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SEED-BORNE AND SEED-TRANSMITTED VIRAL DISEASES AND THEIR EFFECT ON YIELD OF DIFFERENT SOYBEAN GENOTYPES

SUMMARY

In 21st century soybean is a strategic legume for world agriculture. The key factor in increasing soybean production is the average yield growth, which in 2018 and 2021 in Ukraine was 3 t/ha as in the EU. From 2007 to 2017 the soybean yield in Ukraine was 2 t/ha; from 1997 to 2006 it was only 1 t/ha. Unlike, in the EU since 1999 the yield was 3 t/ha, and only in 2003, 2007 and 2012 it was 2 t/ha. For Ukraine not the least role in this situation is played by the reduction of quality of soybean sowing material due to seed infections. It is known that the quality of soybean seeds is significantly deteriorating due to infection by soybean mosaic virus (SMV), which circulates in all soybean growing regions worldwide. The aim of the study was to investigate SMV seed infection in sowing material and its effect on the yield of different soybean genotypes. DAS-ELISA showed SMV presence in a small seeds fraction of several soybean genotypes in both seedlings (embryos and cotyledons) and plants grown from this seeds. The weight of virus-infected seeds was 137 mg compared to healthy (204 mg). It was found that the percentage of seed-transmitted SMV infection is from 1.9 to 10.5% in some of investigated genotypes. It has been proven that seed spotting does not always indicate the presence of virus. Phylogenetic analysis of the SMV *CP* gene fragment was performed. Percent of nucleotide and amino acid sequence identity of Ukrainian SMV isolate with isolates from other countries was established.

Keywords: soybean, genotypes, soybean mosaic virus, seed infection

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INTRODUCTION

Today, soybean is a strategic legume crop of world agriculture of the XXI century, which is the focus of world agricultural science and production. The key factor in increasing soybean production is the growth of the average yield, which in Ukraine in 2018 and 2021 was 3 t/ha, ie reached the EU level. From 2007 to 2017, the yield was 2 t/ha, and from 1997 to 2006 was only 1.0 t/ha in Ukraine. Soybean growing technology needs constant improvement. Given the growing demand for soybeans, the modern market of varieties is extremely diverse and attractive. Soybean yield is greatly influenced by the seed quality, namely the defeat of various diseases. For Ukraine, the reducing in soybean seed quality due to seed infections plays an important role in this situation.

It is known that the quality of soybean seeds significantly deteriorates by soybean mosaic virus (SMV), which circulates in all soybean regions of the world (Bowers and Goodman, 1979; 1991; Domier *et al.*, 2007; Hajimorad *et al.*, 2017). SMV infection leads to significant crop losses. The degree of damage to the crop is dependents on the host genotype, virus strain and virulence, time of the infection and the developmental stage at which soybean plants become infected (Shigemori, 1991). SMV is naturally transmitted by aphids in a non-persistent manner and via infected seeds.

Investigations made by Bashar (2015) shown that SMV transmission by seeds occurs due to infection of the embryo. However, SMV was also found to be present in all seed parts: in seed coat, germ root and cotyledons - 23%, 18% and 33% respectively. From 105 viruses transmitted by seeds, 36 (34.3%) were transmitted by seeds from bean hosts. Of the 35 economically important viruses and one viroid transmitted by seeds, 10 belong to potyviruses, including SMV. Many viruses founded in seeds collected from infected plants but are not transmitted to next generation, i.e. they are seed-borne but not seed-transmitted (Pagán, 2022). The question arises why 2021 was not so high in soybean yield, ie did not reach 3 t / ha with relatively sufficient soil moisture in some soybean crops, in particular in the Vinnytsia region. For the first time in Ukraine, we have shown that transgenic soybean varieties are also affected by the soybean mosaic virus (Mishchenko *et al.*, 2019). It was found that, despite genetic modifications, the yield of SMV infected plants was significantly reduced (Mishchenko *et al.*, 2019; 2018a). When plants were infected by SMV, soybean yields decreased in both farms of Kyiv and Poltava regions by 35.0-65.7%, respectively (Mishchenko *et al.*, 2019). Significant reduction in soybean yield (2.6 times) under SMV infection was found in Poltava region in the conditions of very dry 2017 year (hydrothermal coefficient, HTC = 0.53) compared to 2016 (HTC = 0.99). It was found that infection of plants by SMV (isolates SKP-16 and SGP-17) caused changes in biochemical parameters (protein content, basic fractions of spare proteins, etc.) (Mishchenko *et al.*, 2018a). Two unique amino acid substitutions (Ser → Cys and Lys → Ala) have been identified in the fragment of capsid protein gene of seed-transmitted SMV isolate named SKS-18 that may be

involved in seed transmission of the SMV and other important functions of the viral infection cycle (Mishchenko *et al.*, 2018b).

