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BIOCHEMICAL CHANGES IN SOYBEAN PLANTS AND SEEDS CAUSED BY VIRAL INFECTION

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Abstract. Soybean (*Glycine max* L.) is a significant source of protein and nutrients and is used in many healthy food products. It is known that soybean is susceptible to damage by viruses, such as soybean mosaic virus (SMV). Infection by SMV has a negative effect on the soybean productivity and seed quality. The goal of research was to study the impact of infection by soybean mosaic virus on biochemical composition of soybean plants and seed quality. Testing of soybean plants from Odesa region for the presence of the most destructive viruses showed an appearance of symptoms of crinkle and mosaic of the leaf blade. The results of DAS-ELISA and RT-PCR have shown that the plants are infected by SMV. Standard and adapted methods of biochemical analysis were used for research of biochemical composition of soybean plants and seed. Isolation and identification of main soybean storage protein was carried out using methods developed in the laboratory (Pat #42181, 107671). Electrophoresis of proteins was performed in 15% PAGE using Laemmli method. It has been established that infection by SMV isolates Odesytka-24-Ukr and Vasylkivska-24-Ukr caused changes in the biochemical characteristics (content of protein, main storage protein fractions (glycinin and β-conglycinin), fat, carbohydrates, flavonoids, fat acid composition, electrophoretic spectra of glycinin and β-conglycinin, activity of lectin, lipoxygenase, trypsin inhibitor) in the leaves of plants in the flowering phase and seed. These changes depend on the soybean variety. The obtained results can be used for development of the methods of soybean varieties selection with high seed quality and resilience to viral infection) and will be recommended for implementation in breeding program for creation of soybean varieties for production of high-quality food products.

Keywords: *Glycine max L.*, seed and food quality, glycinin, β-conglycinin, fatty acids composition, soybean mosaic virus...

Introduction. Formulation of the problem

Ukraine is one of the five largest exporters of grain on the world market, supplying more than 45 million tons of grain to the world market every year, especially to countries with food shortages. The main source of vegetable protein and other food substances for the population of all countries of the world are also leguminous crops, in particular soybean (*Glycine max* L.). Soybean is strategic legume world crop of XXI century, which is located in the center of attention of world agricultural science and production. It is one of the few plants in which the protein is balanced in terms of amino acid composition much better than many vegetable and animal proteins. At least 10% of global food production is lost to plant diseases, with an economic value of US$220 billion annually. In Ukraine, numerous phytopathogens, including viruses, create significant obstacles to the sustainable production of the most economically important crops and are one of the main threats to our food security. In 2022, this situation became more complicated as a result of the war waged by russia against Ukraine - global supply chains of food products from Ukraine were disrupted; tons of grain stolen by russia from occupied territories, which led to deficit and high prices for agricultural products in the world. As a result, in addition to the decrease in crop yield due to damage by pathogens, the following problems arise in today's wartime realities: 1) decrease in sown areas due to active hostilities, occupied territories and mined fields (by 45% compared to 2021); 2) mined fields cannot be cultivated, therefore they serve as a source (reservoirs) of harmful organisms (insects – viruses vectors) and plants infected by viral diseases and negative effect of virus infection on the crop productivity and seed and food quality.

Analysis of recent research and publications

A significant increase in soybean production in recent years has taken place in Ukraine. The nutrition value of soybean was attributed to its high-quality protein content. Also, soybean is a major oil crop and a rich source of phytochemicals, which have important beneficial effects on human and animal health [1]. Soybean storage proteins mostly consist of globulins, which are classified according to their sedimentation coefficient [2]. 7S and 11S globulins are the most valuable for the production of soybean products and account for 70–75% of the total protein. 11S globulin (glycinin) is a heterogeneous hexameric protein with molecular mass of 300–380 kDa that consists of a basic polypeptide (~20 kDa) and an acidic polypeptide (~35 kDa) linked together via a disulfide crosslink. The 7S globulin is a trimer glycoprotein with molecuar mass of 150–200 kDa composed of α’ (~72 kDa), α (~68 kDa), and β (~52 kDa) subunits. Glycinin subunits are coded by 5 genes of a family, whereas about 15 genes are present for β-conglycinin subunits [3]. Some subunits of soybean glycinin and β-conglycinin are food allergens [4].

The quality of oil, its biological effectiveness and nutritional value are largely determined by the content and ratio of fatty acids in it. The fatty acids composition of soybean oil is quite favorable for human nutrition and animal feeding in terms of the ratio of linoleic and linolenic acids, which brings it closer to sunflower oil. It contains a small (11–15%) amount of saturated (palmitic and stearic) fatty acids, an excess of which is harmful to the body due to the formation of cholesterol in the blood; a lot (50–55%) of valuable monounsaturated linoleic acid, a moderate content (20–30%) of easily digestible oleic acid and a low (6–12%) content of poorly digestible linolenic acid. The presence of a large amount of linoleic and linolenic fatty acids in soybean oil is one of the reasons for the oil's instability to oxidation during its production and storage. But, on the other hand, these fatty acids are important for the body's nutrition due to their physiological activity. In the human body, they play the role of building material for the synthesis of vital compounds – prostaglandins, which affect cholesterol metabolism, prevent thrombosis, reduce inflammation, and stimulate the protective reactions [5].

Soybean seeds contain many antioxidants, including flavonoids. They have a wide range of biological activity. For example, they are common components of food compounds that perform vital functions for human health, such as reducing the incidence of specific cancers and cardiovascular diseases [6,7]. In addition, they play an important role in formation of plant resilience to diseases and abiotic stress factors [8]. The nutritional properties of soybean seeds are associated with the presence of substances such as trypsin inhibitors, lectins, lipoxygenase, which negatively affect the food and feed value of seeds [9, 10]. But on the other hand, these compounds play an important role in plant resilience to unfavorable factors of environment [11–13].

