using computer programs MS EXCEL [15].

Results. In 2015 observation of soybean crops in the fields in Poltava, Sumy and Kharkiv regions showed symptoms of leaves rugosity, clearly marked dark green bloating between the veins (Fig.1).



Fig. 1. Symptoms of rugosity and clearly marked dark green bloating on the leaves:
1 – sample № 27, Sumy region; 2 – sample № 17, Poltava region

Plants with specified symptoms (29 samples) were selected for further studies.

According to the scientific literature symptoms similar to we have identified may cause *Soybean mosaic virus*, *Alfalfa mosaic virus* and *Bean yellow mosaic virus* [4, 10].

In order to identify a pathogens detected in soybean was preformed ELISA using antisera to viruses that are common to this culture in the world and are found in Ukraine, as well as similar morphological characteristics (*Soybean mosaic virus* (SMV), *Alfalfa mosaic virus* (AMV) and *Bean yellow mosaic virus* (BYMV)).

The analysis showed that 48% of selected samples contained antigens of

Soybean mosaic virus (SMV), the largest number of the tested samples were selected in the Poltava region (samples №№ 2, 3, 5, 9, 10, 12-15, 17-19 and 21) (Fig. 2). Only the one sample (№ 27), containing SMV antigens was detected on the territory of Sumy region (Fig. 3).

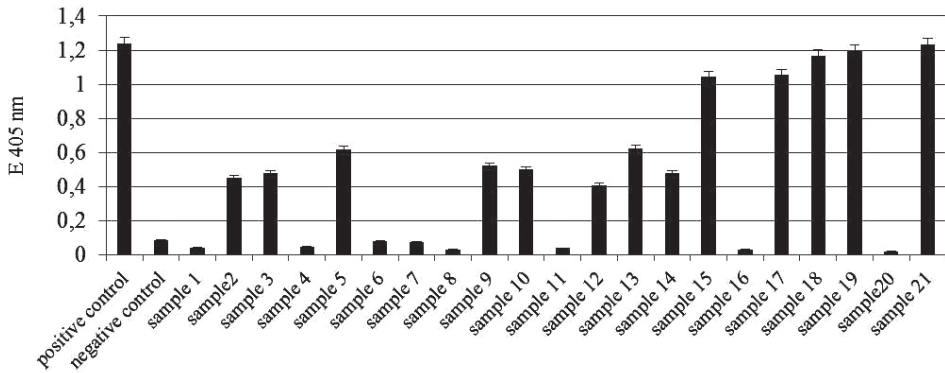


Fig. 2. Content of the *Soybean mosaic virus* antigens in soybean samples, Poltava region

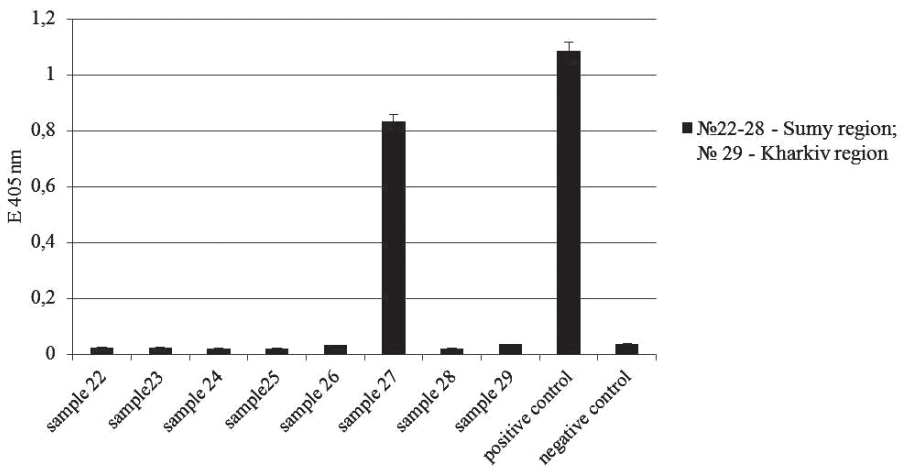


Fig. 3. Content of the *Soybean mosaic virus* antigens in soybean samples, Sumy and Kharkiv region

This is the first report about SMV affecting of soybeans grown in left-bank forest of Ukraine.