Therefore, the aim of this study was to estimate the presence of seed transmission of SMV to plants next generation, to determine "seed-transmitted" or "seed-borne" infection and impact of the virus on productivity and yield of different soybean genotypes.

MATERIAL AND METHODS

Seed SMV infection was determined in the seed of 2021 yield. Various soybean genotypes, grown on the fields of Vinnytsia region (Kordoba, Kea, Kofu, Medok, Viola, Volta, Avatar, Ezra) and Poltava region (Niagara and Neptun of different reproductions, AKAE, Kordoba 3, Kofu-1, Kofu-2) were investigated. It should be noted that incorrect seed sampling at this stage can lead to erroneous test results. In our experiment, the seeds were divided into fractions by size, degree of spotting and 100 seeds of each fraction of the studied soybean samples were selected.

Viruses detection was performed in germinated for three day at 24° C soybean seeds and plants grown from them in greenhouse (growing-on test) by DAS-ELISA using commercial antibodies against SMV, BYMV, AMV manufactured by Loewe (Germany) in three replicates. Samples of healthy soybean were used as negative controls. Commercial SMV, BYMV, AMV preparations (Loewe, Germany) were used for positive controls. The reaction results were recorded on the Thermo Labsystems Opsi MR (USA) reader with Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive (Crowther, 1995).

RT-PCR was used to confirm the SMV presence in samples. GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, USA) was used to extract total RNA. cDNA synthesis was performed using RevertAid Reverse Transcriptase (Thermo Scientific, USA) and SMV-specific oligonucleotide primers for amplifying a 469bp fragment of viral coat protein gene were used. Amplification steps using Dream Taq Green PCR Master Mix (Thermo Scientific, USA) were performed using a Genetic Research Instrumentation LTD thermocycler (UK).

The obtained sequence of the *CP* gene of Ukrainian SMV isolates from soybean (MG940992) was compared with the sequences available in the NCBI GenBank database using the BLAST program. 33 SMV isolates from different countries were used for the analysis. Nucleotide and amino acid sequences were aligned using Clustal W. Phylogenetic trees for the 469 nt fragment of *CP* gene of SMV isolates were constructed in MEGA 7 by the Neighbor Joining method (Kumar *et al.*, 2018) using the Jukes–Cantor model with 1000 bootstrap replicates to estimate the statistical significance of each node.

To accurately determine the possible seed transmission of SMV, ELISA was performed in soybean germinated seeds and leaves of 14-day soybean plants

grown from these seeds. In the first stage of SMV detection was performed in 3-day seedlings. Then these seedlings were sown in the soil in a laboratory greenhouse. Phenological observations of plants were carried out throughout the growing season until the formation of beans, namely, the number of plants with symptoms of SMV infection on the leaves and plant productivity. In the second stage, ELISA was used to determine SMV antigens in the leaves of two-week-old soybean plants. The results of SMV antigens in the leaves of two-week-old soybean plants grown from SMV-infected seeds were considered reliable. Percent of virus seed transmission (ST) calculated using the formula: $ST = (n \times 100) / N$, where n - number of virus infected plants (confirmed with symptoms presence, ELISA and RT-PCR), pcs; N - total number of plants grown from virus infected seeds under laboratory conditions, pcs. Several series of experiments with different soybean genotypes and in different years were carried out in order to accurately establish seed transmission of SMV, namely "seed-transmitted" and "seed-borne" and their impact on plant productivity and yield.

Germination, seed weight and plant productivity were determined by the conventional ISO weighting method (Pask *et al.*, 2012). In previous years (2016-2019), 30 soybean cultivars from five regions of Ukraine were studied.