It is known that soybean is susceptible to damage by many viruses, such as soybean mosaic virus (SMV), alfalfa mosaic virus, bean yellow mosaic virus, and others [14]. SMV is the most prevalent virus and is recognized as the most serious, long-standing problem in many soybean producing areas in the world. Infection by SMV usually results in severe yield losses, seed quality and seedling reduction [15]. The strains SKP-16 and SGP–17 of SMV are present in the fields of Ukraine. It was found that the studied SMV isolates differ in virions size from the previously identified Ukrainian SMV isolates. Phylogenetic analysis of the nucleotide sequence of the capsid protein gene of SMV showed a 100% level of phylogenetic relatedness between the Ukrainian representative isolates and Chinese, Iranian isolates, American isolate 452, and Polish isolate M, which testifies to their common origin [16]. Also investigation of isolate SKS-18 that has ability to transmission by soybean seeds was conducted. It was shown that studied isolate has amino acid substitutions in the CP gene which can be involved to its transmission via seeds [15].

The aim of the work is to study the impact of soybean mosaic virus on biochemical composition of soybean plants and seeds of different varieties grown in Odesa region of Ukraine.

To achieve this goal, it is necessary to solve the following tasks:

– compare the results of the biochemical analysis of healthy soybean plants and infected by SMV;

– characterize the main biochemical parameters of seed quality in healthy seeds and infected by SMV;

– study the content and component composition of 11S and 7S globulins in healthy seeds and infected by SMV;

– research of fatty acids composition of healthy seeds and infected by SMV.

Materials and methods.

The seeds of the soybean varieties (*Glycine max* L.), created by classical breeding methods and grown in Odesa region (‘Yatran’, ‘Aryadna’, ‘Syaivo’, ‘Fenix’, ‘Melpomena’, ‘Symphony’, ‘Antares’, ‘Aurora’, ‘Odesytka’, ‘Serenada’, ‘Taverna’, ‘Zmina’, ‘Evridika’, ‘Vasylkivska’) were used in research.

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) [17] and two step RT-PCR were used to identify SMV. Total RNA was extracted from fresh leaves using GeneJet Plant RNA Purification Kit (Thermo Fisher Scientific, USA). The reverse transcription was performed using RevertAid Reverse Transcriptase (Thermo Scientific, USA) according to the manufacturers’ instructions using specific oligonucleotide primers to part of SMV capsid protein gene (CP) (amplification product size 469 bp) [16]. Sanger sequencing was performed on 3130 Genetic analyzer (Applied Biosystems-HITACHI, USA) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sample-file analysis was performed with Sequencing Analysis Software v5.2.0 (Thermo Fisher Scientific, USA). The sequencing data of two SMV isolates named as Odesytka-24-Ukr and Vasylkivska-24-Ukr was deposited to the NCBI GenBank under Accession numbers PV646567 and PV646568, respectively.

Protein content was measured by the Kjeldahl method using automatic analyzer Kjeltec Auto-1030 (FOSS, Höganäs, Sweden) [18]. The isolation and identification of 7S and 11S soybean globulins was carried out by method developed and improved in the laboratory (Patent No 107671). Protein electrophoresis was carried out in 15% polyacrylamide gel containing 0,1% SDS at pH 8,3 according to the method Laemmli [19] using equipment for vertical electrophoresis of “Hem-Hoff” (USA). Proteins-markers produced by “Serva” (Germany) were used to plot the calibration graph (phosphorylase B (97 kDa), bovine serum albumin (67 kDa), albumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), L-lactalbumin (14.4 kDa).The percentage of components in the electrophoretic spectra of the proteins was determined using “Imagel” image analysis software.

The oil content was determined by extraction method [20]. The fatty acids composition was determined by gas-liquid chromatography in the form of methyl esters using a gas chromatograph GC-16A “Shimadzu” (Japan) with a flame ionization detector, a thermostat with a temperature not lower than 200˚C, an evaporator with a temperature not lower than 300˚C, software “GC solution”, column THERMO TR-FAME 30m x 0,25mm with filler (70% Cyanopropyl (equiv) Polysiphenylene-siloxane), carrier gas with nitrogen not lower than 99,99% purity. The content of fatty acids was expressed as % of the total fatty acid (DSTU ISO 5508-2001).

The sugar content was measured using the anthrone method [21]. 100 mg of sample was added to 5 mL of boiling 2,5 N HCl and incubated for three hours in a boiling water bath. After the solution cooled down to room temperature, sodium carbonate powder was added to neutralize the acid until the effervescence ceased. The total volume of the reaction was adjusted to 100 mL, and samples were centrifuged at 5000 rpm for 15 min at 4 ◦C. Then 0,5 mL of the supernatant was mixed with 0.5 mL of distilled water and 4 mL of anthrone reagent. The mixture was heated for 8 min in a boiling water bath, rapidly cooled down, and absorbance of the reaction was measured at 630 nm in a UV-VIS spectrophotometer UVmini-1240 (Shimadzu, Kyoto, Japan).

