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# THE IMPACT OF SOYBEAN MOSAIC VIRUS INFECTION ON BIOCHEMICAL COMPOSITION OF SOYBEAN SEED

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*It is known that infection caused by soybean mosaic virus (SMV) has a negative effect on soybean yield, productivity and seed quality. The main indicators of the nutritional value of soybean seed are the content of main storage proteins glycinin and β-conglycinin, as well as flavonoids, carbohydrates and fat. The goal of the research was to study the impact of SMV infection on the biochemical composition of the seeds of the soybean varieties created by classical breeding methods ('Kano', 'Kuban', 'Kordoba') and varieties of transgenic soybean ('Grimo' and 'Monro'). DAS-ELISA and two step RT-PCR were used to identify SMV. Protein electrophoresis was carried out in 15% polyacrylamide gel containing 0.1% SDS at pH 8.3 according to the Laemmli method. We detected an increase in the activity of trypsin inhibitor and lectins and a decrease flavonoids content in the infected seeds of studied varieties in comparison with the healthy seeds. Seeds of all infected varieties showed a decrease in glycinin content and glycinin/β-conglycinin ratio, with the exception of 'Monro' infected seeds in which both glycinin and β-conglycinin contents were increased or at the level of healthy seeds. We can distinguish the variety 'Kano' with a high content of flavonoids, varieties 'Kuban' and 'Grimo' with increased or maintained levels of healthy seed content of carbohydrates, fat and proteins under infection conditions. The differences in the relative content of the components of β-conglycinin and glycinin in infected seeds depending on the soybean variety were revealed. The obtained results can be used for the selection of soybean varieties resistant to viral infection and with high seed quality.*

*Keywords: soybean mosaic virus, seed quality, glycinin, β-conglycinin, flavonoids.*

Leguminous crops are the main source of vegetable protein in human and animal nutrition and one of the biological factors of restoring soil fertility, due to the high productivity of symbiotic nitrogen fixation. Soybean has a special place among these crops. On the world market, soybean is considered a strategic crop of the 21st century, and more and more countries in Europe, Asia, and America, using modern technologies for extracting and purifying soybean proteins, use them for the production of food products [1]. Soybean proteins contain all the essential amino acids necessary for the normal functioning of the body. Soybean is an important source of protein and oil and most commonly used in the diets of both humans and animals [2]. Soybean has a rare combination of protein and oil content with a valuable set of vitamins, ash elements and biologically active substances. For example, soybean contains a significant amount of polyphenolic compounds, in particular flavonoids, which are natural antioxidants and have a wide range of biological activity. They are involved in many important processes related to germination, growth, pollination and reproduction of plants. One of their important functions is the protection of plants from the influence of various adverse environmental factors. The antioxidant properties of these compounds are responsible for the stress-protective effect [3]. The nutritional properties of legume seeds are associated with the presence of substances such as trypsin inhibitors, lectins, lipoxygenase, which negatively affect the nutritional and feed value of seeds [4]. On the other hand, these compounds play an important role in plant immunity [5].

It was found that the most promising proteins for the production of food from soybean are globulin fractions with sedimentation constants of 7S and 11S, and their content and ratio in total protein determine its quality, since they are unbalanced in amino acid composition. 11S globulin (glycinin) is a hexamer of six subunits, and each glycinin subunit is known to consist of two protomers. The molecular weight of soybean glycinin is 350 kDa. Soybean glycinin subunits are divided into groups: group I (G1, G2, G3 or A1aB2, A2B1a, A1bB1b) is encoded by the genes *Gy1*, *Gy2*, *Gy3*; group IIa (G4 or A5A4B3) is encoded by the *Gy4* gene; group IIb (G5 or A3B4), encoded by the *Gy5* gene. Two additional glycinin genes have been identified and mapped, namely the pseudogene gy6 and the functional gene *Gy7*, encoding the sixth subunit of glycinin G7 [6].