Statistical analysis of experimental data was performed according to the parametric criteria of the normal distribution of variants, the standard deviation of the mean values - according to the generally accepted method using the computer database management program MS EXCEL.

RESULTS AND DISCUSSION

Soybean seeds of two varieties (KEA and Kordoba) were divided into different fractions by color and size. In fig. 1 presents soybean seeds of KEA variety.

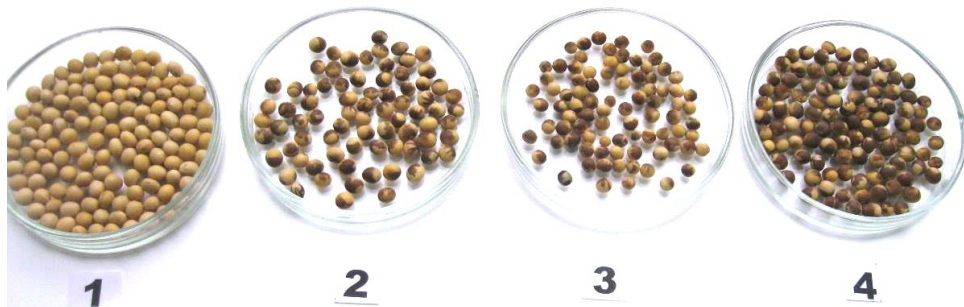


Figure 1. Seeds of variety KEA: 1 –without spotting; 2 – “butterfly” spotting; 3 – small size and spotted; 4 –big spots

Average weight of seeds was 0.205 g, 0.196 g, 0.137 g, and 0.197, respectively. In germinated seeds of Kordoba variety there were no antigens of SMV (data not shown). DAS ELISA and RT-PCR confirmed SMV presence in germinated KEA seeds #3, 4 and in dry germinated seeds (Fig. 3). To determine seed borne or seed-transmitted infection we revealed the growing-on test was

done. The remaining germinated seeds were planted in the soil in laboratory greenhouse and observations of plant growth and the appearance of viral symptoms were done.

On soybean KEA plants grown in greenhouse from seeds with big spots (Fig. 1, #4), we observed symptoms of SMV infection (Fig. 2a).

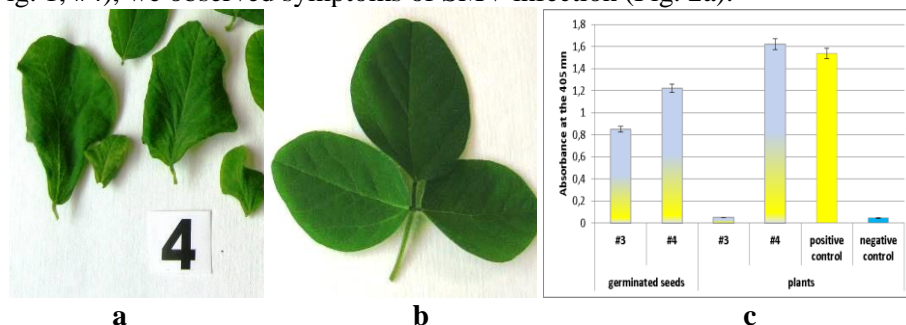


Figure 2. SMV symptoms on leaves of soybean plants var. KEA grown from infected spotted seeds #4 (a) and healthy (b); c - detection of SMV by DAS ELISA in germinated seeds and plants of var. KEA

There no symptoms of SMV infection in soybean KEA plants grown in greenhouse from small size and spotted seeds (Fig. 1,#3). DAS-ELISA confirmed the absence of the virus in this sample (Fig. 2c). So, this was seed-borne infection. So, DAS ELISA showed the presence of SMV antigens in soybean KEA plants grown from SMV infected seeds with big spots (Fig. 2c). Thus, experiment shown that SMV was presented in sprouts and cotyledons of small size and spotted seeds (seed-borne infection) and seeds with big spots (seed-transmitted infection). The presence of root nitrogen-fixing nodules in healthy variants of KEA soybeans in contrast to the SMV infected was quite important and interesting, which is clearly visible visually in Fig. 3. The total weight of a healthy plant exceeded twice that of virus infected, namely: for a healthy plant 0.338 g, and 0.164 g for the SMV-infected. Each variant had 50 plants.



