DOI: 10.17707/AgricultForest.65.4.02

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INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON PRODUCTIVITY OF TRANSGENIC SOYBEAN AND MOLECULAR PROPERTIES OF DISEASE PATHOGEN

SUMMARY

For the first time in Ukraine, it has been shown that transgenic soybean cv. Grimo and Monro is affected by various pathogens, including viral diseases. Negative effect of the *Soybean mosaic virus* (SMV)-infection on the productivity and yield structure of soybean plants is proved. Also, significant role for the cultivation of transgenic soybeans (Kyiv and Poltava regions, Ukraine) is revealed for growth conditions. Cultivation of the cv. Monro in different weather and climatic conditions has shown that the growth limiting factor is moisture. In 2018, harvest of soybean cv. Monro in farm "Mriya" in Kyiv region was 3.1 t/ha, but in the farm "Mir" in Poltava region it was almost twice lower. We analyzed the coefficient of significance of deviations of the agrometeorological regime in 2018 comparing with the average perennial metadata. It was 1.14 - 2.30 and demonstrated that the conditions in 2018 are very different comparing with the average perennial parameters and close to the rare. SMV named SGK-17 (Ac No MG940988), isolated from transgenic soybean plants cv. Monro from Kyiv region, was studied in the detail. Nucleotide and amino acid sequences of the SGK-17 coat protein gene region (430 nt) were compared with sequences of SMV isolates from different countries. SGK-17 has the highest identity level (97.9% nt and 97.2% aa) with isolates from China, Poland, Iran, USA, Ukraine and is belonging to the one cluster with them. Four unique substitutions in CP gene of SGK-17 are revealed, which can be involved in its ability to infect transgenic soybean.

Keywords: transgenic soybean, soybean pathogens, sequencing, productivity, weather and climatic conditions

INTRODUCTION

Plants can be damaged by infectious pathogens such as viruses, bacteria, and fungi. They can also be damaged by noninfectious (abiotic) factors such as temperature and moisture extremes, chemical toxicity etc. Weather conditions

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Paper presented at the 10th International Scientific Agricultural Symposium "AGROSYM 2019".

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

influence on plants, phytopathogens and their vectors. There are two distinct mechanisms by which climate change can impact the relationship between pests and crop plants. Firstly, changes in climate have a direct impact on the biology of insects, including vectors, leading to differences in their survival, reproduction and spread. Secondly, there are the likely changes in agricultural practice that will take place as a result of climate change, and the influence of these changes on the availability of host plants for the pest species; e.g. the introduction of new crop species and plant genotypes, and changes in husbandry practice (Roos al., 2011). It was shown that factors like CO_2, elevated temperature and rainfall-related parameters influence on plant viruses, eg, CMV,PVY, PVX (Del Torro et al., 2015) TYLCV, and TuMV (Chung et al., 2015), Potato leaf roll virus and Potato yellow vein virus (Jones, 2014), BYDV (Nancarrow et al., 2014; Rua et al., 2013), BYMV (Guerret et al., 2016), and several important their vectors (Gillespie et al., 2012). Increased soil moisture and temperature in temperate regions, including northern Europe, are expected to increase the activities of zoospores and nematodes that transmit viruses (Jones 2014; Roos al., 2011). Potato mop-top virus (PMTV) and Beet necrotic yellow vein virus (BNYVV) are transmitted by the plasmodiophorids *Spongospora subterranea* and *Polymyxa betae*, respectively (Santala et al. 2010). The most important factor for this spread is probably the movement of infected plant material and soil, but climate factors may also have been involved. Bymoviruses and furoviruses (transmitted by *Polymyxa graminis*) are of great economic importance in autumn-sown cereals in large parts of Europe. In Canada increasing of PLRV incidence was detected due to increased vectors amount due to high winter temperatures (Boland et al., 2004).). In Germany, a clear relation was recently found between the number of infection days in autumn and BYDV-attack in winter barley fields (Habekuß et al., 2009). It was shown that rainfall less than the 30-year average caused a difference in the relative detection rate of BYDV-PAV and Cereal yellow dwarf virus, RPV strain in Alabama (Bowen, 2003).

In the future, the frequency of new encounters between viruses and plant species is likely to increase even more rapidly because of the major alterations in cultivated plant distributions anticipated from climate change. Climate change has been shown to cause emergence of a new viruses and strains in certain countries. So, many new PVY strains (PVYNTN, PVYNW) have appeared in the Netherlands over the last 12 years due to significant climate change (Lal et al., 2018). In Brazil and Azerbaijan the occurrence of BYDV was first reported only in 2013 (Mar et al., 2013; Mustafayev et al., 2013). Most of the scientific papers are review and dedicated to the prediction and development of a scenario of the impact of climate change on plant viruses. However, experimental works that would be directly focused on the influence of abiotic factors with SMV on genetically modificated (GM) soybean are absent.

