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## Phylogenetic analysis of two Ukrainian isolates of *Wheat streak mosaic virus*

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The study of molecular characteristics and, in particular, the nucleotide (nt) and amino acid (aa) sequences of the viral genomes, is necessary to know the changes in their geographical range, phylogenetic relationships, viruses' evolution, and their emergence as new epidemics. **Aim.** Phylogenetic analysis of the coat protein (*CP*) gene sequences of two new Ukrainian isolates of *Wheat streak mosaic virus*: Ukraine-Mal-18 and Ukraine-Ep-18. **Results.** The nucleotide sequences of 676 nt region of the *CP* gene of two Ukrainian WSMV isolates were compared with the sequences of 72 WSMV isolates/strains from GenBank. The phylogenetic analysis showed that the Ukrainian WSMV isolates cluster with the clade B or *WSMV-ΔE* isolates (originating from Europe and Asia) and have a typical for this clade triplet deletion at the position 8412-8414 nt in the *CP* gene. The isolate Ukraine-Mal-18 has the highest level of the sequence identity (93.5 %-95.9 % nt and 93.6-95.0 % aa) with the clade B isolates. The Ukraine-Ep-18 isolate shares 89.2 %-91.4 % (nt) and 88.6-87.1 % (aa) identity with the clade B isolates. Additionally, both Ukrainian WSMV isolates have a number of unique aa substitutions in the central *CP* gene domain. **Conclusions.** Ukrainian WSMV isolates belong to the clade B. But Ukraine-Mal-18 and Ukraine-Ep-18 have some differences from other members of the clade: i) a higher divergence compared to other B isolates (the Ukraine-Mal-18 has 12 aa substitutions, the Ukraine-Ep-18 has 25 aa substitutions, whereas the other clade B isolates have no more than 2 aa substitutions); ii) have aa substitution identical with the B1 non-crop isolates of this virus, many aa substitutions are in the same motifs as the substitutions of B1 grass WSMV isolates.

**Keywords:** *Wheat streak mosaic virus*, *Triticum aestivum*, phylogenetic analysis, sequencing, coat protein

## Introduction

In recent years, not only the number and prevalence of cereal viruses have increased expressively, but also their economic significance [1, 2]. *Wheat streak mosaic virus* (WSMV) is the most harmful and widespread virus of cereals in all wheat grown areas in the world. WSMV can cause significant yield losses of wheat – up to 60 % [3] and in some cases – up to 100 %. That is why a detail investigation of WSMV is warranted. WSMV is spread and detected in USA, Canada, Argentina, Australia, Asia, and Europe. In Europe, WSMV was found in Romania, Austria, Czech Republic, France, Hungary, Turkey, Italy, Poland, Russia, Slovakia, and Ukraine. In Lithuania and Germany, this virus was first detected only in 2013 [4, 5]. The WSMV hosts are plant species of the family *Poaceae*. The main of them are: wheat, barley, maize, oat and many other grasses [6-8]. These species are reservoirs of the virus and its vector.

The study of molecular characteristics and in particular, the nucleotide sequences of the genome, provides an opportunity to follow the virus phylogenetic relationships with others, to elucidate its evolutionary history and prospects. Furthermore, based on these data it is possible to predict the changes and emergence of new properties in the strains or isolates circulating in a specific area.

Based on the coat protein (CP) gene sequences, WSMV isolates have been divided into four clades, named A–D [4, 9, 10].

Clade A represents one isolate from Mexico, known as El-Batán. Clade B or WSMV- $\Delta$ E includes Asian and European isolates [4, 11]. B isolates have a deletion of triplet codon GCA

(Glycine amino acid) in the CP gene sequence [11]. Except the CP gene, the differences between isolates from different clades were revealed in the putative protein P1/helper-component-proteinase (HC-Pro) protease cleavage site [4, 12].

Recently, Singh & Kundu [8] proposed a new clade B1 for the WSMV isolates infecting grasses. This clade includes Czech isolates: ar1 detected in *Agropyron repens* (KY419572), pp1 from *Phleum pratense* (KY419573), and pp2 from *Poa pratensis* (KY419574). It was shown that these grass WSMV isolates are more similar to the B isolates rather than to A and D isolates. Moreover, the Czech grass isolates are characterized by the presence of a triplet deletion at the position 8412-8414 nt in the CP gene as well as the clade B isolates. On the other hand, the B1 isolates were slightly different from previously described the WSMV isolates from Czech Republic and had several aa substitutions located in the central domain of the CP gene [8].

Clade C comprises isolate from Iran (Ac. No AF454458) [13].

Clade D includes isolates from USA, Canada, Argentina, Australia, Canada, Turkey (AF454455), and Iran (EU914917) [13, 14]. Interestingly, some USA WSMVs have Gly deletion in the CP gene as well as B isolates [14]. The clade D isolates are divided into four subclades, D1-D4 [15, 16]. This clade also includes WSMV described as isolate Ger (Ac. No AJ889242). Isolate Ger actually represents the strain PV57 from North America which had been maintained in the virus collection of the former Institute for Resistance Research and Pathogen Diagnostics in Aschersleben, Germany [4].