Due to the reports of soybean infection in Ukraine with *Alfalfa mosaic virus* and *Bean yellow mosaic virus* studied samples were tested on the presence of these pathogens. ELISA results showed no antigens of BYMV and AMV in all tested samples.

The electron microscopy method revealed that the studied isolates of *Soybean mosaic virus* are filamentous virus particles. However, they differ greatly in virions length depending on the soybean sample. It was found three modal lengths of viral particles: 530-550 nm, 620-650 nm and 800-820 nm with a width of 14-17 nm (Fig. 4).

Morphology of investigated SMV isolates is similar to viruses from genus *Potyvirus*, *Potyviridae*, which are filamentous virions (680-900 × 11-13 nm). According to the literature previously isolated Ukrainian SMV isolates have 650-750 × 17-18 nm [10] and 700-760 nm [4].

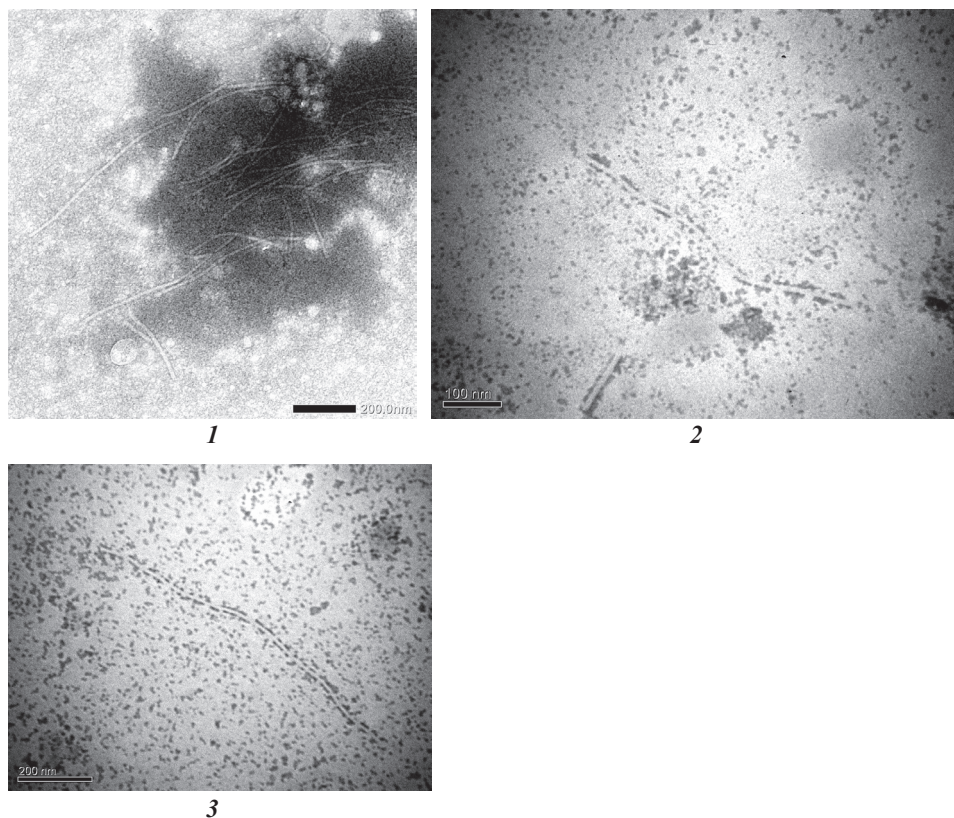


Fig. 4. Electron microscopy picture of Soybean mosaic virus isolates detected in soybean samples from left-bank Ukraine: 1 – 620-650 nm (JEM-1400); 2 – 530-550 nm; 3 – 800-820 nm (JEM-1230)

To investigate the belonging to strain clusters and genetic relatedness of studied SMV isolate with already known and described in the literature, we have conducted the RT-PCR. RT-PCR showed the presence of *Soybean mosaic virus* in the sample №17 from Poltava region (Pol-17), as evidenced by the presence in agarose gel amplicons of appropriate size (194 bp) (Fig 5).