7S globulins are glycoproteins and consist of three subunits: α′ – 76 kDa, α – 72 kDa and β – 52 kDa. Three genes encoding the synthesis of β-conglycinin subunits have been described. The *Cgy1* gene is represented by 3 alleles: two active – *Cgy1-α* for the electrophoretic variant of the α′ subunit, *Cgy1-b* – for the α′-slow subunit, and zero – *cgy1* in the absence of these subunits in the electropherogram. Using molecular genetics methods, it was revealed that the absence of the α' subunit, which is determined by the *cgy1* null allele, is associated with the deletion of a DNA fragment that contains almost the entire coding sequence for this polypeptide and the 5′-flanking end [7]. It was established that 7S and 11S soybean globulins affects nutritional value of products and human health. For example, it was showed that soybean 7S and 11S globulins had a protective effect on the stability of cyanidin-3-O-glucoside (C3G) during simulated digestion and improved the antioxidant capacity of C3G after simulated digestion. Soybean 11S globulin had a better effect than soybean 7S globulin in protecting the stability and antioxidant capacity of C3G against simulated gastrointestinal environment [8]. But some subunits of glycinin and β-conglycinin cause an allergic reaction [9]. The presence or absence of α, α′, β subunits and A, A3, A5, B components in the component composition, as well as the content and ratio of 7S and 11S globulins in soy protein are the main indicators of the quality and nutritional value of soybean seed.

It is known that soybean is susceptible to damage by many viruses, such as SMV, alfalfa mosaic virus, bean yellow mosaic virus, and others [10]. SMV is the most harmful to soybean. Yield reduction caused by SMV ranges from 8 to 50-70% [11]. Significant losses of the soybean crop are observed under drought conditions. It is shown that the lack of moisture during the flowering, bean formation and seeding phases sharply reduces soybean productivity. It is established the great variations in seed transmission of SMV in different soybean germplasms [12]. There is a 100% presence of SMV in flowers, immature seeds and green pods. But in the case of the dry pods, there is mostly no detection of SMV [13].

In previous studies it was established that strains SKP-16 and SGP–17 of SMV are present in the fields of Ukraine. The yield of soybean in the SMV infected plants was reduced by 2,6 times, compared with healthy ones. 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 soybean mosaic virus showed a 100% level of phylogenetic relatedness between the Ukrainian representative isolate and Chinese, Iranian isolates, American isolate 452, and Polish isolate M, which testifies to their common origin [14]. 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) [14]. It was found that the percentage of seed-transmitted SMV infection in some Ukrainian soybean varieties was from 1.9 to 10.5% [15]. 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 [16]. When plants were infected by SMV, soybean yields decreased in both farms of Kyiv and Poltava regions by 35.0-65.7%, respectively [17].

The goal of present research was to study the impact of soybean mosaic virus on biochemical composition of soybean seed. The research was focused on the content and component composition of glycinin and β-conglycinin.

## Materials and Methods

The seeds of the soybean variety (*Glicine max* L.), created by classical breeding methods ('Kano', 'Kuban', 'Kordoba') and the seeds of 2 varieties of transgenic soybean 'Grimo' and 'Monro', were obtained from the National Plant Germplasm System.

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and two step RT-PCR were used to identify SMV [18]. Total RNA was extracted from fresh leaves using Genomic DNA purification kit (Thermo Scientific, USA). 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) [19].

Protein content was determined by method [20]. The isolation and identification of 7S and 11S soybean globulins was carried out by method developed and improved in the laboratory [21].

Protein electrophoresis was carried out in 15% polyacrylamide gel containing 0.1% SDS at pH 8.3 according to the method [22] 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 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 pea 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 extract obtained was treated with a small amount of active charcoal and filtered through a folded filter to remove the colour. 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 $\eta^{20} = 1.4698$, 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].

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 Table 1, differences between the infected and healthy seeds were considered reliable at the significance level $P < 0.05$ by the Student criterion.