Figure 3. Root system of soybean var. KEA: a – healthy with nodules; b – seed-transmitted SMV infection

Results about reduction of root nodules in soybean due to SMV infection are also demonstrated in India (Mandhare & Gawade, 2010).

In case with soybean seeds from Poltava region, seeds were also divided into several groups due to size and color. ELISA revealed SMV in germinated seeds of 5 soybean genotypes: Niagara P-35 (spotted big seeds); Niagara 34 (white seeds of various sizes); Neptun 24 (white seeds of various sizes); Neptun P 25 (both white and spotted seeds of various sizes) (Fig. 4).

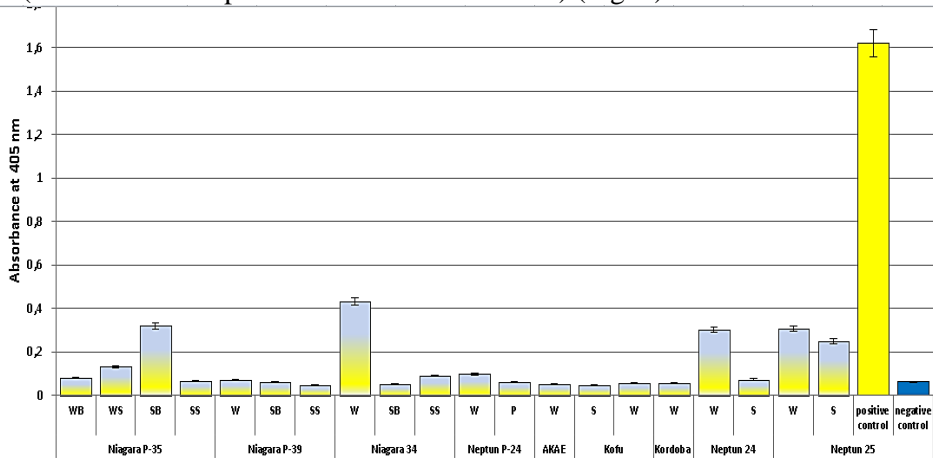


Figure 3. Detection of SMV in soybean seed by DAS-ELISA: WB – white big seeds; WS – white small seeds; SB –spotted big seeds; SS – spotted small seeds; W - white seeds of various size; S - spotted seeds of various size

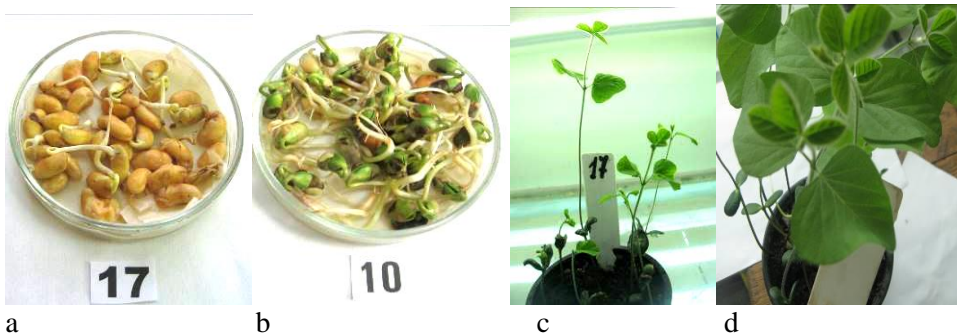


Figure 4. Seeds germination (a,b) and grown soybean plants (c,d): a,c - var. Neptun 24, white (seed-borne infection); b, d – var. Niagara P39, spotted (healthy), 25th February, 2022

There are no SMV was detected in plants grown from these infected seeds (data not shown). So, the infection is seed-borne. Despite this, in some samples seed-borne infection was revealed to reduce seed germination as seed-transmitted (Fig. 4). In Neptun 24, seed germination under SMV-infection was reduced in 28% -30 %.

In 2016-2019 seeds were collected by the presence of characteristic spotting of various degrees (Fig. 5a, 5b). In some cases seeds were without spotting (i.e. varieties Sultana, Ustyia) but in field conditions we observed SMV infection symptoms.