Phylogenetic analysis of the nucleotide sequence of the part of capsid protein gene (positions 9505-9699 bp) of SMV isolate from Poltava region

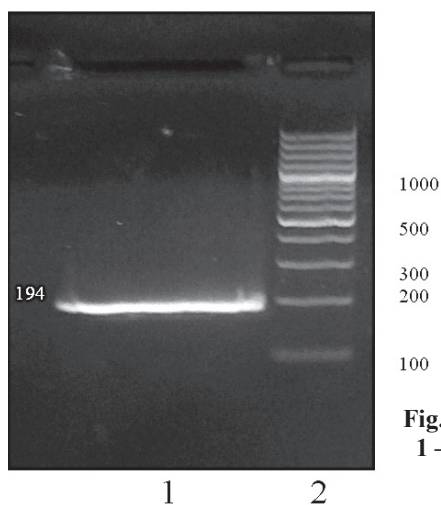


Fig. 5. The electrophoregram of RT-PCR products: 1 – cDNA, soybean sample № 17, Poltava region. Product size - 194 bp; 2 – markers (100 bp, Fermentas)

(Pol-17) and 36 isolates and strains of this virus (Table 1) showed a 100% level of homology between the Ukrainian representative isolate with a number of Asian, European and American isolates (Fig. 6).

Table 1.

Identity of CP gene nucleotide sequences between Ukrainian SMV isolate Pol-17 and known strains of this virus

	SMV Isolate/strain	GenBank accession No	Country	Host	References	Similarities to Pol-17 isolate, %
1.	HB-S19	KR065491	China	<i>Glycine max</i>	Lin et al., Unpublished	100
2.	HB-S23	KR065495	China	<i>Glycine max</i>	Lin et al., Unpublished	100
3.	HB-S25	KR065497	China	<i>Glycine max</i>	Lin et al., Unpublished	99,3
4.	HB-S27	KR065499	China	<i>Glycine max</i>	Lin et al., Unpublished	100
5.	XFQ001	KP710871	China	<i>Glycine max</i>	[16]	97,9
6.	XFQ010	KP710874	China	<i>Glycine max</i>	[16]	99,3
7.	XFQ014	KP710876	China	<i>Glycine max</i>	[16]	100
8.	LJZ002	KP710865	China	<i>Glycine max</i>	[16]	95,6
9.	LJZ010	KP710866	China	<i>Glycine max</i>	[16]	95,6
10.	SC7-N	KP710868	China	<i>Glycine max</i>	[16]	95,6
11.	ChS	AH008450	China	<i>Glycine max</i>	[17]	100
12.	SX-Z	KP710870	China	<i>Glycine max</i>	[16]	99,3
13.	A	KM886930	Poland	<i>Glycine max</i>	[18]	95,6
14.	M	KM886929	Poland	<i>Glycine max</i>	[18]	100
15.	Ar33	KF297335	Iran	<i>Glycine max</i>	[19]	100
16.	Go11	KF135491	Iran	<i>Glycine max</i>	[19]	100
17.	Lo3	KF135490	Iran	<i>Glycine max</i>	[19]	100
18.	strain 452	AY216485	USA	<i>Glycine max</i>	[17]	100
19.	VA2	AF200584	USA	<i>Glycine max</i>	[17]	99,3
20.	strain 1083	AY216481	USA	<i>Glycine max</i>	[17]	99,3
21.	G1	FJ640977	USA	<i>Glycine max</i>	[20]	95,6
22.	G2	S42280	USA	<i>Glycine max</i>	[20]	94,1
23.	G3	FJ640978	USA	<i>Glycine max</i>	[20]	95,6
24.	G6	AF200569	USA	<i>Glycine max</i>	[17]	95,6
25.	L	EU871724	Canada	<i>Glycine max</i>	[21]	94,1
26.	L-RB	EU871725	Canada	<i>Glycine max</i>	[21]	93,3
27.	NP-C-L	HQ166265	Canada	<i>Glycine max</i>	[22]	91,8
28.	NP-L	HQ166266	Canada	<i>Glycine max</i>	[22]	94,1
29.	G5H	FJ807701	South Korea	<i>Glycine max</i>	[23]	95,6
30.	strain G6H	FJ640981	South Korea	<i>Glycine max</i>	[23]	95,6
31.	WS101	FJ640957	South Korea	<i>Glycine soja</i>	[23]	95,6
32.	WS32	FJ640954	South Korea	<i>Glycine soja</i>	[23]	95,6
33.	severe strain	AJ312439	China	<i>Pinellia ternata</i>	[24]	97,9
34.	Am	KC845322	China	<i>Atractylodes macrocephala</i>	Niu Y.B., 2013 Unpublished	88,5
35.	NN	KF982784	China	<i>Pinellia pedatisecta</i>	Lu Unpublished	80,7
36.	HZ1	AJ628750	China	<i>Pinellia ternata</i>	[25]	85,2

