Lidiya MISHCHENKO*, Alina DUNICH,
Ivan MISHCHENKO, Olga MOLODCHENKOVA [1]

MOLECULAR AND BIOLOGICAL PROPERTIES OF SOYBEAN MOSAIC VIRUS AND ITS INFLUENCE ON THE YIELD AND QUALITY OF SOYBEAN UNDER CLIMATE CHANGE CONDITIONS

SUMMARY

Soil and climatic conditions of Ukraine provide the obtaining of ecologically sound yields of grain crops and legumes, among which the soybean occupies the forefront. In 2017, the average soybean yield was significantly (by 3.4 - 13 centner/ha) lower than in 2016.

The aim of the work was to investigate the molecular and biological properties of the Soybean mosaic virus (SMV) isolate SKP-16, its effect on soybean yield and grain quality under agro-climatic change conditions. In 2015-2017, monitoring inspections of soybean crops showed that viral infections, occupied a significant place, mainly SMV. Also, seed transmission of SMV infected several soybean varieties was proved. In 2017, a very dry year for Ukraine, and especially for Poltava region, we found a significant infecting of many varieties with SMV.

Hydrothermal coefficient of G.T. Selyaninov (HTC) was less than 1, only 0.53. This indicator takes into account simultaneously the actions of the main climate elements – temperature and precipitation. The low HTC provided a significant number of aphids – vectors of SMV.

We have studied in detail the SMV isolate SKP-16 from soybean variety Kophy (GenBank Accession No MG940990). The yield of soybeans in the SMV-infected plants was reduced by 2.6 times, compared with healthy ones in 2017. But in 2016, the difference was considerably smaller, indicating a synergistic harmful effect of both factors - the virus and climatic conditions. The analysis of the nucleotide and amino acid sequences of the SKP-16 capsid protein gene revealed the highest percentage of identity (97.9% and 97.2% respectively) with the isolates UA1Gr, Ar33, Lo3, VA2. Four amino acid substitutions were found in position 1 (Ser → Cys), position 2 (Lys → Ser), position 3 (Gly → Leu), and position 5 (Val → Leu).

Keywords: soybean, Soybean mosaic virus, sequencing, yield, quality.

[1]Lidiya Mishchenko[*](corresponding author: tarasuniv@ukr.net), Alina Dunich, Virology Department, ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv, UKRAINE; Ivan Mishchenko, National University of Life and Environmental Sciences of Ukraine, Kyiv, UKRAINE; Olga MolodchenkovA, Plant Breeding & Genetics Institute –National Center of Seed and Cultivar Investigation, Odessa, UKRAINE

INTRODUCTION

Soil and climatic conditions of Ukraine provide the obtaining of ecologically sound yields of grain crops and legumes, among which the soybean occupies the forefront. Since 1990 the area of the collected culture has been constantly growing (from 87.8 thousand hectares in 1990 to 2 million hectares in 2017). However, soybean suffers from a lot of species of fungal, bacterial and viral diseases. To date, over 30 fungal, 10 bacterial and about 67 viral diseases are known which cause significant damage and can occur at different stages of soybean plant growth and development: from seed germination to the end of the vegetation. Particularly acute is the problem of viral diseases, which can affect soybean throughout the growing season, taking into account its ability to branch and appearance of young leaves that are very attractive to many aphid species. It was noted that the average soybean yield in 2017 was significantly (by 3.4 centner/ha, in many farms by 10-13 centner/ha) lower than in the previous year (23.4 centner/ha). This is due to a significant drought in 2017, since the soybean is needs moisture. It was investigated that Soybean mosaic virus (SMV) is affect transgenic soybean (Mishchenko et al., 2018a). But biochemical studies of soybean grain and harvest under the influence of viral infection under conditions of climate change and molecular biological properties of the SMV (isolate SKP-16) was not conducted, which was the aim of our study.