## Results and Discussion

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, carbohydrates, flavonoids, activity of trypsin inhibitor, lectins, lipoxygenase) under the influence of SMV (Table 1). These changes depend on studied variety.

The protein content decreased by 5.78% in case of viral infection only in the variety 'Kano'. But in other varieties the protein content increase by 4.41-6.38%. A similar pattern was observed for the change in carbohydrate content. There was an insignificant decrease in the content of fat in all varieties at the viral infection (except for the variety 'Kuban'). Similar results were obtained by us in pre-

*Table 1. Influence of SMV infection on the biochemical characteristics of soybean seed*

| Variety | Content of protein, % per abs. dry substance | Content of fat, % per abs. dry substance | Content of carbohydrates % per abs. dry substance | Trypsin inhibitor activity, g/kg | Lipoxygenase activity, CU/min/mg of protein | Lectins activity, (µg/g protein/ml)$^{-1}\times10^{-3}$ | Content of flavonoids, µg/g |
|---|---|---|---|---|---|---|---|
| Kano, healthy | 40.49 ± 0.02 | 23.75 ± 0.02 | 14.81 ± 0.02 | 36.64 ± 0.40 | 0.975 ± 0.005 | 1.281 ± 0.009 | 79.0 ± 0.5 |
| Kano, SMV | 38.15 ± 0.02* | 22.85 ± 0.03 | 13.75 ± 0.02 | 54.96 ± 0.50* | 0.888 ± 0.005 | 2.746 ± 0.07* | 93.0 ± 0.3* |
| Kuban, healthy | 42.06 ± 0.05 | 22.49 ± 0.03 | 13.59 ± 0.03 | 41.31 ± 0.50 | 0.697 ± 0.003 | 0.561 ± 0.002 | 44.0 ± 0.3 |
| Kuban,SMV | 44.08 ± 0.03* | 22.40 ± 0.03 | 15.13 ± 0.02* | 46.26 ± 0.70* | 0.611 ± 0.002 | 0.982 ± 0.005* | 41.0 ± 0.4* |
| Grimo, healthy | 30.49 ± 0.02 | 27.01 ± 0.01 | 14.79 ± 0.03 | 45.30 ± 0.40 | 0.925 ± 0.005 | 0.304 ± 0.002 | 79.0 ± 0.3 |
| Grimo, SMV | 35.82 ± 0.03* | 24.22 ± 0.02* | 15.04 ± 0.03 | 46.92 ± 0.50 | 0.812 ± 0,008 | 1.325 ± 0.009* | 73.0 ± 0.5* |
| Monro, healthy | 43.04 ± 0.05 | 22.38 ± 0.03 | 14.26 ± 0.04 | 45.81 ± 0.50 | 0.827 ± 0.006 | 0.249 ± 0.001 | 51.0 ± 0.4 |
| Monro, SMV | 44.94 ± 0.04 | 21.71 ± 0.02 | 16.76 ± 0.05* | 50.83 ± 0.80* | 0.742 ± 0.005 | 0.285 ±0.002 | 42.0 ± 0.5* |

Note. *Differences are significant at $P < 0.05$

vious studies on soybean and wheat plants affected by viruses [14, 15, 25]. We established an increase in activity of trypsin inhibitor and lectins and an insignificant decrease of lipoxygenase activity in all studied varieties under the influence of a viral infection. The content of flavonoids increased in the infected seeds of variety 'Kano'. In other studied varieties the content of flavonoids decreased in infected seeds compare to healthy seeds. It is known about an important function of flavonoids, trypsin inhibitors and lectins in the protection of plants from external unfavorable abiotic and biotic factors, in particular viruses [26-28].

The next step in our research was isolation of 7S and 11S globulins and investigation of its content, ratio and component composition in seed of studied soybean varieties at the infection by SMV. Content of glycinin is from 11.78 to 16.36 % per content of protein in healthy seeds. Content of β-conglycinin is from 10.49 to 13.69% per content of protein depends on variety (Table 2).