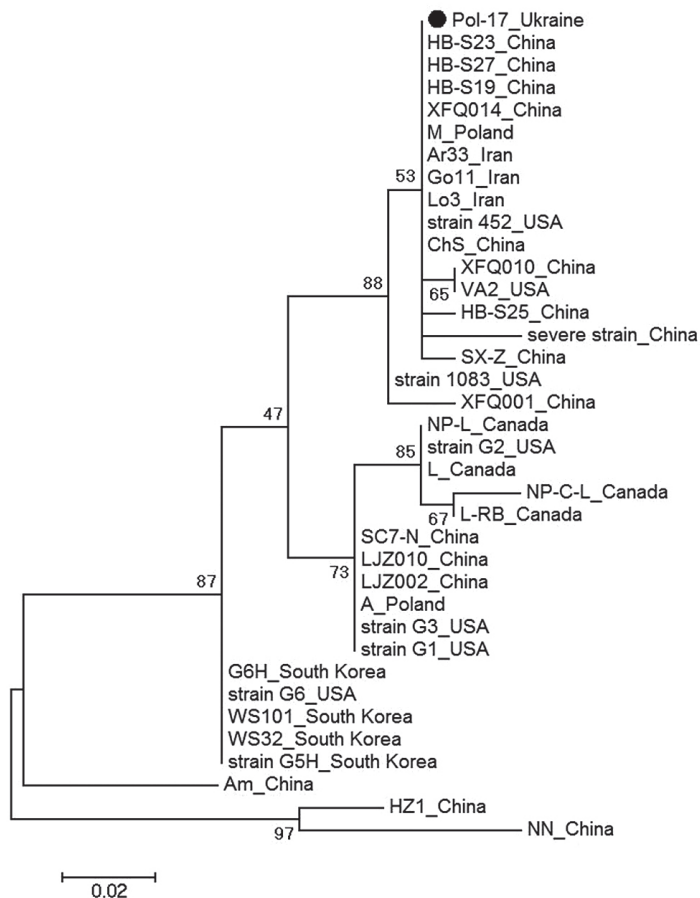


Fig. 6. Maximum likelihood (ML) tree resulting nucleotide sequences of part of the CP gene of Ukrainian isolate Pol-17 and different isolates from other countries (names and GenBank accession numbers are given in Table 1). The phylogenetic tree was constructed applying Jukes-Cantor model

As is evident from the phylogenetic tree, isolate Pol-17 belong to the one cluster and has a common origin with Chinese isolates HB-S19, HB-S23, HB-S27, SX-Z, XFQ014, ChS, Iranian and M isolate of Polish and American strain 452 (Fig. 6).

Topology of the tree constructed using nucleotide sequences of the part of SMV capsid protein gene was very similar to that recently described for complete genome sequences of SMV isolates [16]. According to this classification, 83 isolates were divided into four clades – from I to IV. According to it, isolate Pol-17, that has 100% homology with the Chinese isolate XFQ014, belongs to clade IV, together with Iranian isolates Ar33, Go11, Lo3 and Canadian isolates L, L-RB, NP-C-L, NP-L.

This is the first phylogenetic analysis of SMV isolate from the left bank of Ukraine. Earlier Sherepitzko et al. isolated SMV from soybean on the right bank of the country (Vinnitsya region). It was investigated that Ukrainian isolate SMV (UA1Gr) for sequences of the coat protein coding region and P1 coding region has strong genetic relationships with SMV-VA2 isolate which together were sorted in one clade with G2 strain [5]. However, we cannot include Ukrainian isolate UA1Gr to our analysis because studied areas of CP gene do not overlap.