Analysis of the WSMV whole genomes showed that the clustering of some of them into different clades is based not only on geographical origin. Isolate Saadat-Shahr (Ac. No EU914918) is in the clade B, isolate with Ac. No AF454458 is in the clade C, and one more Iranian isolate Naghadeh (Ac. No EU914917) has high genetic similarity with the clade D isolates [4]. Similar situation is with Turkish isolates. Whereas the isolate *Turkei* (Ac. No FJ606886) clustered with European isolates from the clade B, another Turkish isolates *Turkey1* (Ac. No AF454455) and *Turkey2* (Ac. No AF454457) clustered with isolates from the clade D [17]. Isolate *Agdia* (FJ695510) from Czech Republic is also grouped with the clade D isolates.

Noteworthy, considerable attention is paid to the study of wheat viruses in Ukraine, in particular, WSMV. The WSMV investigation was started in 1968 [18]. In the 1990s the disease was marked with different intensity on winter wheat crops in many regions [19]. Further, study on the WSMV circulation in Ukraine has shown that the virus is distributed in the central and eastern regions of the country. Later, an increase in the impact of climate change on the manifestation and circulation of this virus has been marked [3, 20]. Monitoring of wheat crops in the Poltava, Kyiv and Kharkiv regions of Ukraine in the last years has shown minor damage with WSMV. Earlier, some molecular properties of Poltava isolate of WSMV were investigated [3]. In 2018, we noted the streak symptoms in the fields of winter wheat in the Kharkiv region. So, this study describes the phylogenetic analysis of the *CP* gene sequences of two new Ukrainian isolates of *Wheat streak mosaic virus*.

## Materials and Methods

**Samples collection.** Winter wheat leaf samples with streak symptoms were collected in May 2018 in the fields of Kharkiv region (east part of Ukraine).

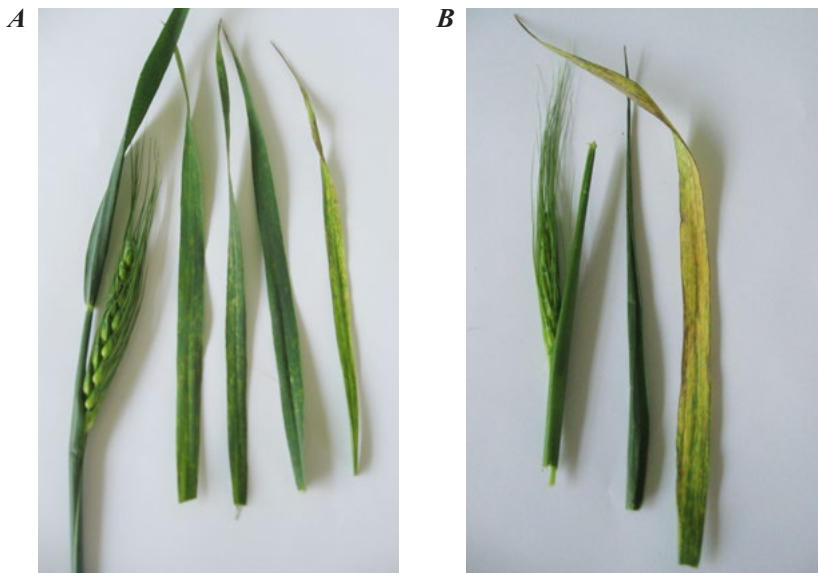
**Enzyme-linked immunosorbent assay.** Identification of the viruses in sap of wheat leaves was performed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Specific antibodies against *Wheat streak mosaic virus* (WSMV) and *Barley yellow mosaic virus* (BYDV-PAV) (Loewe, Germany) were used. Antigen samples were prepared by grinding leaf tissue in PBS buffer, pH 7.4, at the ratio 1:2 (w/V). Leaf samples from healthy wheat were also included as negative controls. Positive controls were commercial (Loewe, Germany). The results were recorded on Thermo Labsystems Opsi MR reader (USA) with Dynex Revelation Quicklink software at the wavelength of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher than those of negative controls [21].

**RNA extraction, RT-PCR and sequencing.** Total RNA was extracted from fresh leaves using RNeasy Plant Mini kit (Qiagen, Great Britain) following the manufacturer's instructions. Two-step RT-PCR was performed. The reverse transcription was performed using RevertAid Reverse Transcriptase (Thermo Scientific, USA) according to the manufacturer's instructions. Amplification was performed using thermocycler (Genetic research instrumentation LTD, Great Britain). WSMV CP-specific primers were used: WS-8166F 5' GAGAGCAATACTGCCGTGTACG 3' and WS-8909R 5' GCATAATGGCTCGAAGTGATG

3' [22]. DNA product of 750 bp was amplified. Amplification was performed in 12.5 µl of Dream Taq PCR Master Mix (2x) buffer (containing Dream Taq DNA polymerase, 2x Dream Taq buffer, 0.4 mmol/l of each dNTP and 4 mmol/l of MgCl<sub>2</sub>), 7.5 µl of nuclease-free water, 1 µl of each primer (10 µmol/l), and 3 µl of cDNA. The temperature regime for amplification reactions was as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The final extension was at 72°C for 10 min. PCR products were separated on a 1.5 % agarose gel with DNA markers MassRuler DNA Ladder Mix ready-to-use (SM 0311, Thermo Scientific, USA), stained with ethidium bromide, and visualized under UV light. The PCR products were purified from the agarose gel using a QIAquick Gel Extraction Kit (Qiagen, Great Britain). Sequencing of the purified amplified DNA

fragments was carried out with the 3130 Genetic Analyzer (Applied Biosystems, USA).