It is known that infection caused by Soybean mosaic virus (SMV) has a negative effect on the soybean productivity, structure yield, and seeds quality in Ukraine (Mishchenko et al., 2018a; Mishchenko et al., 2018b). Given the harmful effects of the virus, new soybean varieties that are resistant to various phytopathogens, including viruses are intensively developing and introducing. However, mostly soybean varieties have complex resistance against abiotic factors and fungal or bacterial diseases. Varieties of genetically modified (GM) soybeans appeared in Ukraine that should be characterized by high productivity and resistance to diseases. Soybean varieties Grimo, Monroe, Apollon, etc already grown. Also, significant role for the cultivation of transgenic soybeans (Kyiv and Poltava regions, Ukraine) is revealed for growth conditions (abiotic factors). Therefore, the aim of our work was to investigate the possibility of infecting GM soybean by SMV, to study its effect on productivity and yield of plants, as well as to establish the molecular genetic features of the SMV isolate that has ability to affect GM plants.

MATERIAL AND METHODS

During 2017-2018 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).

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.

Analysis of weather conditions compared to the average multi-year indicators were conducted on the basis of the coefficient of deviations significance (Cs) of agrometeorological regime elements of each of the studied years from average multi-year values by the formula: (Xi-X-)/S, where Xi- element of current weather; X- - average annual value; S - mean square deviation; i- number of the year (Vyshnivskyi, 2013). Level of the coefficients of deviations significance was determined by gradation: 0-1 – conditions are close to the ordinary; 1-2 – conditions that are very different from the average perennials; 2 – conditions close to the rare (extreme).

Hydrothermal coefficient of Selyaninov (HTC) calculated using the formula: $r/(0{,}1\cdot\Sigma\, t>10)$, where r - total rainfall during the growing season (May-August), mm; $\Sigma t>10$ - 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 405/630 nm. Samples were considered positive when their absorbance values were at least three times higher those of negative controls (Crowther, 1995).

Total RNA was extracted from fresh leaves using RNeasy Plant Mini kit (Qiagen) 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. Specific oligonucleotide primers to the part of SMV CP gene (469 bp were used (Mishchenko et al., 2018b)

For sequencing DNA fragments were purified from the agarose gel using a QIAquick Gel Extraction Kit (Qiagen, Great Britain) following to the manufacturers' instructions. CP gene sequences (430 nt) of the Ukrainian SMV isolate were compared with SMV sequences in the NCBI database with the BLAST program. SMV isolates using in this study are presented in Fig. 3. Nucleotide and amino acid sequences were aligned using Clustul W in MEGA 7 (Kumar et al., 2016). 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.

To calculate the dN/dS ratio, an indicator of the evolutionary direction, the CP nucleotide sequences of all SMV isolates were codon-aligned. The ratio of the rate of nonsynonymous (dN) to the rate of synonymous (dS) mutations was calculated using the Nei-Gojoboori method in the SNAP program (Korber, 2000).

Statistical analysis

Five biological repeats were conducted per each ELISA measurement. Each biological repeat contained leaves from five individual plants pooled together prior to grinding. Three technical repeats were conducted for each biological repeat. When appropriate, the technical repeats data was averaged to get the mean value for each biological repeat.

RESULTS AND DISCUSSION

Our previous studies of 30 soybean varieties in the Poltava region showed the presence of wrinkle and mosaic symptoms on leaves.

Figure 1. Symptoms caused by SMV on GM soybean varieties Monroe, Apollon and Grimo

The results of ELISA and RT-PCR showed that plants infected by *Soybean mosaic virus* (Mishchenko et al., 2017a). Among the inspected varieties, our attention was drawn to the fact that the SMV was affecting the transgenic varieties Monroe, Apollon and Grimo. Symptoms of wrinkle and blistering, deformation and reduction of leaf size (Fig. 1). Authors have been noticed that cv. Monroe soybean seeds in 2018 were characterized by significant spotting (Fig. 2).

a b

Figure 2. SMV symptoms on GM soybean cv. Monroe in 2018: a – severe wrinkle of leaves; b – seeds spotting from SMV-infected plants, at the right – seeds from healthy plants

The next stage was to determine effect of SMV isolated from these soybean plants cv. Monroe (named as SGK-17) on the yield. The results showed harmful effect of the virus and significant yield reducing of GM-soybean plants of the Monroe variety infected by SMV SGK-17 (Table 1).