Discussion. For the first time observation of soybean crops in the fields of left-bank forest-steppe of Ukraine (Poltava, Sumy and Kharkiv region) on the presence of viral diseases is carried out. Pathogen that causes symptoms of rugosity and clearly marked dark green bloating on the leaves was identified (ELISA). It was found that 48% of tested samples are affected with *Soybean mosaic virus*. This is the first report about detection of this virus on soybean in a left-bank of Ukraine. Due to the reports about previously detection of mixed infection of SMV with *Alfalfa mosaic virus* [10] or *Bean yellow mosaic virus* [4] in Kyiv, Cherkassy and Vinnitsa regions, 29 of soybean samples were tested on their presence. ELISA results showed no antigens BYMV and AMV in all tested samples.

Our research showed that SMV isolates from the Poltava and Sumy regions had a size of $500 \text{ to } 850 \times 13\text{-}17 \text{ nm}$. So, the length of virions differs from previously isolated SMV in Ukraine. According to the literature, the Ukrainian SMV isolates have sizes of $750 \pm 25 \text{ nm}$ [3], $650\text{-}750 \times 17\text{-}18 \text{ nm}$ [10], $700\text{-}760 \text{ nm}$ [4].

Phylogenetic analysis of the nucleotide sequence of the capsid protein gene of *Soybean mosaic virus* showed a high level of phylogenetic relatedness between the representative Ukrainian isolate and Chinese, Iranian isolates, American isolate 452, and Polish isolate M, which testifies to their common origin.

Results of our phylogenetic analysis as the data of many other authors [5, 17, 18, 20, 23] testifies about absence of clear correlation between genetic differentiation and geographical origin that can be explained with the processes described for the SMV recombination between its various strains.

So, Seo et al. [20, 23] analyzed the 44 SMV isolates and strains and did not provide clear clues for classifying the SMV population according to geographical origins. All known isolates/strains they are largely divided into two geographic subpopulations, Far East Asia and North America.

Soybean originated in East Asia and has been cultivated for several thousands of years, and it was recently suggested that SMV, like soybean itself, originated in South East Asia when it diverged from WMV around 1500 years ago, and further diverged into the soybean and *Pinellia* lineages around a 1000 years ago [26]. However, in North America, soybean was first introduced in 1765 and has been widely spread there in the early twentieth century. As soybean is the natural host of SMV in fields, the history of the virus in North America is believed to be similar to that of soybean [20, 23]. Comparison of nucleotide diversities of within- or between-population also revealed that little genetic differentiation has occurred between two geographical subpopulations. Thus, it is likely that few sequence diversity have yet accumulated in SMV population of North America. This, taken together with our phylogenetic results, also suggests that not much genetic drift has occurred in the SMV population. On the other hand, the involvement of such human activities as trade of SMV-infected soybean seed or relatively recent spread of the virus from Asia on a global scale can be another possible reason for phylogenetic inconsistency on geographical clustering [20, 23].

Results show that SMV isolates have a high level of homology among themselves and the most significant difference was found between strains from Far East Asia and North America, which made it possible to divide them into

two geographical subpopulations. It should be noted that some authors speak of a great genetic variability of isolates form, a variety of symptoms and severity of the disease than North American isolates that may be associated with a much older cultivation of soybean in China (more than 2 thousand years ago), and then for a long time and the evolution of SMV strains in the region.

Obtained in this study data about affecting of soybeans in three regions of Ukraine by SMV indicate about detection of the pathogen in large soybean producing areas of the country that can be a significant danger to agriculture, given that the virus can cause epiphytotic and reducing of seed quality of this crop.