Flavonoid content of the extracts was quantified using the aluminum chloride assay method [22]. The C-4 keto group and the hydroxyl group of either the C-3 or C-5 flavonoids react with aluminum chloride to form an acid-stable complex. In brief, 0,5 mL of extract was mixed with 1,5 mL of ethanol (95%) and 0,1 mL of 10% aluminum chloride; then 0,1 mL of 1 M sodium acetate was added and the final volume, adjusted to 5 mL with distilled water. After 30 min, the absorbance of the mixture was measured at 420 nm. Rutin was used as a standard. The flavonoid content of the plant extracts was expressed as milligrams of rutin equivalents per gram of dry weight. All the experiments were carried out in three replicates

The activity of lipoxygenase was determined by the spectrophotometric method of coupled oxidation of β-carotene in the presence of linoleic acid at 440 nm. Lipoxygenase was extracted from ground soybean seeds with petroleum ether (boiling point 40–60°C). After removal of the ether, the material was mixed with 50 ml of water, shaken for one hour and centrifuged to obtain a clear solution. The obtained extract was treated with a small amount of active charcoal and filtered through a folded filter to remove the color. The filtrate containing the lipoxygenase solution was stored in toluene at 5°C under vacuum. The substrate used was linoleic acid obtained from fresh linseed oil by cold saponification with 10% KOH, followed by distillation of unsaturated fatty acids in a vacuum at 160°C under 4 mm Hg and freezing at −20°C. The resulting linoleic acid, with a refractive index of η20 = 1.4698, was stored in ampoules under vacuum at −5°C. A solution of the sodium salt of linoleic acid was prepared immediately before the experiment by dissolving the calculated amount of linoleic acid in 0,1 N NaOH, based on a linoleic acid content of 1 mg per ml. The carotene solution contained 1,5 mg of crystalline carotene in 100 ml of a mixture consisting of 75 ml of twice distilled acetone and 25 ml of alcohol. Lipoxygenase activity was inhibited with a 20% aqueous NaOH solution. To carry out the experiment, 47 ml of H_2O and 3 ml of phosphate buffer at pH 6,5 were added to two 250 ml flasks, one of which was the control flask. Then 2 ml of 20% NaOH was added to the control flask. Then 1 ml of Na salt of linoleic acid, 5 ml of carotene solution and 0,1 to 1 ml of aqueous lipoxygenase extract were added to the flasks. After a fixed time, 2 ml of 20% NaOH was added to the test flask to stop the action of lipoxygenase. Lipoxygenase activity was expressed in units of optical density at 440 nm per 1 mg per minute.

Trypsin inhibitor activity was determined by the decrease in the rate of casein hydrolysis in the presence of the inhibitor [23]. Lectin activity was determined by the method [24]. Chlorophyll a and b content was determined using spectrophotometric method [25].

Statistical processing of the investigation results was made using the pack of programs "Analysis of the Data of Electron Tables Microsoft Excel", image analysis program "Imagel". The experiments were performed in triple biological and analytical repetitions. Mean values and their standard errors are presented in Tables 1-5, differences between the infected and healthy seeds were considered reliable at the significance level $P < 0.05$ by the Student criterion.

Results of the research and their discussion

Research of soybean plants in the Odesa region of Ukraine showed the presence of symptoms of leaf wrinkling and mosaic (Fig. 1).

The results of ELISA and RT-PCR showed that the plants were infected with soybean mosaic virus (SVM). According to meteorological conditions, the area where the study was conducted belongs to the Steppe (hydrothermal coefficient (HTC) 0.7-1.0) and dry Steppe (HTC 0.4-0.7).

It is established that the protein content in leaves of virus-infected plants in the flowering phase decreased by 9-12% relative to healthy plants, with the exception of the plants of variety 'Taverna' (an increase by 9%), the chlorophyll a content decreased by 25,3-86,3%, the chlorophyll b content by 9,3-76,8%, and the carotenoid content by 8,58-83,6%, depending on variety. In the plants of variety 'Evridika' the carotenoid content practically did not change relative to the control. A decrease in protein and chlorophyll content was also observed in susceptible plants of rice and wheat infected by viral infection [26, 27]. The obtained results showed that the sugar content increased by 1,23-7,98% in leaves of SMV-infected plants relative to healthy ones, but in some varieties ('Taverna', 'Serenada') a decrease of sugar content by 9,97-20,26% was observed (Table 1). These changes in the functioning of the photosynthetic apparatus affect subsequent biochemical reactions, as the decrease of protein content and, in some varieties, the sugar content. It is shown that content of flavonoids increased by 11-39% relative to control in plants of varieties "Aryadna', 'Vasylkivska' and 'Zmina' at the infection by SMV. A decrease of flavonoids content by 7,8-33,8% at the infection by SMV was established in the plants of varieties 'Avrora', 'Evridika', 'Serenada', Syaivo'. It has been established that flavonoids are phytochemicals which have antiviral activity. The flavonoids act at different stages of viral infection, such as viral entrance, replication and translation of proteins [28].

The study of the biochemical composition of virus-infected soybean seed showed the presence of changes in the main biochemical parameters characterizing seed quality (content of protein, fat, carbohygrates, flavonoids, activity of trypsin inhibitor, lectins, lipoxygenase) under the influence of SMV (Table 2). These changes depend on studied variety. The protein content decreased by 2-2,4% at the viral infection in the varieties 'Serenada','Zmina'. But in other varieties the protein content increases by 2,2-9,2% or was at the level of healthy plants.

a

b

Fig. 1. Symptoms of SMV on soybean plants of the 'Fenix' and 'Antares' varieties:
a – leaf wrinkling; b – seed spotting

There was a decrease in the content of fat in seeds at the viral infection (except for the varieties ‘Odesytka’, ‘Taverna’, ‘Evridika’). Under the influence of SMV, the content of total carbohydrates in seeds decreased by 2,1-29,9% relative to the control in almost all studied varieties. In most of the studied varieties, a decrease in the content of flavonoids by 17,3-55,2% was observed in seeds infected with SMV relative to the control, with the exception of the variety ‘Syaivo’ (an increase in content by 6,6%). We established an increase in activity of trypsin inhibitor by 2,2-12,1% % relative to control in the seeds of three varieties (‘Aryadna’, ‘Evridika’, ‘Vasylkivska ‘).