*Phylogenetic analysis.* Sequences of Ukrainian WSMV isolates were compared with WSMV sequences in the NCBI database with the BLAST program (<http://www.ncbi.nlm.nih.gov>). WSMV isolates used in this study are listed in Table 1. Nucleotide and amino acid sequences were aligned using Clustal W in MEGA 7 (<http://www.megasoftware.net/>). Phylogenetic tree for the part of coat protein gene of 2 Ukrainian WSMV isolates and 72 WSMV isolates from different countries was constructed by the Neighbor-Joining method [23] using the best-fitting model (Jukes – Cantor). To check the reliability of the constructed tree we used bootstrap test with 1000 bootstrap replications. Multiple alignments of the coat protein amino acid sequences (225 aa) of WSMV isolates were performed by BioEdit program.



**Fig. 1.** Winter wheat with symptoms of WSMV-infection: a – cv. Malynivka; b – cv. Epokha Odeska

## Results and Discussion

In 2018, winter wheat plants cv. Malynivka and Epokha Odeska with WSMV-like mild and severe streak symptoms were found near Kharkiv in the east part of Ukraine (Fig.1).

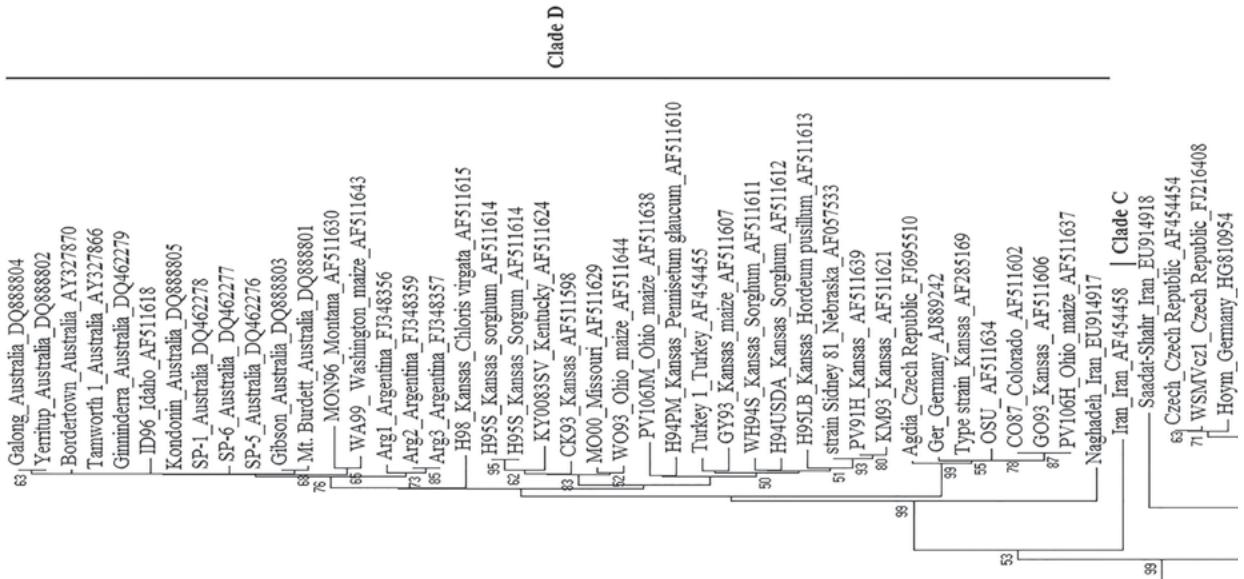
DAS-ELISA and RT-PCR showed that both wheat samples were infected with WSMV. BYDV-PAV antigens were not detected. These two samples were taken for this study. The WSMV isolate from wheat cv. Malynivka was