MATERIAL AND METHODS

Inspections of soybean plants were conducted by visual diagnostics method (Peresypkin et al., 2000). Biometrics, crop and its structure were carried out by generally accepted methods (Dospekhov, 1985).

Content of protein, fat, isoflavones in soybean seeds was investigated by (Molodchenkova et al., 2016). Meteorological data (the amount of precipitation, the amount of active temperatures, and the HTC for vegetation) were provided from the agrometopost of the Ustimovka Experimental Station of Plant Production, Plant Production Institute nd. a. V. Ya. Yuryev of NAAS, Poltava region.

Hydrothermal coefficient of Selyaninov (HTC) calculated using the formula: $r/ (0,1 \cdot \sum t > 10)$, where r - total rainfall during the growing season (May-August), mm; $\sum t > 10$ - the average daily air temperature more than 10 ° C for the same period. Identification of the viruses was performed by DAS-ELISA using commercial antibodies against Soybean mosaic virus (Loewe, Germany). The results were recorded on Termo Labsystems Opsis MR reader (USA) with Dynex Revelation Quicklink software at wavelengths of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher those of negative controls (Crowther, 1995). Statistical data processing was carried out according Dospekhov (1985).

Viral particle morphology was studied by transmission electron microscopy. Negative staining of virions was performed with the 2% solution of phosphotungstic acid and studied by electron microscope JEM 1400, JEOL,

Japan (Center for collective use, Danylo Zabolotny Institute of Microbiology and Virology of National Academy of Science of Ukraine). Total RNA was extracted from fresh leaves using Genomic DNA purification kit (Thermo Scientific, USA) following the manufacturer's instructions.

Two step RT-PCR was performed. The reverse transcription was performed using RevertAid Reverse Transcriptase – genetically modified MMuLV RT (Thermo Scientific, USA) according to the manufacturers' instructions using specific oligonucleotide primers to part of SMV CP gene (469 bp) (Mishchenko et al., 2018 a,b) CP gene sequences of the Ukrainian SMV isolate were compared with SMV sequences in the NCBI database with the BLAST program. Nucleotide and amino acid sequences were aligned using Clustul W in MEGA 7 (Kumar et al., 2016).

Phylogenetic trees for the part of SMV coat protein gene were constructed by the maximum-likelihood method (ML) (Huelsenbeck JP, Rannala, 1997) using the best-fitting evolutionary models. To check the reliability of the constructed trees used bootstrap test with 1000 bootstrap replications. The values at the nodes indicate the percentage of replicate trees in which associated taxa clustered together (number of bootstrap trails: 1000 replicates). The scale bar shows the number of substitutions per base. Aligned CP amino acid sequences were visualized and compared using BioEdit sequence alignment editor.

The percentage of the nucleotide sequences identity was presented as color blocks using the software package SDT v.1 (Sequence Demarcation Tool Version 1.1). Statistical analysis of experimental data was carried out according to the parametric criteria of the normal distribution option, the standard deviation of the mean values - according to the generally accepted method.

RESULTS AND DISCUSSION

The analysis of annual gross of soybean gross showed that in the last four years it was almost stable due to the increase of harvested areas and the highest yield in 2016, which was 2.34 t / ha. The following yield was noted: in 2000 – 1.06 t / ha, in 2010 – 1.62; 2012 – 1.72; 2013 – 2.05; 2014 - 2.16; 2015 - 1.84 t / ha, 2016 - 2.34, and 2017 - 2.00 t / ha. Beginning in 2010, soybean yield was less than 2.0 t / ha in 2010, 2012, and 2015 (Fig.1).
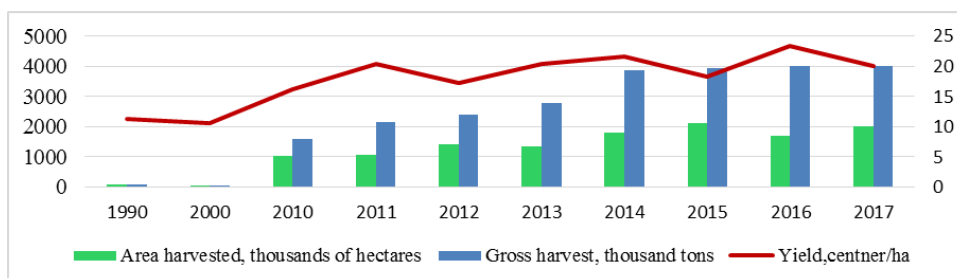