Table 1 – **Biochemical characteristics in leaves of soybean varieties affected by SMV (per abs. dry substance)**

Sample	Content of protein, %	Content of fat, %	Content of carbohydrates, %	Content of flavonoids, μg/g	Lipoxygenase activity, CU/mg of protein	Lectin activity, (μg of protein/ml)$^{-1}$ x 10^{-3}	Trypsin inhibitor activity, g/kg
Aryadna, control	44,6±0,05	20,5±0,03	24,2±0,5	123,0±0,8	0,76±0,003	1,064±0,004	41,3±0,5
Aryadna, infected by SMV	47,0±0,07*	16,9±0,05*	23,7±0,3*	90,4±0,5*	1,05±0,008*	1,285±0,002	46,3±0,7*
Syaivo, control	41,8±0,04	21,6±0,03	25,8±0,4	95,7±0,8	1,98± 0,002	1,064±0,006	53,9±0,5
Syaivo, infected by SMV	44,9±0,06*	19,5±0,04*	25,5±0,3	102,0±0,7*	1,59± 0,003*	1,354±0,008	38,4±0,6*
Fenix, control	42,4±0,06	20,4±0,04	16,3±0,4	116,8±0,3	2,57±0,005	5,031±0,007	52,4±0,6
Fenix, infected by SMV	46,3±0,08*	17,9±0,08*	11,8±0,3*	92,8±0,5*	2,59±0,004	5,325±0,009	43,2±0,3*
Avrora, control	41,7±0,06	22,7±0,04	28,6±0,4	56,0±0,1	1,76±0,004	1,167±0,003	27,5±0,1
Avrora, infected by SMV	41,6±0,05	21,1±0,03*	26,9±0,3*	48,0±0,2*	2,34±0,006*	1,254±0,006	19,4±0,2*
Odesytka, control	44,3±0,05	17,4±0,02	30,9±0,5	112,2±0,7	1,75±0,003	3,121±0,007	15,5±0,1
Odesytka, infected by SMV	44,0±0,07	17,6±0,03	30,5±0,5	92,8±0,5*	2,05±0,006*	2,325±0,009*	15,3±0,2
Serenada, control	45,1±0,08	18,5±0,05	20,9±0,3	65,5±0,2	2,37±0,002	2,538±0,004	22,2±0,3
Serenada, infected by SMV	44,0±0,04*	18,2±0,04	19,8±0,4*	56,2±0,1*	2,32±0,003	2,827±0,005	16,8±0,1*
Taverna, control	43,8±0,04	19,2±0,05	20,7±0,5	86,0±0,4	2,16±0,002	3,145±0,004	22,2±0,1
Taverna, infected by SMV	43,9±0,05	19,7±0,04	17,1±0,2*	81,0+0,3*	2,28±0,003	3,782±0,005	16,8±0,2*
Zmina, control	44,5±0,07	19,6±0,05	25,2±0,3	76,3±0,3	2,11±0,002	1,685±0,002	44,5±0,7
Zmina, infected by SMV	43,6±0,05*	18,1±0,03*	17,6±0,3*	64,0±0,4*	2,74±0,003	1,864±0,003	43,65±0,6*
Evridika, control	44,6±0,06	19,3±0,03	15,9±0,4	67,0±0,3	2,57±0,004	2,619±0,002	44,57±0,5
Evridika, infected by SMV	45,6±0,07*	19,8±0,04	15,3±0,3	59,0±0,2*	2,59±0,003	1,145±0,003*	45,56±0,7*
Vasylkivska, control	44,2±0,04	22,1±0,06	19,0±0,2	64,0±0,2	3,61±0,004	3,613±0,004	44,18±0,5
Vasylkivska, infected by SMV	46,3±0,06*	21,7±0,05*	18,0±0,2*	62,0±0,1*	2,06±0,003*	2,059±0,003*	46,34±0,6*

Note.*– differences are significant at $P < 0,05$

Table 2 – Biochemical characteristics in seeds of soybean varieties affected by SMV (per abs. dry substance)

Sample	Content of protein, %	Content of chlorophyll *a* mg/100g	Content of chlorophyll *b* mg/100g	Content of carotenoids mg/100 g	Content of sugar, %	Content of flavonoids, mg/g
Avrora, control	15,9±0,2	22,9±1,0	82,2±2,3	146,5±5,5	6,6±0,4	2,2±0,01
Avrora, SMV-infected plants	14,3±0,2*	28,0±0,8*	68,8±1,5*	126,4±3,4*	6,2±0,3	1,45±0,05*
Aryadna, control	19,6±0,1	161,8±3,8	103,3±2,6	128,4±4,8	7,4±0,3	1,44±0,03
Aryadna, SMV-infected plants	14,7±0,2*	33,7±0,7*	45,0±1,9*	82,6±1,0*	6,8±0,3*	2,00±0,02*
Vasylkivska, control	17,6±0,2	108,2±2,2	60,7±2,6	127,3±2,5	8,4±0,2	1,4±0,03
Vasylkivska, SMV-infected plants	16,4±0,1*	31,9±0,2*	26,2±1,7*	89,0±1,9*	7,6±0,1*	1,5±0,02
Zmina, control	14,8±0,1	69,4±0,7	37,9±1,8	120,9±2,2	8,6±0,3	1,5±0,02
Zmina, infected by SMV	13,1±0,1*	12,9±0,2*	19,8±1,3*	19,8±1,5*	8,6±0,3	1,8±0,01*
Evridika, control	15,2±0,1	87,2±3,2	45,2±2,2	127,4±2,5	7,5±0,4	1,7±0,02
Evridika, infected by SMV	13,4±0,1*	65,1±2,2*	36,4±1,7*	127,2±1,9	8,1±0,4*	1,5±0,03*
Serenada, control	17,2±0,2	142,6±5,7	64,9±2,6	132,8±3,2	8,2±0,2	1,5±0,03
Serenada, infected by SMV	15,2±0,1*	65,5±3,2*	36,3±1,7*	121,4±1,3*	7,4±0,03*	1,3±0,01*
Syaivo, control	15,3±0,2	87,1±1,4	51,5±2,3	122,9±2,3	7,4±0,4	1,6±0,01
Syaivo, infected by SMV	13,9±0,2*	11,9±1,8*	11,9±0,9*	86,6±1,2*	7,9±0,5	1,3±0,03*
Odesytka, control	16,2±0,1	86,8±2,8	43,2±1,9	119,2±2,1	9,1±0,3	1,9±0,04
Odesytka, infected by SMV	15,0±0,1*	74,9±3,2*	38,8±1,4*	120,3±1,9	8,2±0,1	1,8±0,03
Taverna, control	14,2±0,1	69,4±1,8	44,6±1,0	115,1±1,4	12,5±0,2	1,7±0,02
Taverna, infected by SMV	15,5±0,2*	11,4±0,9*	46,5±1,0	73,9±0,4*	9,9±0,2*	1,6±0,01
Fenix, control	17,9±0,1	135,2±2,3	104,8±3,7	125,2±1,5	8,5±0,1	1,5±0,02
Fenix, infected by SMV	16,2±0,2*	61,2±3,2*	38,3±1,4*	120,9±1,2*	7,4±0,1*	1,4±0,01