Table1. Influence of SMV infection on the yield structure and productivity of GM-soybean cv. Monroe

Variant	Branching, pcs	Stem height to 1^{st} bean, cm	Plant height, cm	Amount of beans per plant, pcs					Total of seds, pcs	Weight of seeds from1 plant, g	Weight of 1000 grains, g
				Total	With 1 seed	With 2 seeds	With 3 seeds	With 4 seeds			
Healthy plants	3.5 ±0.5	9.6 ±1.5	110.3 ±1.8	95.0 ±4.0	47.0 ±1.0	18.0 ±2.0	18.0 ±2.0	4..0 ±0.4	153.0± 6.0	19.65 ±2.13	181 ±12
Infected plants	2.2 ±0.2	8.1 ±1.3	94.3 ±1.4	58.0 ±3.0	39.0 ±1.0	14.0 ±2.0	12.0 ±2.0	–	103.0 ±4.0	14.01 ±1.10	139 ±10

It was found that in the virus infected plants, branching was 1.6 times lower than in healthy ones. In addition, the SMV infection reduces the amount of beans per plant by 1.6 times and amount of seeds by 1.5 times. Weight of seeds from one plant is reduced by 1.4 times, the weight of 1000 grains - by 1.3 times. In healthy plants, even a small number of beans (4.0) with four seeds in the bean were noted. Infection of soybean plants by Soybean mosaic virus (SMV) leads to significant crop losses - from 8% to 50% in natural conditions and even up to 100% - in cases of epiphytosis (Liao et al., 2002). SMV infection can cause changes in the biochemical composition of seeds, reduce the viability of seedlings (El-Amretz et al., 1987; Mishchenko et al., 2018b).

The yield of transgenic soybean cv. Monroe in 2017 -2018, grown in farm ‘Mir’ (Poltava region) was 1.3 - 1.4 t / ha while in the farm Mriya (Kyiv region), where there was more precipitation, the yield of soybean Monroe ranged from 2.18 t / ha. up to 3.0 t / ha. But the harvest in 2016, more moisture-proof and susceptible for soybean growing, was 2.6 and 2.8 t / ha, respectively. Weather in 2016 in the Poltava region was considered very dry because the hydrothermal coefficient of Selyaninov (HTC) was 0.99. HTC in 2017 was only 0.53 that means that 2017 was very dry year. Therefore, in most of the farms in the region, soybean production was at a level of 1.3 t / ha. In case of damage by SMV infection, soybean yield decreased in both farms of Kyiv and Poltava regions on 35.0 – 65.7%, respectively. More significant yield reduction (in 2.6 times) in case of viral infection was noted in the Poltava region under conditions of very dry climate (in 2017 HTC=0,53; in 2018 HTC= 0,5). (Mishchenko et al., 2018b).

We have calculated coefficient of deviations significance (Cs) for the period 1955-2018 for all months (Table 2).

Table 2. Coefficient of deviations significance of the agrometeorological regime indicators of the current year from the average perennials, 2018

Indicator	April	May	June	July	August	September
Air temperature	1,84	1,14	1,34	1,34	2,96	2,30
Precipitation amount	-0,82	-0,65	-0,65	-0,48	-1,55	0,30

The results of the table indicate that during the entire period of soybean growth in the Poltava region in 2018, the conditions for its cultivation were far from ordinary, namely: from April to July they were so much different from the average perennials. In August and September they approached the rare ones. Yield reducing of transgenic soybean cv. Monroe in the farm ‘Mir’ in the Poltava region was almost twice, in comparison with farm "Mriya" in Kyiv region, where the conditions of cultivation were close to ordinary and yield was 3.0 t / ha. Such yield decreasing can be explained by influence of such important abiotic factor as the coefficient of the significance of deviations of the agrometeorological regime indicators.

Climate change affects the level of damage from plant diseases, because the environment significantly affects not only plants, but also pathogens and their vectors. The appearance, prevalence, harmfulness of plant virus diseases, as well as the subsequent co-evolution of plants and their pathogens, which leads to changes in the species composition of viruses in a certain region, the appearance of differences in the properties of viral isolates, the emergence of epiphytothies directly depend on changes associated with global warming (increase in temperature, change of precipitation, increase of CO2 and ozone, drought, etc)

(Bowen,et al., 2003; Nancarrow et al., 2014;Mishchenko et al., 2017b). It is worth noting that the SMV is transmitted by vector - aphids. In this regard, climate change can indirectly affect the intensity and prevalence of SMVs through their reproductive / survival effects of its vectors. Thus, in the conditions of mild winter, there is a high migration rate of aphids in the spring and a high incidence of plant damage.