We found that under the influence of SMV there was a decrease in the content of glycinin in all varieties, except of variety 'Monro'. Content of β-conglycinin increased by 1.38 and 1.2 times at the infection by SMV in varieties 'Kano' and 'Kuban', respectively, and decreased by 1.2 times in the variety 'Grimo'. Also, It was established that ratio of glycinin/β-conglycinin decreased at the influence of SMV in all studied varieties, except variety 'Monro'. 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 of syrah curds, such as tofu, with divalent cations, as well as in the imitation of syrah and brinzy. The thermoplastic characteristics of 7S globulin fraction positively blend into soybean milk [29]. 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. 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 7S and 11S globulins is polymorphic and had a different number of protein components depending on

*T a b l e 2. Content of 7S and 11S globulins in the soybean seed at the action of SMV*

| Variety | Content of protein, % per abs. dry substance | Content of glycinin (11S globulin) % per content of protein | Content of β-conglycinin (7S globulin) % per content of protein | Ratio of glycinin/ β-conglycinin |
|---|---|---|---|---|
| ‘Kano’, healthy | 40.49 ± 0.02 | 13.03 ± 0.04 | 10.49 ± 0.08 | 1.24 |
| ‘Kano’, SMV | 38.15 ± 0.02* | 11.83 ± 0.05* | 14.54 ± 0.03* | 0.81 |
| ‘Kuban’, healthy | 42.06 ± 0.05 | 16.36 ± 0.02 | 11.81 ± 0.05 | 1.38 |
| ‘Kuban’,SMV | 44.08 ± 0.03* | 15.81 ± 0.05 | 13.64 ± 0.06* | 1.16 |
| ‘Grimo’, healthy | 30.49 ± 0.02 | 15.79 ± 0.04 | 13.69 ± 0.07 | 1.15 |
| ‘Grimo’, SMV | 35.82 ± 0.03* | 13.07 ± 0.04* | 11.75 ± 0.05* | 1.11 |
| ‘Monro’, healthy | 43.04 ± 0.05 | 11.78 ± 0.03 | 10.87 ± 0.04 | 1.08 |
| ‘Monro’, SMV | 44.94 ± 0.04 | 12.64 ± 0.04 | 10.81 ± 0.05 | 1.17 |

Note. *Differences are significant at $P < 0.05$

the variety: 7S – from 12 to 19, 11S – 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, in the seed of the variety ‘Kano’, under the infection by SMV, the intensity of glycinin components with a molecular weight of 30, 18, 14.4, 12 kDa increased and the intensity of components with a molecular weight of 65, and 45 kDa decreased compare to the healthy seed (Fig. 1). In the electrophoretic spectrum of β-conglycinin under the influence of viral infection, an increase in the intensity of the components with a molecular mass of 67, 65, and 45 kDa and a decrease in the intensity of the bands of components with a molecular mass of 60, 35, and 20 kDa were observed. An increase in the intensity of glycinin components with a molecular weight of 30 and 18 kDa and a decrease in the intensity of β-conglycinin components with a molecular weight of 65, 60, 35, and 20 kDa were established in the seeds of soybean plants of the variety ‘Monro’ under infection by SMV. In the virus-infected seeds of the variety ‘Grimo’, an increase in the intensity of the bands of the glycinin component with a molecular weight of 45 kDa and a decrease in the quantitative content of β-conglycinin components with a molecular weight of 67, and 65 kDa were observed in comparison with the healthy seeds (Fig. 2). In the virus-infected seeds of the variety ‘Kuban’, an increase in the intensity of the bands of the glycinin component with a molecular weight of 45, 47, and 65 kDa and a decrease in the intensity of β-conglycinin components with a molecular weight of 45, and 33 kDa and an increase the content of component with molecular weight 67 kDa were established in comparison with the healthy seeds. An increase in the intensity of glycinin components with a molecular weight of 45, 28, 20.1, and 18 kDa were established in the seeds of soybean plants of the variety ‘Kordoba’ under infection by SMV (Fig. 3).