Note.*– differences are significant at $P < 0,05$

Other studied varieties have decreased activity of trypsin inhibitor or at the level of control. In the seeds of seven varieties, an increase in lectin activity by 5,8-27,3% was found, and a decrease in lectin activity by 25,5-56,9% relative to the control were established in three varieties (‘Odesytka’, ‘Vasylkivska ‘, ‘Evridika’). An increase of lipoxygenase activity by 5,6-38,2% was found in the five studied varieties under the influence of SMV. A decrease of activity of this enzyme by 19,7 and 43% compared to the control was shown in the varieties ‘‘Syaivo’, ‘Vasylkivska’. It is known about an important function of trypsin inhibitors and lectins in the plant protective reactions against phytopathogens, in particular viruses [11, 29].

Content of glycinin is from 12,75 to 18,26 % per content of protein in healthy seeds. Content of β-conglycinin is from 10,86 to 15,29 % per content of protein depends on variety (Table 3). We found that under the influence of SMV the glycinin content in all varieties decreased by 2,2-24,1%. Content of β-conglycinin increased by 4,1; 15,1% and 7,8% at the infection by SMV in the varieties ‘Aryadna’, ‘Serenada’, ‘Taverna’, respectively, and decreased by 6,9-13,3% in the varieties ‘Zmina’, ‘Syaivo’, ‘Evridika’, ‘Fenix’, ‘Vasylkivska’ by 4,9-10,6%. Also, It was established that ratio of glycinin/β-conglycinin decreased at the influence of SMV in the seeds of varieties 'Aryadna', 'Taverna', 'Serenada'. The ratio of these fractions determines the functional properties of these proteins and thus the quality of the products and their technological properties. It has been established that the globulin fraction, enriched with 11S globulins, plays an important role in the formation with divalent cations of cheese curds such as tofu, as well as in the imitation of cheese and brinzen.The thermoplastic characteristics of 7S globulin fraction positively blend into soybean milk [30]. So, the content and ratio of 7S and 11S globulins in soybean protein, as well as the presence or absence of α, α′, β subunits and A, A3, A5, B components in the component composition of these proteins are the main indicators of the quality and nutritional value of soybean seeds.

Table 3 – Content of 7S and 11S globulins in the soybean seed at the infection by SMV (per abs. dry substance)

Sample	Content of protein, %	Content of glycinin (11S globulin), % from protein content	Content of β-conglycinin (7S globulin) % from protein content	Ratio glycinin/ β-conglycinin
Aryadna, control	44,65±0,05	17,45±0,02	12,81±0,03	1,36
Aryadna,infected by SMV	46,98±0,07*	13,25±0,01*	13,34±0,02*	0,99*
Avrora, control	41,70±0,06	15,92±0,04	11,94±0,04	1,33
Avrora, infected by SMV	41,62±0,05	15,61±0,05	11,85±0,03	1,31
Odesytka, control	44,33±0,05	18,23±0,04	14,29±0,05	1,27
Odesytka, infected by SMV	44,03±0,07	17,83±0,05*	14,14±0,03	1,26
Serenada, control	45,11±0,08	13,63±0,04	11,49±0,08	1,19
Serenada, infected by SMV	43,98±0,04*	12,75±0,05*	13,54±0,03*	0,94*
Taverna, control	43,83±0,04	17,36±0,02	12,81±0,02	1,35
Taverna, infected by SMV	43,95±0,05	17,21±0,03	12,74±0,06	1,35
Zmina, control	44,46±0,07	15,49±0,02	14,69±0,04	1,05
Zmina, infected by SMV	43,65±0,05*	14,07±0,04*	12,75±0,05*	1,10
Syaivo, control	41,76±0,04	18,25±0,03	15,29±0,04	1,19
Syaivo, infected by SMV	44,87±0,06*	16,37±0,04*	13,75±0,05*	1,19
Evridika, control	44,57±0,06	14,24±0,03	11,67±0,04	1,08
Evridika, infected by SMV	45,56±0,07*	13,64±0,04*	10,86±0,03*	1,17
Fenix, control	42,44±0,06	17,79±0,04	14,69±0,07	1,21
Fenix, infected by SMV	46,35±0,08*	16,17±0,03*	12,75±0,05*	1,26
Vasylkivska, control	44,18±0,04	18,26±0,02	14,81±0,05	1,23
Vasylkivska,infected by SMV	46,34±0,06*	16,81±0,04*	13,24±0,06*	1,26