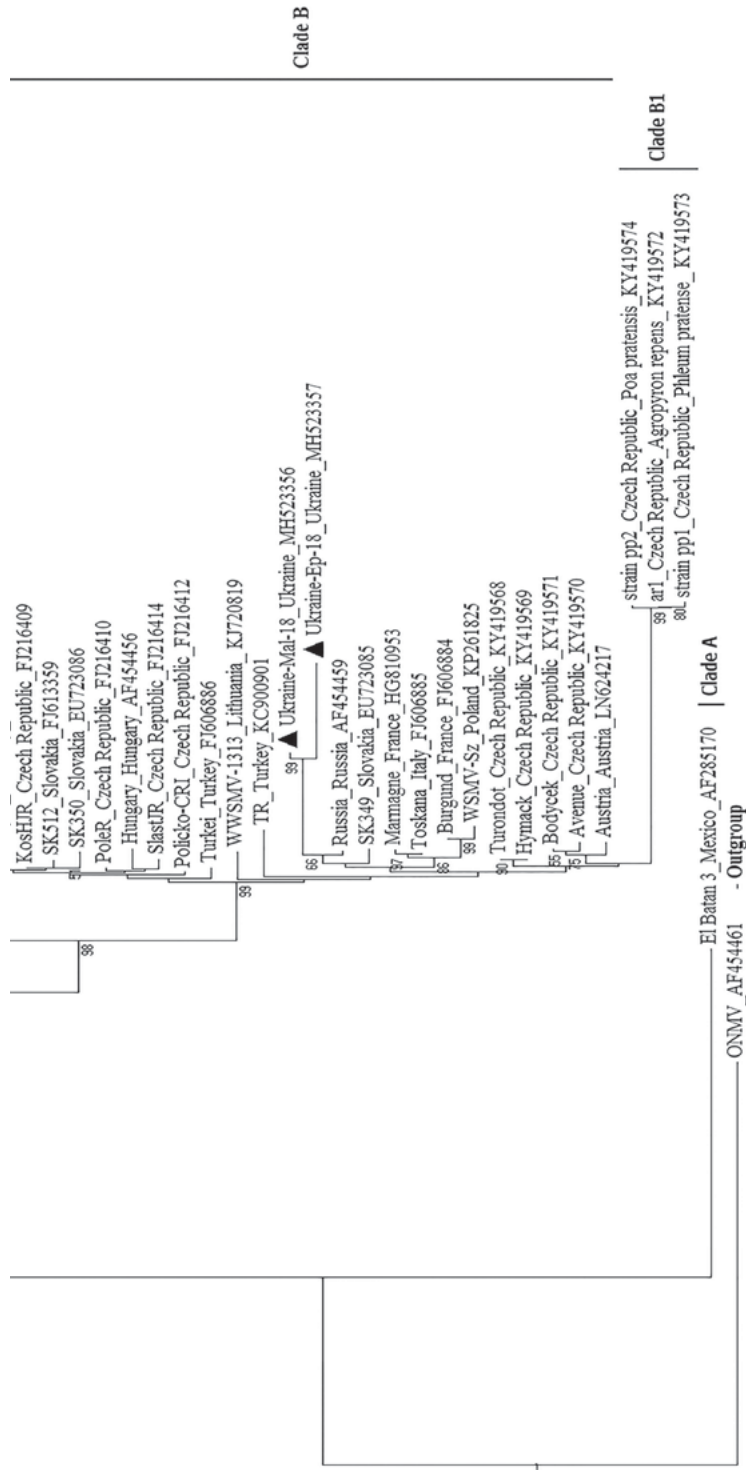
**Table 1. Nucleotide and amino acid sequence identity of part of the CP gene of the Ukrainian WSMV isolates with isolates/strains from other countries (%)**

No.	Isolate / strain name	Accession No in GenBank	Host	Country of origin	Ukraine-Mal-18		Ukraine-Ep-18		Clade
					nt	aa	nt	aa	
1	El Batan 3	AF285170	wheat	Mexico	66.0	76.7	62.6	70.3	A
2	Ukraine-Mal-18	MH523356	wheat	Ukraine	-----	-----	95.7	93.6	B
3	Ukraine-Ep-18	MH523357	wheat	Ukraine	95.7	93.6	-----	-----	B
4	Russia	AF454459	wheat	Russia	95.9	95.0	91.4	88.6	B
5	Marmagne	HG810953	wheat	France	94.9	95.0	91.2	88.6	B
6	Czech	AF454454	wheat	Czech Republic	94.9	95.0	90.8	88.6	B
7	Austria	LN624217	wheat	Austria	94.2	94.1	90.3	87.6	B
8	Hoym	HG810954	wheat	Germany	94.7	94.6	90.6	88.1	B
9	Hungary	AF454456	wheat	Hungary	94.4	94.6	90.3	88.1	B
10	Saadat-Shahr	EU914918	wheat	Iran	88.8	94.6	84.8	88.6	B
11	Toskana	FJ606885	wheat	Italy	95.0	95.0	91.4	88.6	B
12	Burgund	FJ606884	wheat	France	94.0	95.0	90.6	88.6	B
13	WSMV-1313	KJ720819	wheat	Lithuania	94.7	94.6	90.6	88.1	B
14	SK512	FJ613359	wheat	Slovakia	95.0	94.6	91.0	88.1	B
15	SK349	EU723085	wheat	Slovakia	95.0	95.0	90.8	88.6	B
16	SK350	EU723086	wheat	Slovakia	94.7	95.0	90.6	88.6	B
17	WSMV-Sz_	KP261825	wheat	Poland	93.5	94.1	90.1	87.6	B
18	Turkei	FJ606886	wheat	Turkey	95.0	95.0	91.0	88.6	B
19	TR	KC900901	wheat	Turkey	93.5	94.6	89.2	88.1	B
20	KosHJR_	FJ216409	wheat	Czech Republic	95.4	95.0	91.4	88.6	B
21	Policko-CRI	FJ216412	wheat	Czech Republic	95.2	95.0	91.2	88.6	B
22	Turondot	KY419568	wheat	Czech Republic	95.0	95.0	91.2	88.6	B
23	Hymack	KY419569	wheat	Czech Republic	95.0	95.0	91.2	88.6	B
24	PoleR	FJ216410	wheat	Czech Republic	95.0	95.0	91.0	88.6	B
25	SlastJR	FJ216414	wheat	Czech Republic	94.5	94.6	90.5	88.1	B
26	WSMVcz1	FJ216408	wheat	Czech Republic	94.7	94.6	90.6	88.1	B
27	Bodycek	KY419571	wheat	Czech Republic	94.2	94.6	90.3	88.1	B
28	Avenue	KY419570	wheat	Czech Republic	94.0	93.6	90.1	87.1	B
29	ar1	KY419572	<i>Agropyron repens</i>	Czech Republic	84.4	82.2	81.0	76.7	B1
30	Strain pp1	KY419573	<i>Phleum pratense</i>	Czech Republic	84.2	81.7	80.8	76.2	B1
31	Strain pp2	KY419574	<i>Poa pratensis</i>	Czech Republic	84.2	81.7	80.8	76.2	B1
32	Iran	AF454458	wheat	Iran	85.6	94.1	81.6	87.6	C
33	Turkey 1	AF454455	wheat	Turkey	84.8	93.1	81.0	86.6	D
34	Ger	AJ889242	wheat	Germany	85.2	91.6	81.6	85.1	D