Thus, the obtained results shown that the SMV infection causes changes in biochemical indicators that characterize seed quality and take part in the protective reactions of plants, the revealed features of changes of these characteristics depended on the variety.

Today, soybean is one of the factors of the “healthy nutrition” system, which is gaining more and more supporters. Soybean milk is obtained from seed, which is used to prepare formulas for feeding infants – the future of the nation. Regular use of soybean products reduces cholesterol synthesis, regulates proper metabolism, and reduces fat accumulation. In addition, soybean products are very useful for cardiovascular, allergic and oncological diseases, as well as diabetes, obesity, pathology of the musculoskeletal system [30]. Soybean globulins such as 7S and 11S have become the major source of plant proteins in recent years because of their desirable digestibility and functionalities. Due to various structures, each soybean globulin possesses significant differences in nutritional and functional properties for food industry. For example, 7S globulin is better soluble in aqueous system and 11S globulin has

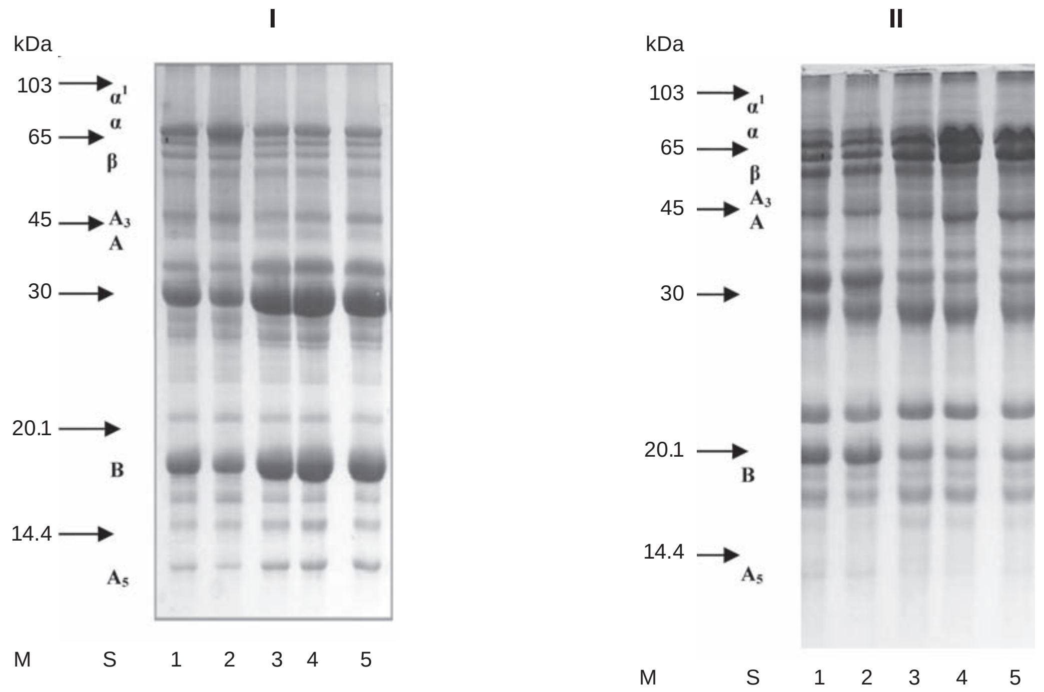


*Fig. 1. Electropherogram of the components of 11S and 7S globulins in the seeds of soybean variety 'Kano': M – molecular weight markers; S – glycinin and β-conglycinin subunits, I – glycinin, II – β-conglycinin, 1,2 – control, 3-5 – SMV-infected seed*