Note.*– differences are significant at $P < 0,05$

Therefore, the next stage of our research was to study the component composition of glycinin and β-conglycinin at the action by SMV. According to the data of electrophoretic analysis, the spectrum of the component composition of glycinin and β-conglycinin is polymorphic and had a different number of protein components depending on the variety: β-conglycinin – from 12 to 19, glycinin – from 14 to 17 components. The study of the component composition of glycinin and β-conglycinin of the seeds of soybean plants infected by SMV showed the presence of changes in the intensity of individual components of these proteins in different varieties. Thus, the intensity of high-molecular (67-103 kDa) and medium-molecular (45-20,1 kDa) components of β-conglycinin and glycinin decreased and the intensity of low-molecular (14,4 kDa and lower) components increased in the seed of the variety ‘Avrora’, under the infection by SMV, compare to the healthy seed (According to the data of electrophoretic analysis, the spectrum of the component composition of glycinin and β-conglycinin is polymorphic and had a different number of protein components depending on the variety: β-conglycinin – from 12 to 19, glycinin – from 14 to 17 components. The study of the component composition of glycinin and β-conglycinin of the seeds of soybean plants infected by SMV showed the presence of changes in the intensity of individual components of these proteins in different varieties. Thus, the intensity of high-molecular (67-103 kDa) and medium-molecular (45-20,1 kDa) components of β-conglycinin and glycinin decreased and the intensity of low-molecular (14,4 kDa and lower) components increased in the seeds of the variety ‘Avrora’, under the infection by SMV, compare to the healthy seeds (Fig. 2). An increase in the intensity of most components of β-conglycinin and glycinin and detection of a new components of glycinin with a molecular weight of 25 kDa and 18 kDa were established in the seeds of soybean plants of the variety ‘Aryadna’ under infection by SMV. A decrease in the intensity of the high-molecular and medium-molecular components of β-conglycinin and glycinin and increase in the intensity of components with a molecular weight of 12 kDa and lower were observed in the virus-infected seeds of the variety ‘Vasylkivska’, compare with the healthy seeds (Fig. 3). In the virus-infected seeds of the variety ‘Zmina’, an decrease in the intensity of the bands of the high-molecular and medium-molecular components of β-conglycinin and glycinin, an increase in the intensity of components with a molecular weight of 12 kDa and lower and absence of β-conglycinin component with molecular weight of 100 kDa were observed in comparison with the healthy seeds (Figure 3). A reduce in the intensity of all components of 11S and 7S globulins were established in the seeds of soybean plants of the variety ‘Evridika’ under infection by SMV. In the seeds of the variety ‘Syaivo’, under the infection by SMV, the intensity of high-molecular and medium-molecular components of β-conglycinin and glycinin decreased and the intensity of low-molecular (12 kDa and lower) components increased compare to the healthy seeds and new components of glycinin with molecular weight of 47 kDa, 22 kDa, 18 kDa and 12 kDa were detected (Fig. 3).Thus, the obtained results shown that the SMV infection causes changes in biochemical indicators that characterize protein quality of seeds which depended on the variety.

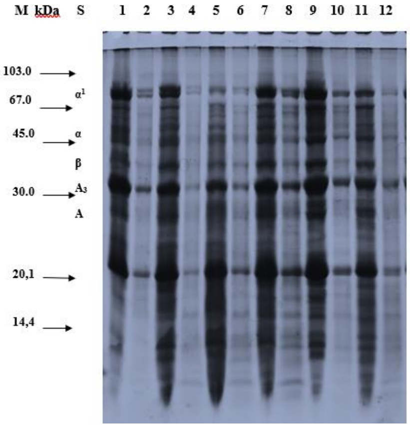

Fig. 2. Electropherogram of the components of 11S and 7S globulins in the seeds of soybean varieties:

M – molecular weight markers; S – glycinin and β-conglycinin subunits, 1 – Avrora, healthy (7S globulin);

2 – Avrora, healthy (11S globulin); 3 – Avrora, SMV-infected (7S globulin); 4 – Avrora, SMV-infected

(11S globulin); 5 – Aryadna, healthy (7S globulin); 6 – Aryadna, healthy (11S globulin); 7 - Aryadna,

SMV-infected (7S globulin); 8 – Aryadna, SMV-infected (11S globulin); 9 – ‘Vasylkivska’, healthy (7S globulin); 10 – ‘Vasylkivska’, healthy (11S globulin); 11 – ‘Vasylkivska’, SMV-infected (7S globulin); 12 – ‘Vasylkivska’,

SMV-infected (11S globulin)

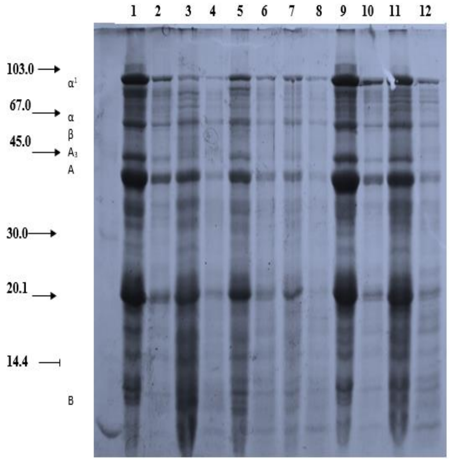

Fig. 3. Electropherogram of the components of 11S and 7S globulins in the seeds of soybean varieties:

M – molecular weight markers; S – glycinin and β-conglycinin subunits, 1 – ‘Zmina’, healthy (7S globulin);