**Міщенко Л.Т.¹, Дуніч А.А.¹, Шевченко Т.П.¹, Будзанівська І.Г.¹,
Поліщук В.П.¹, Андрійчук О.М.¹, Молчанець О.В.¹, Антіпов І.О.²**

¹ ННЦ «Інститут біології та медицини»,
Київський національний університет імені Тараса Шевченка,
вул. Володимирська, 64/13, Київ, 01601, Україна,

² Національний університет біоресурсів і природокористування,
вул. Героїв оборони, 15, Київ, 03041, Україна

ВИЯВЛЕННЯ ВІРУСУ МОЗАЇКИ СОЇ В ДЕЯКИХ ОБЛАСТЯХ ЛІВОБЕРЕЖНОГО ЛІСОСТЕПУ УКРАЇНИ

Резюме

Соя (*Glycine max* L.) є стратегічною зернобобовою культурою світового землеробства XXI століття, яка перебуває в центрі уваги світової аграрної науки і виробництва. Вірус мозаїки сої (ВМС) є збудником однієї з основних та найбільш шкочинних вірусних хвороб, яка поширена в агроценозах усіх сусідніх регіонів світу. Інфікування рослин сої ВМС призводить до значних втрат врожаю та спричиняє зниження якості насіння та життєздатності сходів. Дослідження вірусів сої в Україні були проведені виключно на Правобережній її частині. Однак, виявлення та можливого поширення і циркуляції цих вірусів в умовах Лівобережного Лісостепу дотепер не проводилося. **Мета.** Провести діагностику та ідентифікацію вірусів, які уражують сою на території Лівобережної України, та дослідити деякі з їх властивостей. **Методи:** візуальна діагностика, трансмісійна електронна мікроскопія, імуноферментний аналіз, виділення тотальної РНК із рослинного матеріалу, полімеразна ланцюгова реакція, сиквенування, філогенетичний аналіз. **Результати.** Досліджено 29 зразків рослин сої на території Лівобережного Лісостепу України (Полтавська, Сумська і Харківська області) на наявність вірусної інфекції. Методом ІФА встановлено, що 48% зразків (13 зразків із Полтавської області та 1 – з Сумської) уражені вірусом мозаїки сої. Це перше повідомлення про ураження ВМС сої, вирощеної в умовах Лівобережної України. Зважаючи на раніше представлені дані щодо циркуляції на території Правобережної частини країни змішаної інфекції цього вірусу із вірусом жовтої мозаїки квасолі (ВЖМК) та вірусом мозаїки люцерни (ВМЛ), досліджувані зразки були нами перевірені на наявність і цих збудників. Результати аналізу показали відсутність антигенів ВЖМК і ВМЛ. Встановлено, що досліджувані ізоляти ВМС відрізняються за розмірами віріонів від виділених раніше в Україні ізолятів вказаного збудника. Філогенетичний аналіз нуклеотидних послідовностей ділянки гена капсидного білка вірусу мозаїки сої виявив 100% рівень філогенетичної спорідненості між репрезентативним українським ізолятом та китайськими, іранськими

ізолятами, а також американським ізолятом 452 і польським ізолятом М, що свідчить про їх спільне походження.

Ключові слова: *Glucine max*, вірус мозаїки сої, вірус мозаїки люцерни, вірус жовтої мозаїки квасолі, імуноферментний аналіз, ПЛІР, сиквенування, морфологія вірусів, філогенетичний аналіз.

**Мищенко Л.Т.¹, Дунич А.А.¹, Шевченко Т.П.¹, Будзанивская И.Г.¹,
Полищук В.П.¹, Андрійчук Е.Н.¹, Молчанец О.В.¹, Антипов И.А.²**

¹ ОНЦ «Институт биологии и медицины», Киевский национальный университет имени Тараса Шевченко, ул. Владимирская, 64/13, Киев, 01601, Украина

² Национальный университет биоресурсов и природопользования, ул. Героев Оборонь, 15, Киев, 03041, Украина