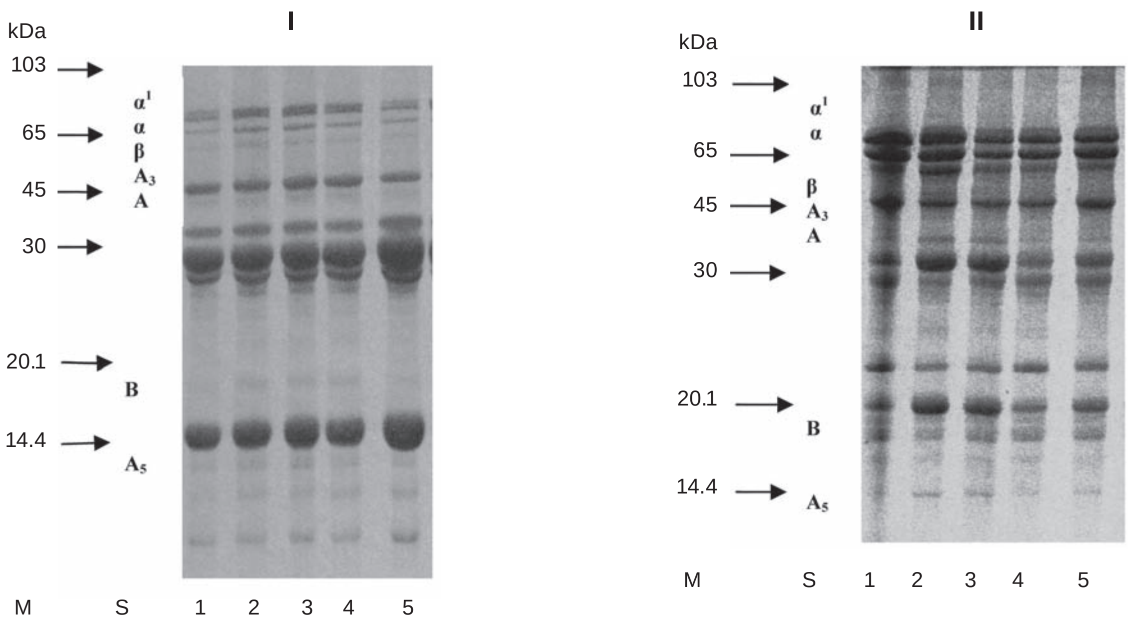


*Fig. 2. Electropherograms of the components of 11S and 7S globulins in the seeds of soybean variety 'Monro': M – molecular weight markers; S – glycinin and β-conglycinin subunits, I – glycinin, II – β-conglycinin, 1-3 – control, 4,5 – SMV-infected seed*