2 – ‘Zmina’, healthy (11S globulin); 3 – ‘Zmina’, SMV-infected (7S globulin); 4 – ‘Zmina’, SMV-infected

(11S globulin);5 – ‘Evridika’, healthy (7S globulin); 6 – ‘Evridika’, healthy (11S globulin); 7 – ‘Evridika’,

SMV-infected (7S globulin); 8 – ‘Evridika’, SMV-infected (11S globulin); 9 – ‘Syaivo’, healthy (7S globulin);

10 – ‘Syaivo’, healthy (11S globulin);11 – ‘Syaivo’, SMV-infected (7S globulin); 12 – ‘Syaivo’, SMV-infected

(11S globulin)

It was showed that an increase in the amount of saturated fatty acids is observed in almost all varieties due to stearic acid and in some varieties (‘Aryadna’, ‘Taverna’) due to palmitic acid at the infection by SMV (Table 4, 5). Under the influence of SMV, an increase in the amount of unsaturated acids was found due to content of oleic, linoleic and linolenic acids in the seeds of variety ‘Aryadna’ and due to content of linoleic and linolenic acids in the seeds of the variety ‘Aurora’. A decrease in the amount of unsaturated fatty acids was detected due to linolenic acid content in the varieties ‘Serenada’, ‘Taverna’, and due to oleic acid content in the variety ‘Fenix’, and due to content of linoleic and linolenic acids in the seeds of variety ‘Vasylkivska’ at the infection by SMV. In almost all studied varieties a tendency to decrease the unsaturation coefficient of fatty acids in the lipid composition and increase the level of saturated fatty acids, in particular stearic and palmitic acids was established under the influence of viral infection (Table 5). It is believed that the ability to regulate the degree of unsaturation of plant cell membrane lipids underlies one of the mechanisms of plant adaptation to adverse environmental factors.

Soybean is a major crop in the Ukraine and one of the important factors of the “healthy food”. Infection by SMV usually results in severe yield losses, seed quality, seedling viability reduction of soybean [16]. Having data on the quantitative content of total protein, 7S globulin (β-conglycinin) and 11S globulin (glycinin) and their ratio, component composition, content of fat and antinutrient compounds (trypsin inhibitors, lectins, lipoxygenase activity), it is possible to carry out specific studies on the creation of soybean varieties for food use. Particularly important is the selection of varieties that maintain seed quality characteristics at the infection by SMV. Among the studied varieties, we can distinguish varieties that have high levels of protein, fat, carbohydrates and flavonoids at the infection by SMV. This is, for example, the varieties ‘Syaivo’, ‘Evridika’, ‘Odesytka’, ‘Taverna’, ‘Vasylkivska’. The differences in the content, composition, and structure of glycinin and β-conglycinin affect both the nutritional and functional properties of soybean seeds. The varieties ‘Avrora’, ‘Odesytka’, ‘Taverna’, ‘Fenix’, ‘Vasylkivska’ had increased or at the level of healthy seeds the content of protein, glycinin, β-conglycinin and the ratio 11S/7S at the SMV infection. It was established a tendency to decrease the unsaturation coefficient of fatty acids in the lipid composition and increase the level of saturated fatty acids, in particular stearic and palmitic acids in the soybean seeds under the influence of viral infection. Also changes in fatty acids composition, decrease of the linolenic acid content in soybean seeds at the infection by SMV were established [31]. Furthermore, these authors showed the negative correlations between SMV incidence and stearic and linolenic acid contents.

Changes of fatty acids profiles in soybean seeds infected by CCMV, TRSV and SVNV, with significant differences in palmitic, linoleic, linolenic and stearic fatty acids were found [32]. Our research has shown the significant impact of SMV on seed quality components, which are important factors in current soybean production systems. These findings provide a foundation for future studies on the impact of SMV on seed quality.

Table 4 – Fatty acids content in soybean seeds at the infection by SMV, (% from general content of fatty acids)

Sample	Palmitic acid content, $C_{16:0}$	Stearic acid content, $C_{18:0}$	Oleic acid content, $C_{18:1}$	Linoleic acid content, $C_{18:2}$	Linolenic acid content, $C_{18:3}$	Eicosapentaenoic acid content, $C_{19:0}$
Aryadna, control	10,3	4,8	32,0	41,6	5,2	0,5
Aryadna, infected by SMV	11,1*	5,3*	33,7*	42,9*	5,4	0,7*
Avrora, control	10,8	4,7	35,6	42,2	4,8	1,2
Avrora, infected by SMV	10,9	5,4*	33,8*	44,1*	5,1*	0,6*
Odesytka, control	11,2	4,8	36,8	41,8	4,8	0,2
Odesytka, infected by SMV	10,6*	5,6*	34,9*	42,9*	5,3*	0,2
Serenada, control	11,3	4,7	35,8	42,3	5,4	0,3
Serenada, infected by SMV	11,1	5,7*	36,4*	40,8*	5,4	0,4
Taverna, control	11,4	4,6	34,1	43,3	5,6	0,2
Taverna, infected by SMV	11,8	5,7*	34,6	41,9*	5,7	0,2
Zmina, control	11,6	4,7	32,7	43,2	5,5	0,8
Zmina, infected by SMV	11,4	5,5*	32,9	43,8	5,4	0,8
Syaivo, control	10,8	4,6	36,0	41,8	5,2	0,6
Syaivo, infected by SMV	10,1	5,8*	35,8*	42,4*	4,8*	0,6
Evridika, control	11,1	4,5	34,1	43,6	5,4	0,3
Evridika, infected by SMV	10,9*	5,6*	34,9	43,3	5,4	0,6*
Fenix, control	11,0	4,7	34,7	42,8	5,7	0,6
Fenix, infected by SMV	11,0	5,4*	33,2*	44,0*	5,2*	0,6
Vasylkivska, control	10,9	4,8	33,6	44,7	6,0	0,5
Vasylkivska,infected by SMV	11,1	5,7*	33,8	43,5*	5,4*	0,5