Continued Table 1

No.	Isolate / strain name	Accession No in GenBank	Host	Country of origin	Ukraine-Mal-18		Ukraine-Ep-18		Clade
					nt	aa	nt	aa	
35	Agdia	FJ695510	wheat	Czech Republic	85.4	92.1	81.8	85.6	D
36	Arg1	FJ348356	wheat	Argentina	85.8	93.6	82.0	87.1	D
37	Arg2	FJ348359	wheat	Argentina	85.8	93.6	82.0	87.1	D
38	Arg3	FJ348357	wheat	Argentina	85.8	93.6	82.0	87.1	D
39	PV91H	AF511639	wheat	Kansas, USA	85.0	92.6	81.2	86.1	D
40	MO00	AF511629	wheat	Missouri, USA	85.2	93.6	81.4	87.1	D
41	KM93	AF511621	wheat	Kansas, USA	84.6	91.6	80.8	85.1	D
42	Strain Sidney 81	AF057533	wheat	Nebraska, USA	85.2	93.1	81.4	86.6	D
43	ID96	AF511618	wheat	Idaho, USA	84.8	94.1	81.0	87.6	D
44	MON96	AF511630	wheat	Montana, USA	84.6	93.1	80.8	86.6	D
45	OSU	AF511634	unknown	unknown	84.4	91.1	80.8	84.7	D
46	CO87	AF511602	wheat	Colorado, USA	84.6	90.6	81.0	84.2	D
47	GO93	AF511606	wheat	Kansas, USA	84.6	90.6	81.0	84.2	D
48	CK93	AF511598	wheat	Kansas, USA	85.0	93.1	81.2	86.6	D
49	KY0083SV	AF511624	wheat	Kentucky	84.4	92.1	80.6	85.6	D
50	H95S	AF511614	sorghum	Kansas, USA	85.2	93.6	81.6	87.1	D
51	WA99	AF511643	maize	Washington, USA	85.6	93.6	81.6	87.1	D
52	WO93	AF511644	maize	Ohio, USA	85.2	93.1	81.4	86.6	D
53	PV106JM	AF511638	maize	Ohio, USA	84.2	92.6	81.0	86.1	D
54	Type strain	AF285169	wheat	Kansas, USA	85.0	91.6	81.4	85.1	D
55	PV106H	AF511637	maize	Ohio, USA	84.6	90.6	81.0	84.2	D
56	GY93	AF511607	maize	Kansas, USA	85.0	93.1	81.2	86.6	D
57	H94PM	AF511610	<i>Pennisetum glaucum</i>	Kansas, USA	85.0	93.6	81.4	87.1	D
58	WH94S	AF511611	sorghum	Kansas, USA	86.0	93.1	82.2	86.6	D
59	H94USDA	AF511612	sorghum	Kansas, USA	85.2	93.1	81.4	86.6	D
60	H95LB	AF511613	<i>Hordeum pusillum</i>	Kansas, USA	84.6	93.6	80.8	87.1	D
61	H95S	AF511614	sorghum	Kansas, USA	85.2	93.6	81.6	87.1	D
62	H98_Kansas	AF511615	<i>Chloris virgata</i>	Kansas, USA	85.8	93.6	82.2	87.1	D
63	Naghadeh	EU914917	wheat	Iran	85.4	94.6	81.4	88.1	D
64	Gibson	DQ888803	wheat	Australia	85.4	93.6	81.6	87.1	D
65	Mt. Burdett	DQ888801	wheat	Australia	85.2	93.6	81.4	87.1	D
66	Tamworth 1	AY327866	wheat	Australia	85.6	94.1	81.8	87.6	D
67	Bordertown	AY327870	wheat	Australia	85.6	94.1	81.8	87.6	D
68	Ginninderra	DQ462279	wheat	Australia	85.6	94.1	81.8	87.6	D
69	SP-1	DQ462278	wheat	Australia	85.6	94.1	81.8	87.6	D
70	SP-6	DQ462277	wheat	Australia	85.4	93.6	81.6	87.1	D
71	SP-5	DQ462276	wheat	Australia	85.6	94.1	81.8	87.6	D
72	Kondonin	DQ888805	wheat	Australia	85.6	94.1	81.8	87.6	D
73	Galong	DQ888804	wheat	Australia	85.4	93.6	81.8	87.6	D
74	Yerritup	DQ888802	wheat	Australia	85.4	93.6	81.8	87.6	D