The severity of viral diseases is determined in large part by the amount of inoculum and the time of infection. Infection of plants at an early developmental stage usually leads to more severe disease symptoms, e.g., SMV (Ren et al. 1997). For some viruses, higher temperatures also cause more severe symptoms development. Insects such as aphids are expected to have increased survival with milder winter temperatures, and higher spring and summer temperatures will increase their development and reproductive rates and lead to more severe disease. Mild winters increase the survival of weeds that are the reservoirs of viruses. Increases in frequency and intensity of summer storms with high winds, rain, and hail will increase wounding of plants and result in increased transmission of viruses by mechanical means. It is also known that, at elevated air temperatures, the symptoms of viral diseases are often masked, sometimes not noticeable, which significantly complicates the visual diagnosis and control of these pathogens (Svensson, 2010; Jones and Barbetti, 2012).

Molecular investigation of the SMV isolate SGK-17 shown that the highest level of the nucleotide sequences identity (97.9%) and amino acid sequences (97.2%) it has with Ukrainian isolates, Iranian, Chinese isolates, American isolate VA2 and Polish isolate M (Fig 3).

To explore the evolutionary forces acting on the SMV CP gene, the dN/dS values were calculated for all of the SMV CP sequences included to our study. This ratio indicates the amount of nonsynonymous to synonymous mutations. dN/dS ratio for isolate SGK-17 compared to all other isolates was 0,0536, The global dN/dS ratio for all of the sequences taken to the study was 0.014 ($p < 0.01$). This indicates about higher nucleotide diversity of the isolate SGK-17 compared to SMV isolates from other countries.

SGK–17 has four amino acid substituions in the 430 nt region of coat protein gene: at the position 1 (Ser→Trp), at the position 2 (Lys→Phe), at the position 3 (Gly→Arg), and at the position 4 (Lys→Asn) (Fig. 4).

It should be noted that we revealed the same aa substitution for all SMV isolates from GM soybean which we were studied earlier (Mishchenko et al., 2018a). It was the aa Ser→Trp substitution at the position 1. It is known that only few single-amino-acid changes near the C terminus of the CP of certain SMV strains led to the impossibility to seed transmission (Jossey et al., 2013). It was also found several single nucleotide variations (SNVs) in different regions of genome of seed-transmitted SMV (Jo et al., 2017; Mishchenko et al., 2018c). So, such substitution can be involved to the ability of these SMV isolates infect GM soybean.

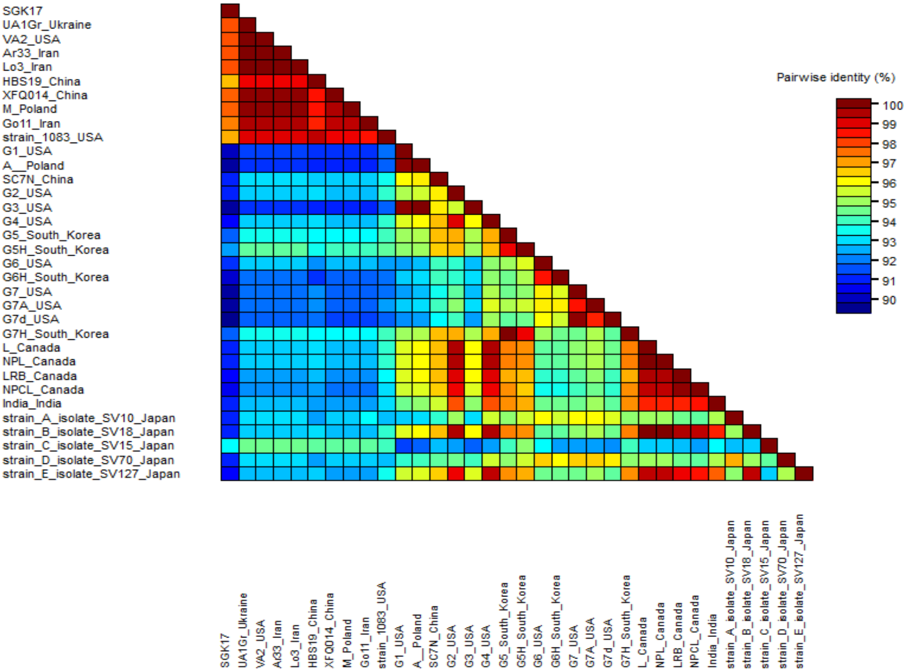
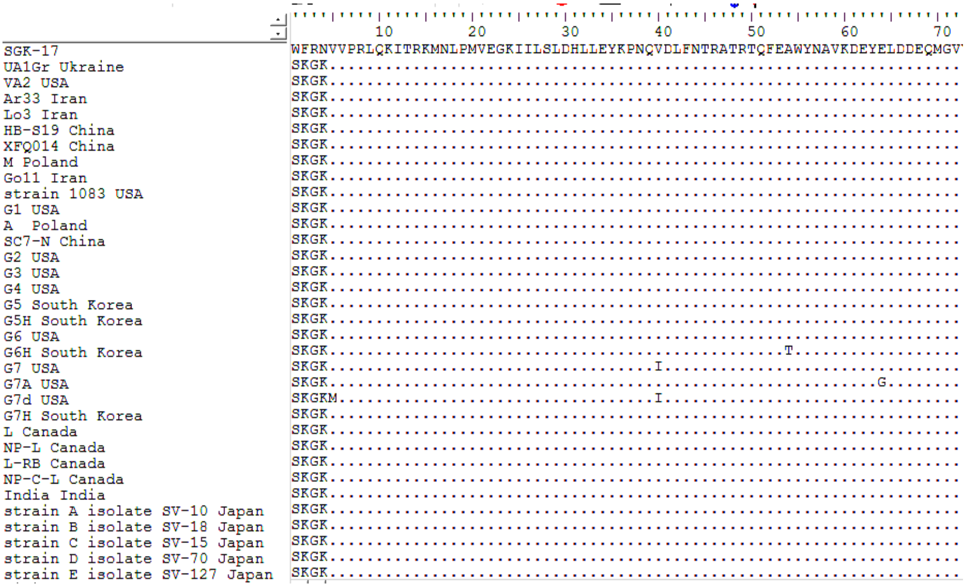