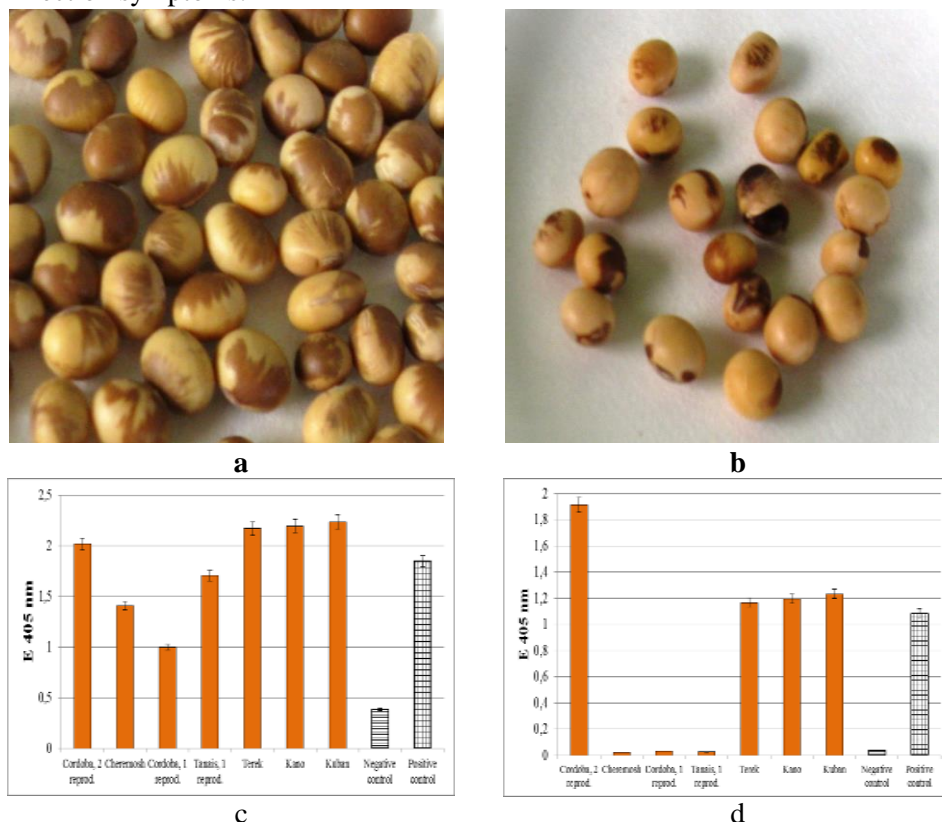


Figure 5. Soybean seeds (a,b) and SMV detection by ELISA (c,d): a – var. Medok (healthy); b –var. Kuban (seed-transmitted SMV infection), Poltava region 2016; c - in 3-days-old seed sprouts; d - in 2-week-old soybean plants, grown from SMV-infected seeds

DAS-ELISA results showed that SMV antigens are presented in 3-days-old sprouts of 7 soybean cultivars: Cheremosh, Tanais 1st reproduction, Cordoba 1st reproduction (Lviv region), Cordoba, 2nd reproduction (Sumy region), Terek, Kano and Kuban (Poltava region) (Fig. 5 b). In plants of 4 of them (Cordoba 2, reproduction, Terek, Kano and Kuban) were presented SMV (seed-transmitted) (Fig. 5d, Table 1). SMV symptoms presented also on the Fig. 6.

Our results showed that SMV seed infection (both seed-transmitted and seed-borne) don't significantly affect the germination rate comparing with virus-free seeds and this parameter was different depending the tested soybean cultivar (Table 1).