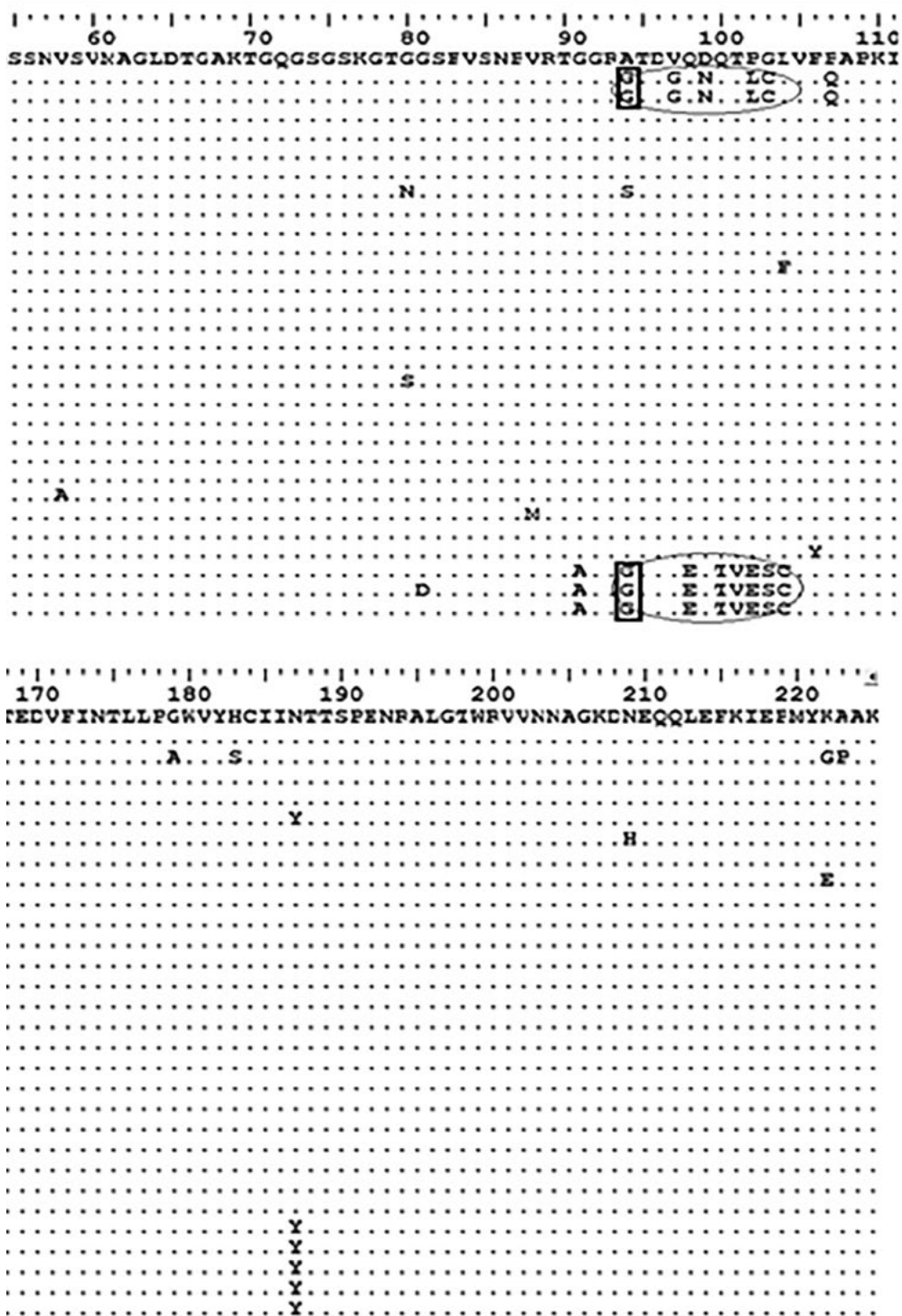


**Fig. 2.** Neighbor-Joining tree based on nucleotide sequences of 676 bp CP gene region of Ukrainian WSMV isolates and WSMV isolates from other countries. Jukes-Cantor model was performed. The scale bar shows the number of substitutions per base. *Oat necrotic mottle virus*, ONMV (Ac. No AF454461) used as an outgroup sequence. Ukrainian isolates are shown in triangles.



	10	20	30	40	50
Russia	AVIAAANAGSGSAPSSGSTQSSLSASTASGSGSSPSGSGSAAAGGSGSGSAQTQ				
Ukraine-Mal-18	ER.				
Ukraine-Ep-18	ER.				
Marmagne					
Czech					
Austria					
Hoym					
Hungary					
Saadat-Shahr					
Toscana					
Burgund					
WWSMV-1313					
SK512	V				
SK349					
SK350					
WSMV-Sz					
Turkei					
TR	N				
KoshJR					
Policko-CRI	M				
Turondot					
Hymack					
PoleR					
SlastJR					
WSMVcz1					
Bodycek					
Avenue					
arl	G				
strain pp1	G				
strain pp2	G				

	120	130	140	150	160
Russia	TTKAIYMPKIVRDKIKPEMINNMIKYQPPAELICNRYATTEQINIWIKASEGLDVT				
Ukraine-Mal-18	F.LFL				
Ukraine-Ep-18	F.LFL E.K.V. DL.TQ Y.I				
Marmagne					
Czech					
Austria	S				
Hoym					
Hungary					
Saadat-Shahr					
Toscana					
Burgund					
WWSMV-1313					
SK512					
SK349					
SK350					
WSMV-Sz	R I				
Turkei					
TR					
KoshJR					
Policko-CRI					
Turondot					
Hymack					
PoleR					
SlastJR					
WSMVcz1					
Bodycek					
Avenue	S				
arl	IYQE NIYS NE.RY.EDFVVFY Y.I				
strain pp1	IYQE NIYS NE.RY.EDFVVFY Y.I				
strain pp2	IYQE NIYS NE.RY.EDFVVFY Y.ID				



**Fig. 3.** Comparative analysis of amino acid sequences of Ukrainian WSMV isolates with they-clade B and B1 strains. Identical aa variations among sequences are represented with boxes. Amino acid variations in the same regions are represented with circles. Numbers on top represent the deduced CP amino acid position. Only the differences are shown. Accession numbers for isolates used for the alignment are shown in Table1.

named Ukraine-Mal-18. The isolate from wheat plants cv. Epokha Odeska was named Ukraine-Ep-18.