Figure 1. Soybean area harvested, yield and gross harvest in Ukraine for the period from 1990 to 2017

Our inspections of the soybean fields showed significant affecting of soybean plants with viral diseases in 2012 in the Kyiv region and in 2015, 2017 under the conditions of Poltava region. It was detected that the most common and wide spread was SMV. Symptoms caused with SMV on soybean plants showed on Fig.2.

a   b

Figure 2. Symptoms caused by SMV on soybean plants cv. Kophy (a) and Kano (b), Poltava region, 2016

In symptom soybeans, filamentous particles with sizes from 500 to 850 $\times$ 13-17 nm were found. It is typical for the viruses of *Potyvirus*, *Potyviridae* family which are have size 680-900$\times$11-13 nm (Virus taxonomy, 2012). All major deficiencies of soybeans are correlated with weather conditions. Analyzing our about viral infections of soybean, it is safe to state that the its productivity depends on climate change that affects both the host and the virus vectors - aphids. Previously, we have shown that the SMV infection leads to a significant decline in the yield of soybean cv. Kano and the transgenic varieties Grimo and Monro (Mishchenko et al., 2018a, b). But biochemical studies of soybean grains under viral infection in Ukraine were not conducted. The studies on the influence of SMV infection on the quality of soybean seeds showed ambiguous results. In general, it is known that grain (seed) quality deteriorates under viral infection. On the variability of the parameters had effect the time when the infection of soybean plants occurred (phases of growth and development of plants), variety, weather conditions, etc. For example, we present 1). Kano soybeans in a very dry 2017, when the HTC in the Poltava region was significantly less than 1; 2). less arid in 2016, when the HTC was about 1; 3). transgenic Grimo soybeans in 2017. There was an insignificant decrease in the content of oil (fat) in all variants with the damage of plants with viral infections. In comparison, protein content decreased by 2.34% in case of viral infection only in 2017 in the variety Kano. But in the variety of transgenic soybean Grimo, protein content increase by 5.33%. This can be explained by the accumulation of lectins and isoflavones (Table 1).

Table1. Influence of SMV infection on the biochemical parameters of soybean seeds

Variant/ sampling year	Protein, % per abs. dry substance	Fat, % per abs. dry substance	Lipoxygenase activity, CU/min/mg protein	Lectins activity, (µg/g protein /ml)$^{-1}$ $\times 10^{-3}$	Isoflavo -nes, µg/g
Kano, healthy/2016	38,15	23, 21	1,067	2, 651	73
Kano, SMV-infected/2016	39,22	21,90	0,588	2,354	102
Kano, healthy /2017	40,49	23,75	0,975	1,281	79
Kano, SMV-infected/2017	38,15	22,73	0,888	2,746	98
Grimo, helthy/ 2017	30,49	27,01	0,923	0,304	79
Grimo, SMV-infected/2017	35,82	24,22	0,812	1,325	73

An important function of flavonoids is the protection of plants from external unfavorable abiotic and biotic factors, in particular viruses (Panche et al., 2016). It is well seen that in the Kano variety infected with SMV, the content of isoflavones increases from 24 to 39%. In 2017, there was also marked increase in lectins content under SMV infection in both varieties (Kano – in 2.1 and Grimo – in 4.4 times).