Note.*– differences are significant at P < 0, 05

Table 5 – The content of saturated and unsaturated fatty acids in soybean seeds at the infection by SMV

Sample	Content of saturated fatty acids, % from the general content of fatty acids	Content of unsaturated fatty acids, % from the general content of fatty acids	Fatty acids unsaturation coefficient
Aryadna, control	15,6±0,5	78,9±1,3	5,05
Aryadna, infected by SMV	17,1±0,3*	82,0±1,4*	4,79*
Avrora, control	16,7±0,4	82,6±1,2	4,94
Avrora, infected by SMV	16,9±0,3	83,0±1,4*	4,91
Odesytka, control	16,2±0,5	83,4±1,6	5,15
Odesytka, infected by SMV	16,4±0,5	83,1±1,2	5,06
Serenada, control	16,3±0,3	83,5±1,2	5,12
Serenada, infected by SMV	17,2±0,4*	82,6±1,1*	4,80*
Taverna, control	16,2±0,5	83,0±1,1	5,12
Taverna, infected by SMV	17,7±0,2*	82,2±1,2*	4,64*
Zmina, control	17,1±0,3	81,4±1,3	4,76
Zmina, infected by SMV	17,7±0,3	82,1±1,4*	4,63
Syayvo, control	16,0±0,2	83,0±1,3	5,18
Syayvo, infected by SMV	16,5±0,3	83,0±1,3	5,03
Evridika, control	15,9±0,4	83,1±1,1	5,22
Evridika, infected by SMV	17,1±0,3*	83,6±1,1	4,88*
Fenix, control	16,3±0,4	83,2±1,3	5,10
Fenix, infected by SMV	17,0±0,3*	82,4±1,2*	4,85*
Vasylkivska, control	16,2±0,2	84,3±1,3	5,20
Vasylkivska,infected by SMV	17,3±0,2*	82,7±1,2*	4,78*

Note.*– differences are significant at P < 0, 05

Approbation of research results. The obtained results were used in the breeding program of Department of Breeding, Genetics and Seed Production of Legumes Crops of PBGI-NCSCI for creation of soybean varieties for food production and farms of institute (for example, State Enterprise Experimental Base "Dachna").

Conclusion

The results of the evaluation of soybean plants grown in the Odesa region of Ukraine showed that infection with SMV caused changes of main biochemical characteristics (content of protein, sugars, chlorophyll a/b, carotenoids, flavonoids) in leaves and content of protein, main fractions of globulins fractions (glycinin and β-conglycinin), fat, carbohydrates, flavonoids, fatty acids composition, activity of lectins, lipoxygenase, trypsin inhibitor) in seeds. These changes depended on the soybean variety. Electrophoretic analysis revealed varietal differences in the relative content of individual protein components on the electropherograms of the electrophoretic spectra of glycinin and β-conglycinin, which affect the food value of soybean seeds. Varietal differences of fatty acids profiles in soybean seeds infected by SMV with significant changes in level of palmitic, linoleic, linolenic, oleic and stearic fatty acids were established. It was shown a tendency to decrease the unsaturation coefficient of fatty acids in the lipid composition and increase the level of saturated fatty acids in the soybean seeds under the influence of viral infection. The obtained results can be used to develop methods for selecting soybean varieties with high seed quality and resilience to viral infection (SMV) for implementation in soybean breeding programs.

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БІОХІМІЧНІ ЗМІНИ У РОСЛИНАХ І НАСІННІ СОЇ, СПРИЧИНЕНІ ВІРУСНОЇ ІНФЕКЦІЄЮ

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Анотація. Соя (*Glycine max* L.) є значним джерелом білка та поживних речовин і використовується в багатьох продуктах здорового харчування. Відомо, що соя схильна до ураження, такими як вірус мозаїки сої (ВМС). Інфікування ВМС негативно впливає на врожайність сої та якість її насіння. Метою дослідження було вивчення впливу ураження вірусом мозаїки сої на біохімічний склад рослин та якість насіння сої. Тестування рослин сої з Одеської області на наявність найбільш шкідливих вірусів показало появу симптомів зморшкуватості та мозаїки листової пластинки. Результати імуноферментного аналізу та ЗТ-ПЛР показали, що рослини уражені вірусом ВМС. Для дослідження біохімічного складу рослин та насіння сої використовували стандартні та адаптовані методи біохімічного аналізу. Виділення та ідентифікацію основних запасних білків сої проводили за допомогою методів, розроблених у лабораторії (патент №42181, 107671). Електрофорез білків проводили в 15% ПААГ методом Леммлі. Встановлено, що інфікування ізолятами вірусу мозаїки сої Odesytka-24-Ukr та Vasylkivska-24-Ukr викликало зміни біохімічних показників (вмісту білка, основних фракцій запасних білків (гліциніна та β-конгліциніна), жиру, вуглеводів, флавоноїдів, жирнокислотного складу, електрофоретичних спектрів гліциніну та β-конгліциніну, активності лектинів, ліпоксигенази, інгібітора трипсину) у листках рослин у фазі цвітіння та насінні. Ці зміни залежали від сорту сої. Отримані результати можуть бути використані для розробки методів селекції сортів сої з високою якістю насіння та стійкістю до вірусної інфекції та будуть рекомендовані для впровадження в селекційні програми по створенню сортів сої для виробництва високоякісних продуктів харчування.

Ключові слова: : соя, якість насіння та продуктів, гліцинін, β-конгліцинін, жирнокислотний склад, вірус мозаїки сої.