Nucleotide (nt) sequences 676 nt region of the *CP* gene of the Ukrainian WSMV isolates, located at the genomic position 8167-8843, were compared with the sequences of 72 WSMV isolates/strains from GenBank. Analysis showed that the Ukrainian WSMV isolates have the highest percentage of identity with all wheat European isolates from the clade B. Isolate Ukraine-Mal-18 has the highest level of the sequence identity (93.5 %-95.9 % nt and 93.6-95.0 % aa) with the clade B isolates. The isolate Ukraine-Ep-18 shares identity of 89.2 %-91.4 % (nt) and 88.6-87.1 % (aa) with the clade B isolates. (Table 1).

Phylogenetic analysis showed that Ukrainian WSMV isolates with all European and Asian wheat isolates (except isolate Agdia from Czech Republic, Ger from Germany, Turkey 1 from Turkey and Iranian isolate Naghadeh) are clustered into the clade B (Fig. 2).

Ukrainian isolates, like all isolates of the B and B1 clades, have a triplet deletion at the position 8412-8414 nt in the *CP* gene in comparison to isolates from the clade D.

Ukraine-Mal-18 and Ukraine-Ep-18 share 80.8 to 82.2 % (nt) and 76.2-82.2 % (aa) identity with isolates from the clade B1, which includes the Czech non-wheat strain ar1 from *Agropyron repens* (KY419572), strain pp1 from *Phleum pratense* (KY419573), and strain pp2 from *Poa pratensis* (KY419574). Clade C isolate, represented by Iranian isolate Iran (Ac. No AF454458), showed similarity of 81.6-85.6 % (nt) and 87.6-94.1 % (aa) to the isolates Ukraine-Ep-18 and Ukraine-Mal-18,

respectively. Pairwise comparisons of the Ukrainian isolates with sequences of WSMV isolates from the clade D indicated respective nucleotide and amino acid sequence identities ranging from 80.6 to 85.8 % and 84.2 to 94.6 %.

Comparative analysis of aa sequences of Ukrainian isolates with the isolates from clades B and B1 revealed significant differences. Thus, the Ukrainian isolate Ukraine-Mal-18 has 12 aa substitutions in the studied region of the *CP* gene and Ukraine-Ep-18 has 25 aa substitutions, whereas all other isolates of group B have 0 to 2 aa substitutions (Fig. 3).

It is necessary to mention that the substitution A→G at position aa 94 is identical among the Ukrainian and B1 isolates (Fig. 3). However, all other substitutions revealed in putative *CP* amino acid sequences are unique. Additionally, amino acid substitutions were observed at positions aa 112-116 (F.LFL motif in the central region of *CP* gene). None of WSMV isolates were characterized by such mutations in this region of the *CP* gene. Noteworthy, many aa substitutions are in the same motifs or near them like substitutions of the B1 grass WSMV isolates (Fig. 3). These are motifs «N<sub>1</sub>» (TVESC, 100-104 aa), «N<sub>4</sub>» (NE.RY.EDPVVIFY, 135-147aa) that were previously found in the non-crop WSMV isolates of the B1 clade [8]. Also, the aa sequence of the Ukraine-Ep-18 isolate revealed more aa substitutions in the N-terminal domain of the *CP* gene, compared with other WSMV isolates (Fig. 3).

## Conclusions

In our study we revealed that Ukrainian WSMV isolates are clustered into the clade B

or WSMV-ΔE. The Ukrainian isolates, like other isolates of the B and B1 clades, have a triplet deletion at the position 8412-8414 nt in the CP gene sequence which led to the absence of Glycine amino acid. Noteworthy, the Ukraine-Ep-18 and Ukraine-Mal-18 isolates are the most divergent among the WSMV-ΔE isolates based on amino acid CP sequence. Besides, it was shown that the Ukrainian wheat WSMV isolates have identical aa substitution (A→G, 94aa) with the B1 non-crop isolates of this virus. Also, many aa substitutions are in the same motifs or near them (motif «№1» or TVESC at positions aa 100-104 and motif «№4» or NE.RY.EDPVVFY at positions aa 135-147) that were previously found in the non-crop WSMV isolates of the B1 clade [8]. Also, more aa substitutions in the CP sequence of the Ukraine-Ep-18 isolate were revealed in the N-terminal domain of the CP gene, than all the WSMV isolates taken to our study. It is known that tritimoviruses N-terminal domain of the CP gene is less conserved than the central and C-terminal regions [24]. Also, it was found that major aa variations between the wheat and grasses WSMVs were in the N-terminal region, namely in the motifs 3 and 4 [8]. However, in the wheat isolate Ukraine-Ep-18 we revealed aa substitutions in the motif 4, in comparison to other European wheat WSMVs. Prendeville *et al.* [25] suggest that mutations in BYDV from wild grass populations can be connected with the virulence factors and can act as positive fitness elements in wild plants. Recently, it has been revealed that the N-terminal region of tritimoviral CP is involved in the host- and strain-specific long-distance movement [26]. Perhaps, aa substitutions in this CP region revealed by us for the

isolate Ukraine-Ep-18 led to more severe symptoms, compared with the Ukraine-Mal-18 isolate but this requires additional research.