ВЫЯВЛЕНИЕ ВИРУСА МОЗАИКИ СОИ В НЕКОТОРЫХ ОБЛАСТЯХ ЛЕВОБЕРЕЖНОЙ ЛЕСОСТЕПИ УКРАИНЫ

Резюме

Соя (*Glucine max* L.) является стратегической зернобобовой культурой мирового земледелия XXI века, которая находится в центре внимания мировой аграрной науки и производства. Вирус мозаики сои (ВМС) является возбудителем одного из основных и наиболее вредоносных вирусных заболеваний, которое распространено в агроценозах всех сосеющих регионов мира. Инфицирование растений сои ВМС приводит к значительным потерям урожая, снижению качества семян и жизнеспособности всходов. Исследования вирусов сои в Украине были проведены исключительно на Правобережной ее части. Однако, исследования возможного распространения и циркуляции этих вирусов в условиях Левобережной Лесостепи до сих пор не проводилось. **Цель.** Провести диагностику вирусов, которые поражают сою на территории Левобережной Украины, и исследовать некоторые из их свойств. **Методы:** визуальная диагностика, трансмиссионная электронная микроскопия, иммуноферментный анализ, выделение тотальной РНК из растительного материала, полимеразная цепная реакция, сиквенирование, филогенетический анализ. **Результаты.** Исследовано 29 образцов растений сои на территории Левобережной Лесостепи Украины (Полтавская, Сумская и Харьковская области) на наличие вирусной инфекции. Методом ИФА установлено, что 48% образцов (13 образцов из Полтавской области и 1 – из Сумской) поражены вирусом мозаики сои. Это первое сообщение о поражении ВМС сои, выращенной в условиях Левобережной Украины. Исходя из ранее представленных данных о циркуляции на территории Правобережной части страны смешанной инфекции этого вируса с вирусом желтой мозаики фасоли (ВЖМФ) и вирусом мозаики люцерны (ВМЛ), исследуемые образцы были нами проверены на наличие и этих возбудителей. Результаты анализа показали отсутствие антигенов ВЖМФ и ВМЛ. Установлено, что исследуемые изоляты ВМС отличаются по размерам вирионов от выделенных ранее в Украине изолятов указанного возбудителя. Филогенетический анализ нуклеотидных последовательностей участка гена капсидного белка вируса мозаики сои показал 100% уровень филогенетического родства между репрезентативным украинским изолятом и китайскими, иранскими изолятами, а также американским изолятом 452 и польским изолятом М, что свидетельствует об их общем происхождении.

Ключевые слова: *Glycine max*, вирус мозаики сои, вирус мозаики люцерны, вирус желтой мозаики фасоли, иммуноферментный анализ, ПЦР, сиквенирование, морфология вирусов, филогенетический анализ.

1. Clinton GP. Reports of the botanist for 1915: Soybeans. Annual Report, Connecticut Agricultural Experiment Station. 1916: 446.
2. Gardner MW, Kendrick JB. *Soybean mosaic virus*. Journal of Agricultural Research. 1921; 22: 111-14.
3. Bilyk LG. [Soybean mosaic in Ukraine]. Thesis PhD. Kyiv; 1967. Ukrainian
4. Kyrychenko AM, Kraeva GV, Kovalenko OG. Biological characteristic and identification of soybean virus isolated from different Ukraine regions. Mikrobiol. Zh. 2012; 74(1): 46-50. PubMed PMID: 22545444.
5. Sherepitko DV, Budzanivska IG, Polischuk VP, Boyko AL. Sequencing and phylogenetic analysis of *Soybean mosaic virus* isolated in Ukraine. Biopolym. Cell. 2011; 27(6):472–79. <http://dx.doi.org/10.7124/bc.00011A>
6. Wang A. Soybean mosaic virus: research progress and future perspectives. In: Proceedings of VIII World Soybean Research Conference; 2009 Ast 10-15; Beijing, China.
7. Arif M, Hassan S. Evaluation of resistance in soybean germplasm to *Soybean mosaic potyvirus* under field conditions. Online Journal of Biological Sciences. 2002; 2:601-604. Doi: 10.3923/jbs.2002.601.604
8. Liao L, Chen P, Buss GR, Yang Q, Tolin SA. Inheritance and allelism of resistance to *Soybean mosaic virus* in Zao18 soybean from China. Buss Journal of Heredity. 2002; 93(6):447-52. PubMed PMID: 12642647.
9. El-Amretz AA, El-Said HM, Salem DE. Effect of *Soybean mosaic virus* infection on quality of soybean seed. Agricultural Research Review. 1987; 63:155-64.
10. Sherepitko DV. Molecular-genetic and biological properties of viruses (soybean mosaic potyvirus, alfalfa mosaic alfamovirus) identified on soybean under environmental conditions of Forest-steppe zone of Ukraine. Thesis PhD. Kyiv; 2012. Ukrainian.
11. Peresyphkin VF, Markov IL, Shelestova VS. [Workshop on the basics of research in plant protection]. K; 2000. Ukrainian.
12. Salyga YuT, Snitynsky VV. Elektronna mikroskopiya biologichnyh obyektiv. Kyiv: Svit; 1999. Ukrainian.
13. Crowther JR. ELISA. Theory and practice. New York: Hamana Press; 1995.
14. Melnichuk MD, Antipov IO, Spiridonov VG. Molekulyarna diagnostyka ta identyfikatsia X-, Y-, M-, S-, L- virusiv kartopli (*Solanum tuberosum* L.) metodom polimeraznoyi lantcyugovoyi reakcii. Metodychni rekomendatsii. Kyiv: Publish center NAU; 2008. Ukrainian.
15. Lakin GF. [Biometry]. M: High school; 1980. Russian.
16. Zhou G-C, Shao Z-Q, Ma F-F, Wu P, Wu X-Y, Xie Z-Y, et al. The evolution of soybean mosaic virus: An updated analysis by obtaining 18 new genomic sequences of Chinese strains/isolates. Virus Res. 2015; 208:189-98. PubMed PMID: 26103098.
17. Domier LL, Latorre IJ, Steinlage TA, McCoppin N, Hartman GL. Variability and transmission of *Aphis glycines* of North American and Asian soybean mosaic virus isolates. Arch Virol. 2003; 148:1925-41. PubMed PMID: 14551816.
18. Jezewska M, Trzmiel K, Zarzyn'ska-Nowak A, Lewandowska M. Identification of *Soybean mosaic virus* in Poland. Journal of Plant Pathology. 2015; 97(2): 357-62. doi: 10.4454/JPP.V97I2.006.