Figure 3. Graphical representation of pairwise nucleotide identity of SMV isolates (percentage of identity is represented on the scale)



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                                   10        20        30        40        50        60        70
SGK-17                          WFRNVVPRLQKITRKMNLPMVEGKIILSLDHLLEYKPNQVDLFNTRATRTQFEAWYNAVKDEYELDDEQMGV
UA1Gr Ukraine                   SKGK....................................................................
VA2 USA                         SKGK....................................................................
Ar33 Iran                       SKGK....................................................................
Lo3 Iran                        SKGK....................................................................
HB-S19 China                    SKGK....................................................................
XFQ014 China                    SKGK....................................................................
M Poland                        SKGK....................................................................
Go11 Iran                       SKGK....................................................................
strain 1083 USA                 SKGK....................................................................
G1 USA                          SKGK....................................................................
A  Poland                       SKGK....................................................................
SC7-N China                     SKGK....................................................................
G2 USA                          SKGK....................................................................
G3 USA                          SKGK....................................................................
G4 USA                          SKGK....................................................................
G5 South Korea                  SKGK....................................................................
G5H South Korea                 SKGK....................................................................
G6 USA                          SKGK....................................................................
G6H South Korea                 SKGK.................................................T..................
G7 USA                          SKGK...................................I................................
G7A USA                         SKGK...........................................................G........
G7d USA                         SKGKM..................................I................................
G7H South Korea                 SKGK....................................................................
L Canada                        SKGK....................................................................
NP-L Canada                     SKGK....................................................................
L-RB Canada                     SKGK....................................................................
NP-C-L Canada                   SKGK....................................................................
India India                     SKGK....................................................................
strain A isolate SV-10 Japan    SKGK....................................................................
strain B isolate SV-18 Japan    SKGK....................................................................
strain C isolate SV-15 Japan    SKGK....................................................................
strain D isolate SV-70 Japan    SKGK....................................................................
strain E isolate SV-127 Japan   SKGK....................................................................
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Figure 4. Comparison of the amino acid sequences of the CP fragment of isolate SGK-17 with other isolates / strains of the SMV. Numbers above represent the positions of amino acids. With dots "." indicated identical amino acids, with letters - amino acids substitutions

CONCLUSIONS

Thus, it has been shown that transgenic soybean varieties are affected by Soybean mosaic virus. It has been found that the SMV significantly reduces the yield and productivity of soybean plants. It has been established that, despite genetic modifications, the productivity of SMV-infected plants is significantly reduced. Molecular investigation of the SMV isolate SGK-17 revealed aa substitutions which can be involved to the ability of the virus infect GM soybean varieties. It was shown that HTC and so coefficient of deviations significance of the agrometeorological regime indicators play an important role in soybean productivity. Weather indicators in 2018 were significant differ from average perennials and were close to the rare conditions, that in complex with viral infection led to reducing of soybean yield.

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