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- Філогенетичний аналіз двох українських ізолятів вірусу смугастої мозаїки пшениці**
- Л. Т. Міщенко, А. А. Дуніч, І. Я. Скрипкіна, Н. О. Козуб
- Дослідження молекулярних характеристик, зокрема, нуклеотидних (нт) та амінокислотних (аа) послідовностей вірусних геномів, є необхідним для з'ясування змін у географічному ареалі, філогенетичних зв'язків, еволюції вірусів та їх появи у вигляді епідемій. **Мета.** Філогенетичний аналіз гену капсидного білка (СР)

двох нових українських ізолятів вірусу смугастої мозаїки пшениці (ВСМП) Ukraine-Mal-18 та Ukraine-Ep-18. **Методи:** імуноферментний аналіз, виділення тотальної РНК із рослинного матеріалу, полімеразна ланцюгова реакція зі зворотною транскрипцією, сиквенування, філогенетичний аналіз. **Результати.** Послідовності гену СР розміром 676 нт двох українських ізолятів ВСМП порівнювали із послідовностями 72 ВСМП ізолятів/штамів із бази даних GenBank. Філогенетичний аналіз показав, що українські ізоляти входять до клади В або *WSMV-ΔE* (походять із Європи та Азії) та мають типову для цієї клади делецію триплету у позиціях 8412-8414 нт у гені СР. Ukraine-Mal-18 має найвищий відсоток ідентичності за нуклеотидною послідовністю 93,5 %-95,9 %, за амінокислотою – 93,6-95,0 % із ізолятами клади В. Ізолят Ukraine-Ep-18 із ізолятами клади В має ідентичність 89,2-91,4 % (нт) та 88,6 %- 87,1 % (aa). Крім того, у двох українських ізолятів відмічено низку унікальних aa заміщень в центральній ділянці гену СР. **Висновки.** Українські ВСМП ізоляти входять до клади В. Але Ukraine-Mal-18 та Ukraine-Ep-18 мають деякі відмінності від них: і) вищу дивергенцію, ніж інші ізоляти групи В (Ukraine-Mal-18 має 12 aa заміщень, Ukraine-Ep-18 має 25 aa заміщень, а інші ізоляти клади В мають 0-2 aa заміщення); ii) мають aa заміщення, ідентичні із непшеничними ізолятами групи В1 цього вірусу, багато aa заміщень знаходяться в тих самих ділянках гену СР, що і заміщення трав'яних В1 ізолятів ВСМП.

**Ключові слова:** вірус смугастої мозаїки пшениці, *Triticum aestivum*, філогенетичний аналіз, сиквенування, капсидний білок.

#### Филогенетический анализ двух украинских изолятов вируса полосатой мозаики пшеницы

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Исследование молекулярных характеристик, в частности, нуклеотидных (нт) и аминокислотных (aa) после-

довательностей вирусных геномов, необходимо для выяснения изменений географического ареала, филогенетических связей, эволюции вирусов и их появления в виде эпидемий. **Цель.** Филогенетический анализ гена капсидного белка (СР) двух новых украинских изолятов вируса полосатой мозаики пшеницы (ВПМП) Ukraine-Mal-18 и Ukraine-Ep-18. **Методы.** Иммуноферментный анализ, выделение тотальной РНК из растительного материала, полимеразная цепная реакция с обратной транскрипцией, сиквенирование, филогенетический анализ. **Результаты.** Последовательности гена СР размером 676 нт двух украинских изолятов ВПМП сравнивали с последовательностями 72 ВПМП изолятов / штаммов из базы данных GenBank. Филогенетический анализ показал, что украинские изоляты входят в кладу В или *WSMV-ΔE* (происходят из Европы и Азии) и имеют типичную для этой клады делецию триплет в позициях 8412-8414 нт в гене СР. Ukraine-Mal-18 имеет наиболее высокий процент идентичности по нуклеотидной последовательности 93,5 %-95,9 % и аминокислотной – 93,6-95,0 % с изолятами клады В. Изолят Ukraine-Ep-18 с изолятами клады В имеет идентичность 89,2-91,4 % (нт) и 88,6 %- 87,1 % (aa). Кроме того, у двух украинских изолятов отмечен ряд уникальных aa замен в центральном участке гена СР. **Выводы.** Украинские ВПМП изоляты входят в кладу В. Но Ukraine-Mal-18 и Ukraine-Ep-18 имеют некоторые отличия от них: i) выше дивергенцию, чем другие изоляты В группы (Ukraine-Mal-18 имеет 12 aa замен, Ukraine-Ep-18 имеет 25 aa замен, а другие изоляты клады В имеют от 0 до 2 aa замен); ii) имеют aa замены, идентичны непшеничным изолятам группы В1 этого вируса, много aa замен расположены в тех же участках гена СР, что и замены травяных В1 изолятов ВПМП.

**Ключевые слова:** вирус полосатой мозаики пшеницы, *Triticum aestivum*, филогенетический анализ, сиквенирование, капсидный белок.

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