19. Ahangaran A, Habibi MK, Mohammadi GH, Winter S, Garcia-Arenal F. Analysis of *Soybean mosaic virus* genetic diversity in Iran allows the characterization of a new mutation resulting in overcoming *Rsv4*-resistance. *Journal of General Virology*. 2013; 94: 2557-68. PubMed PMID: 23939982.
20. Seo JK, Ohshima K, Lee HG, Son M, Choi HS, Lee SH, et al. Molecular variability and genetic structure of the population of *Soybean mosaic virus* based on the analysis of complete genome sequence. *Virology*. 2009b; 393: 91-103. PubMed PMID: 19716150.
21. Gagarinova AG, Babu M, Poysa V, Hill JH, Wang A. Identification and molecular characterization of two naturally occurring *Soybean mosaic virus* isolates that are closely related but differ in their ability to overcome *Rsv4* resistance. *Virus Research*. 2008; 138: 50-56. PubMed PMID: 18793685.
22. Chowda-Reddy RV, Sun H, Chen H, Poysa V, Lin H, Gijzen M, et al. Mutations in the P3 protein of *Soybean mosaic virus* G2 isolates determine virulence on *Rsv4*-genotype soybean. *Molecular Plant Microbe Interaction*. 2011; 24: 37-43. PubMed PMID: 20795856.
23. Seo JK, Lee HG, Choi HS, Lee SH, Kim KH. Infectious *in vivo* transcripts from a full-length clone of *Soybean mosaic virus* strain G5H. *Plant Pathol J*. 2009; 25:54-61.
24. Chen J, Zheng H, Lin L, Adams MJ, Antoniw JF, Zhao MF, et al. A virus related to *Soybean mosaic virus* from *Pinellia ternata* in China and its comparison with local soybean SMV isolates. *Archives of Virology*. 2004; 149: 349-63. PubMed PMID: 14745600.
25. Shi YH, Hong XY, Chen J, Adams MJ, Zheng HY, Lin L, et al. Further molecular characterisation of potyviruses infecting aroid plants for medicinal use in China. *Arch. Virol*. 2005; 150(1): 125-35. PubMed PMID: 15449140.
26. Gibbs AJ, Trueman JW, Gibbs MJ. The Bean common mosaic virus lineage of potyviruses: where did it arise and when? *Arch. Virol*. 2008; 153:2177-87. PubMed PMID: 19030956.

Отримано 12.09.2016