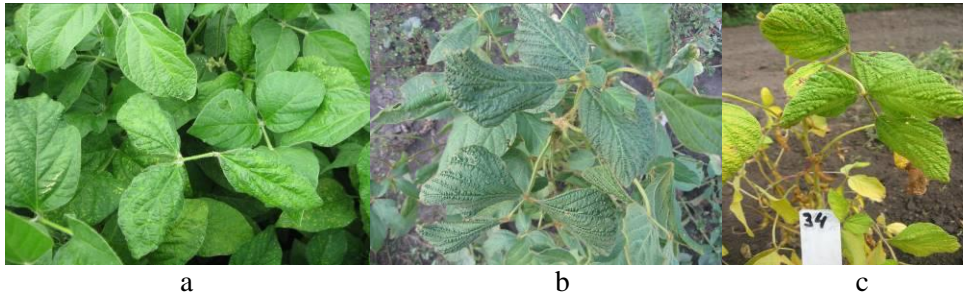


Figure 6. SMV induced symptoms: a –var. Hutoryanochka, 21 July 2016; b – var. Kano, seed-transmitted, 10th August 2017; c – Kano, 6th September 2017

Table 1. Results on SMV seed transmission

Variety	Germination, Lab/field,%	SMV transmission		Dry weight of 1 plant (g)	Soybeans (pcs) per 1 plant (greenhouse)
		type	From seed to plant, %		
Volta*	96/91	seed-borne	0	0.98	2.5
Niagara P 39*	93/90	0	0	1.16	3.5
Neptun P 23*	72 /69	seed-borne	0	0.95	2.3
Neptun P 24*	72/ 70	seed-borne	0	0.94	2.5
Niagara – P 35*	90/88	seed-borne	0	0.97	3.0
Niagara – P 34*	87/82	seed-borne	0	0.89	2.2
Kofu-1*	87/90	0	0	1.15	3.6
Kofu -2*	84/81	0	0	1.23	3.7
Medok*	93/90	0	0	1.20	3.8
Viola *	95/92	0	0	1.20	4.0
Arika*	94/93	0	0	1.20	3.6
Ezra*	95/92	0	0	1.15	3.7
Kordobe, 1 st reproduction, Vinnytsia region*	95/94	0	0	1.20	3,9
KEA, Vinnytsia region*	90/86	seed-transmitted	1.9	0.95	2,0
Kordoba-2, Sumy region**	92/88	seed-transmitted	3.3	7.85	6.0
Kordoba 2 nd reproduction, Poltava region	95/94	0	0	10.05	8.2
Terek, Poltava region, 2016**	89/84	seed-transmitted	10.5	6.15	5.0
Kano, Poltava region**	92/88	seed-transmitted	2.8	7.22	5.5
Kuban, Poltava region**	89/85	seed-transmitted	7.1	7.43	6.0
Tanais, Lviv region**	94/91	seed-borne	0	9.80	9.0
Cheremosh, Lviv region**	94/90	seed-borne	0	9.75	9.0

* winter greenhouse; **spring greenhouse

Liu *et al.* (2022) observed that SMV-infected spotted seed did not change germination rate of susceptible soybean seeds (cultivar NN1138-2). For example, Mandhare and Gawade received the opposite results. They are showed significant

reduction in seed germination (18 to 33%) in SMV infected soybean of different varieties (Mandhare & Gawade, 2010). But it should be mentioned that in our experiment we are showing that despite the fact that SMV doesn't critical affect germination, it reduces dry weight of plants and number of soybeans (pcs) per 1 plant (Table 1). Similar results indicating severe impact of SMV on soybean yield were received by Mandhare and Gawade, which are reported about significant reduction in plant height, number of nodules / plant, 1000 seed weight, seed germination (%) and seedling vigour index in virus infected plants of soybean varieties as compared to control (Mandhare & Gawade, 2010).

Phylogenetic analysis of 459 nt of *CP* gene sequence of soybean isolate named as KHUTP-16 (Ac. No in the NCBI GenBank MG940992) showed that KHUTP-16 clustered with isolates UA1Gr, Ar33, Lo3, VA2 from Ukraine, Iran and USA (Fig. 7) and shares identity 98.1% by nucleotide sequence and 95.8% amino acid sequence.

Thus, our studies (ELISA, RT-PCR, visual symptoms) on seedlings and plants grown from infected seeds showed that a variety of seed spots do not always indicate its infection with SMV. In our vegetation, laboratory and field studies, seed-transmitted SMV infection was detected in Kuban, Cordoba, Kano, Terek and KEA soybean varieties. Seed-borne SMV infection was detected in genotypes Cheremosh, Tanais, Niagara and Neptun of 1st and 2nd reproductions. SMV was not detected in any of the variants in intact plants older than two months.