In the case of a viral infection, decreased activity of lipoxygenase was also noted. Thus, the viral infection contributes to the accumulation of isoflavones in the soybean variety Kano and lectins in Grimo. It was determined that the maximum July temperature, the minimum temperature in August and the total amount of precipitation in July-August are significant factors affecting the productivity of wheat, soybeans and corn (Hatfield at al., 2017), as well as the number of viruses vectors. Therefore, to assess the effect on soybean yield, our attention was focused on the indicators: the amount of precipitation, the amount of active temperatures, and HTC during vegetation (May-August). An analysis of these meteorological data showed that 2017 was characterized by a significantly lower HTC and slightly higher temperatures than in the previous 2016 (Fig. 3).

It is clearly visible from Fig.3 that the HTC was significantly lower than the 1 in 2007 (0.58), 2009 (0.59), 2010 (0.61), 2012 (0.65), 2013 (0.46 - very dry zone), and 2017 (0.53 - very dry zone). It is considered that if the HTC is more than 1, then moisture is good, and for the forest-steppe zone this indicator is 1.2-1.4. Coefficient of variation (V) for the HTC is 32.9%, which indicates its significant variability in the period from 1995 to 2017. But by the sum of active temperatures during vegetation period, the variability was insignificant and the coefficient of variation was only 6.1%. In the amount of precipitation for vegetation in the same period also marked significant variability (V = 29.0%). The results of our research showed that the yield of soybeans of the Grimo

variety in 2016 (HTC about 1) was twice higher than yield of 2017 (a very dry year) in our experiment in the Poltava region. (Mishchenko et al., 2018 a). In Ukraine, soybean yields in 2016 were higher by 3.4 tons / ha compared to 2017.
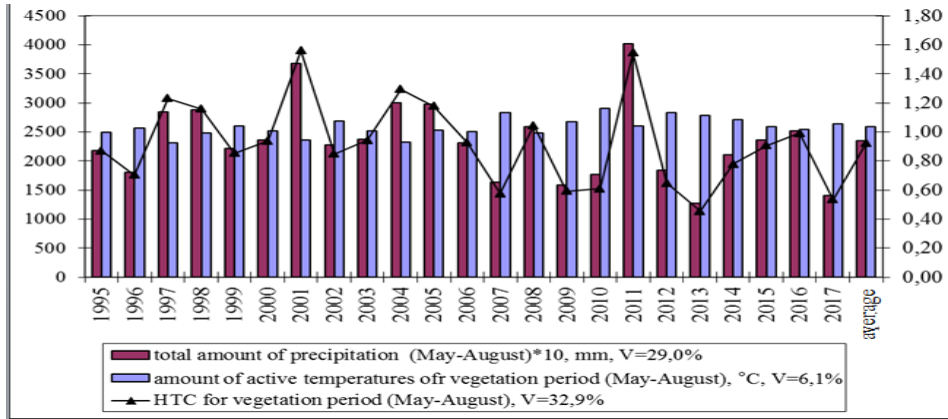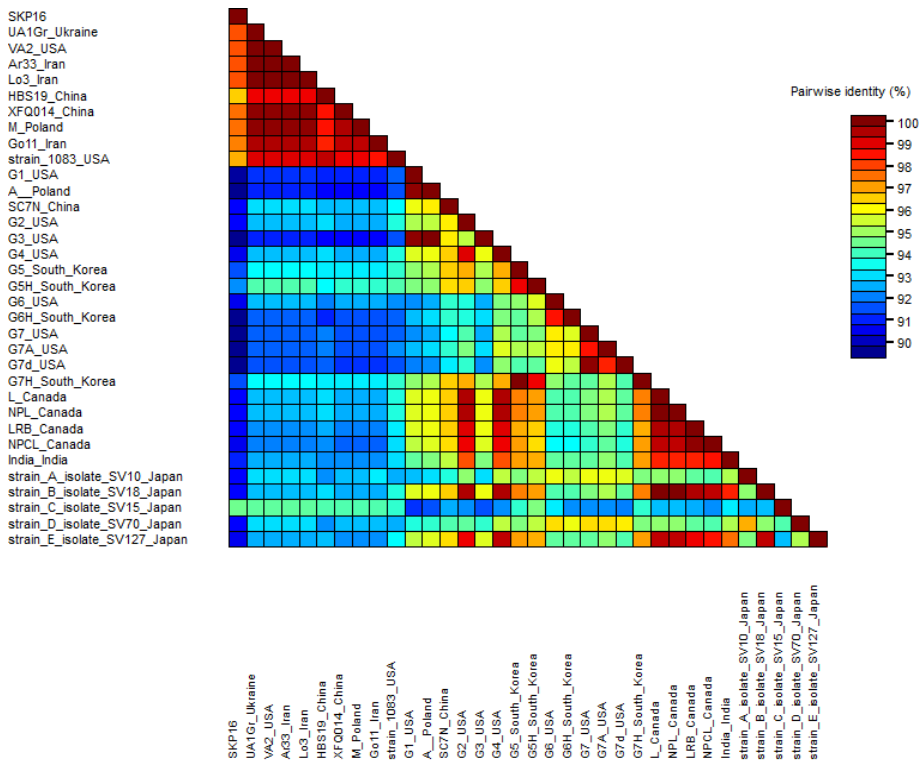

Figure 3. Meteorological data during vegetation periods May-August 1995-2017, Poltava region
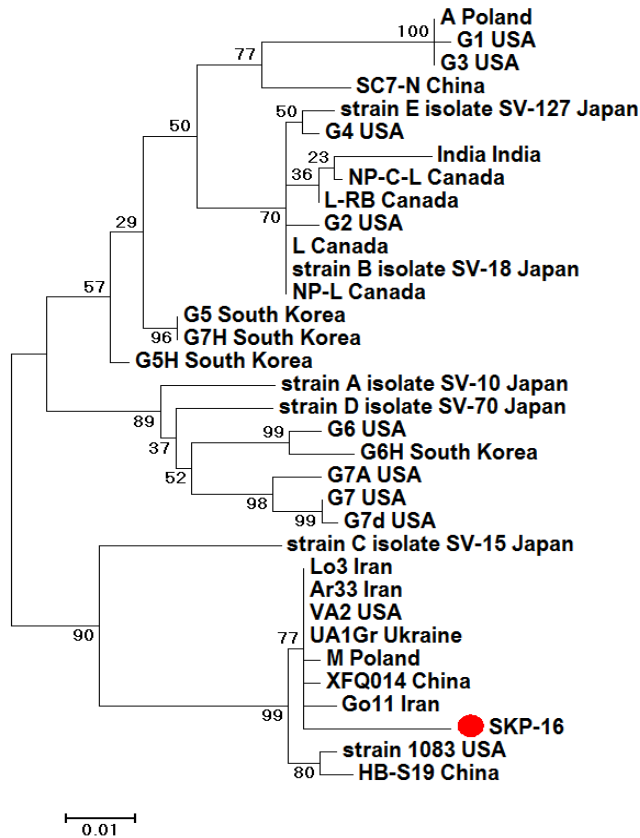

(a)

(b)

Figure 4. Nucleotide identity of SMV isolate SKP –16 and isolates from other countries: (a) -graphical representation of pairwise nucleotide identity of SMV isolates (percentage of identity is represented on the scale); (b) - maximum likelihood (ML) phylogenetic tree resulting nucleotide sequences of 430 bp part of the CP gene of isolates. Tree is constructed using Jukes-Cantor evolutionary model.

Next stage of the work was to study molecular properties of the SMV isolated from soybean cv. Kophy, named as SKP – 16 (GenBank Accession No MG940990). Nucleotide (nt) and amino acid (aa) sequence 430 nt of the coat protein (CP) gene region of the SMV isolate SKP – 16, localizing at the genomic position 8640-9069 were compared with sequences of 33 SMV isolates/strains from GenBank. SKP–16 has the highest percent of the identity with Ukrainian isolate UA1Gr, Iranian Ar33, Lo3 and American VA2: aa – 97.2%, nt – 97.9%. SKP–16 has a high nt identity with other isolates studied in China - XFQ014and HB-S19, Poland – M, Iran - Go11, and in USA - the strain 1083 (96,7-97,6%) (Fig.4a).