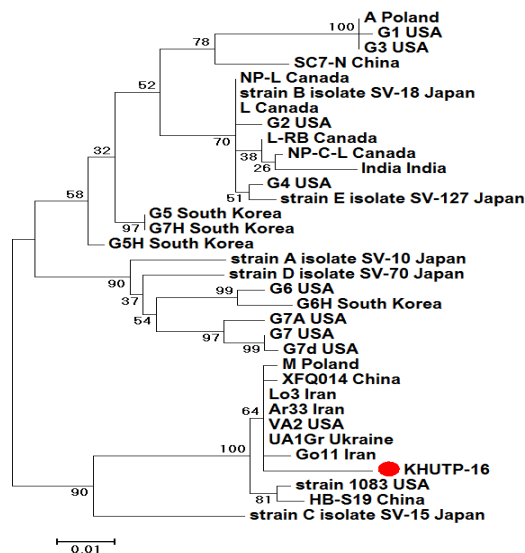


Figure 7. Neighbour-joining tree based on nucleotide sequences of 469 nt *CP* gene fragment of SMV isolates. Jukes-Cantor model was performed. The scale bar shows the number of substitutions per base. The studied Ukrainian isolate is marked with red circle

Data on a significantly different rate of seed transmission of SMV isolates are also shown in other countries: 0-2.78% in Poland (Jezewska *et al.*, 2015), 0-43% in the United States (Domier *et al.*, 2007), 0-67% in Brazil (Porto *et al.*, 1975). Bowers and Goodman have shown that both SMV strains transmitted by seeds and those that do not have this ability penetrate the germ of soybean seeds, but only SMV isolates that remain infectious are transmitted through seeds (Bowers and Goodman, 1979). The involvement of CP in this process has been linked to such different abilities for SMV virions stability. It has also been suggested that changes in the amino acid sequence of the HC-Pro protein may affect the protein's ability to assemble the virion or its ability to suppress post-transcriptional gene silencing (PTGS) (Domier *et al.*, 2007). Summing up the results of multi-series studies, it was found that the highest percentage for seed-transmitted SMV infection have varieties of Poltava region and selection - Terek - 10.5%. Other genotypes (Niagara, Neptun) are also grown in Poltava region but only "seed-borne".

It has been shown that despite the presence of the virus in seeds, isolates do not always have the ability to move from seed to plant, i.e. they are "seed-borne" but not "seed-transmitted". It was found that most SMV isolates do not have the ability to move from seed to plant, the percentage of transmission for them is 0%. Similar results about low per cent of SMV transmission to the leaves of newborn seedlings were demonstrated by Liu and co-authors in China (Liu *et al.*, 2022) comparing to the previous studies which indicated seed transmission of the isolate SMV-SC3 as 0% to 13.68% in various domestic soybean cultivars (Song *et al.*, 2015). Liu *et al.* have showed that isolate SMV-SC3 is replicated in the seed coat (brown spot rate was about 44.98%) and had infected the hypocotyl and cotyledon with rate of 1.67% (n=539) of the seeds in the embryo (Liu *et al.*, 2022). It is shown that 5 SMV isolates circulating in Ukraine are transmitted from seed to plant; the percentage of seed transmission for them is 1.9% - 10.5% depending on the genotype of soybean plants and the properties of the virus isolate. Information about different host genotypes and SMV infection is summarized in the review (Usovsky *et al.*, 2022).

CONCLUSION

Five SMV Ukrainian isolates are transmitted from seed to plant with rate of seed transmission of 1.9% -10.5%. The genotypes of the 2021 harvest from Vinnytsia and Poltava regions have a lower content of "seed-transmitted" compared to 2015. Only 7 isolates from the 60 tested (in winter greenhouse) had a "seed-borne SMV infection", which led to a decrease in germination by 13% - 28% and did not significantly affect plant productivity and yield. Therefore, the harvest in Ukraine has increased to 3 t / ha in recent years. In some farms, the yield was slightly lower due to lack of moisture, especially in the flowering and tying phases of beans, which simply fell off during drought. Based on the results obtained, it is necessary to constantly monitor the presence of soybean mosaic virus in the field for seed collection (seed sowing material).

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