The phylogenetic tree presented in the Fig. 4b is fully consistent with the data in Fig.4a - isolate SKS-18 is located in one cluster with isolates with the

highest nucleotide identity: Ar33 and Lo3, VA2, UA1Gr, XFQ014, HB-S19, M, Go11 strain 1083, as well as strain C, isolate SV-15 that may be due to their similar variability. Isolate SKP –16 have 4 aa substitutions: at position 1 (Ser→Cys), position 2 (Lys→Ser), position 3 (Gly→Leu), and position 5 (Val→Leu) in the 171 aa investigated part of CP gene. It has been established that the aa substitutions of SKP –16 are unique in comparison with all SMV isolates taken for the analysis.

CONCLUSIONS

Analysis of the meteorological data in the agrocenosis was carried out and it was established that the yield of soybean crops is closely related to the ratio of precipitation and air temperature (HTC). From these parameters also depended the frequency of SMV detection, because they have a direct impact on insects – vectors of the viruses and degree of SMV influence on the on the seeds quality. The molecular genetic properties of the SMV isolate SKP-16 were studied.

It is determined that it has the highest percent of nt and aa identity with Iranian, American, Chinese, Polish isolates and isolate from Ukraine. The changes in the molecular genetic level in CP gene of SKP-16 can be involved in the it's harmfulness.

REFERENCES

Crowther J.R. (1995). ELISA. Theory and practice, N.Y.: Hamana Press, 223 p

Dospekhov B.A. (1985). Metodyka polevoho opyta (s osnovamy statystycheskoi obrabotky rezultatov issledovanyi [Methodology of field experience (with the basics of statistical processing of research results)]. Moscow: Agropromizdat, 351 p. [in Russian].

Hatfield J.L., Wright-Morton L., Hall B. (2017).Vulnerability of grain crops and croplands in the Midwest to climatic variability and adaptation strategies. Climate Change, vol. 146, pp. 263–275. doi: 10.1007/s10584-017-1997-x.

Huelsenbeck J.P., Rannala B. (1997). Maximum likelihood estimation of phylogeny using stratigraphic data. Paleobiology, 23(2), pp. 174-180. doi: https://doi.org/10.1017/S0094837300016778

Kumar S., Stecher G., Tamura K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evolution, vol. 33(7), pp. 1870–74.

Mishchenko L.T., Dunich A.A., Dashchenko A.V., Molodchenkova O.O. (2018a). The influence of Soybean mosaic virus on the yield of transgenic soybean and studying of its molecular genetic properties. Scientific reports of NULES of Ukraine, vol. 72 (2), pp. 1-11. [in Ukrainian].

Mishchenko L. T., Dunich A.A., Mishchenko I.A., Budzanivska I.G. (2018b).Viral infections of winter wheat and soybean and their influence on crop yield under climate change conditions. Bulletin of Taras Shevchenko National University of Kyiv. Biology, vol. 75(1), pp. 119-2113. [in Ukrainian].

Molodchenkova O.O., Adamovskaya V.G., Sichkar V.I., Kartuzova T.V., Bezkrovnaya L.Ya., Likhota E.B., Lavrova G.D. (2016). Biochemical characteristics of soybean seed for food direction varieties selection. Breeding and seed, vol.110, pp. 150-161. [in Ukrainian].

Panche A.N. Diwan A.D., Chandra S. R. (2016). Flavonoids: an overview. J Nutr Sci, 2016, vol.5, pp. e47.

Peresypkin V.F., Markov I.L., Shelestova V.S. (2000). Workshop on the basics of research in plant protection, Kyiv. [in Ukrainian].

Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses (2012). eds. A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz: Elsevier, 1327 p.