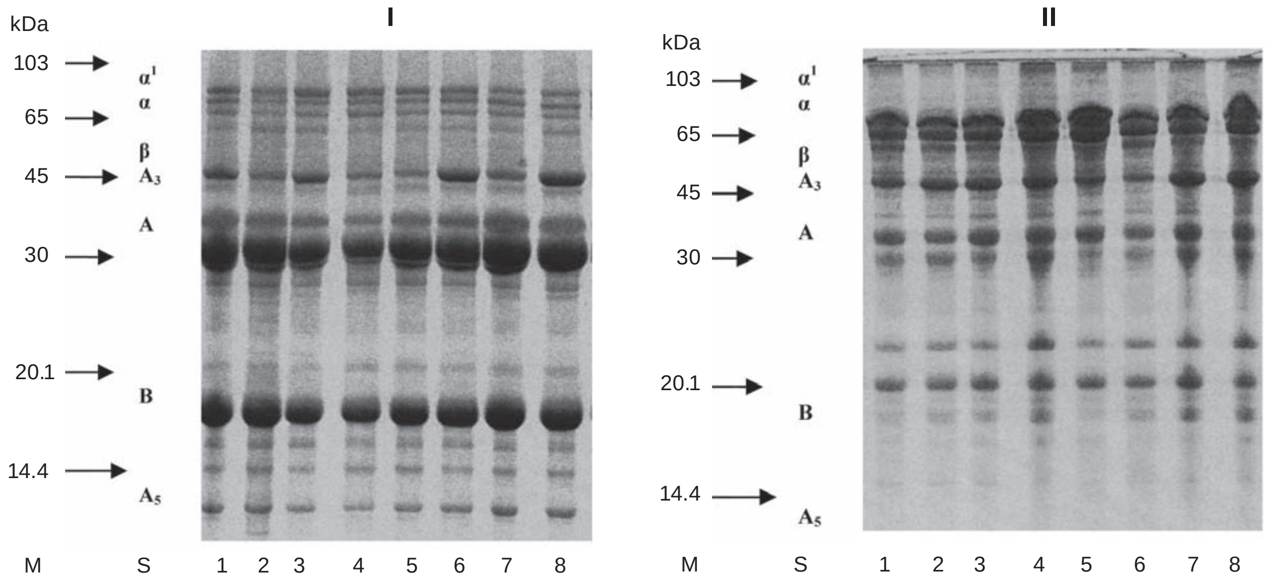


*Fig. 3. Electropherograms of the components of 11S and 7S globulins in the seeds of soybean varieties 'Monro', 'Grimo'. 'Kuban', 'Kano': M – molecular weight markers; S –glycinin and β-conglycinin subunits, 1 – 'Monro', control; 2 – 'Monro', SMV-infected seed; 3 – 'Kordoba', control; 4 – 'Grimo', control; 5 – 'Grimo', SMV-infected seed; 6 – 'Kordoba', SMV-infected seed; 7 – 'Kuban', control; 8 – 'Kuban', SMV-infected seed*

higher gelling properties [31]. Some subunits of 7S and 11S globulins, for example α1,α, and β subunits are all potential food allergens [32].

Having data on the quantitative content of total protein, 7S and 11S proteins and their ratio, component composition, content of carbohydrates, flavonoids, fat and anti-nutrient compounds (trypsin inhibitors, lectins, lipoxygenase activity), it is possible to carry out specific studies on the selection of soybean varieties for food use. Particularly important is the selection of varieties that maintain seed quality indicators after the infection by plant pathogens, for example viral infection (SMV). Among the studied varieties, we can distinguish varieties that have high levels of protein, carbohydrates, flavonoids, and fat during infection. This is, for example, the variety 'Kano' variety with a high content of flavonoids, varieties 'Kuban', 'Grimo', 'Monro' with increased or maintained at the level of healthy seeds content of protein, carbohydrates, fat. Differences in content, composition and structure of glycinin and β-conglycinin are manifested in both nutritional and functional properties. Among the studied varieties, the variety 'Monro' had increased or on the level of healthy seed content of protein, glycinin, β-conglycinin and ratio 11S/7S. Varieties 'Kano' and 'Kuban' had increased content of β-conglycinin in infected seeds compare to healthy ones. These changes studied biochemical characteristics were accompanied by changes in the component composition of these proteins.

*Conclusions.* Therefore, it has been established that infection by SMV caused changes in the biochemical characteristics (content of protein, main storage protein fractions (glycinin and β-conglycinin), fat, carbohydrates, flavonoids, activity of lectin, lipoxygenase, trypsin inhibitor) in the infected seeds that depend on the soybean variety. The electrophoretic analysis revealed varietal differences in the relative content of individual protein components in the electropherograms in the electrophoretic spectra of glycinin and β-conglycinin, which affect the nutritional value of soybean seeds. The use of the biochemical criteria studied allows the selection of food-grade soybean varieties with specific technological parameters.

The obtained results can be used for development of the methods of soybean varieties selection with high seed quality and resilience to the viral infection and will be recommended for implementation in breeding and agricultural practices.

*Conflict of interest.* The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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## ВПЛИВ ІНФІКУВАННЯ ВІРУСОМ МОЗАЇКИ СОЇ НА БІОХІМІЧНИЙ СКЛАД НАСІННЯ СОЇ

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Відомо, що інфекція, спричинена вірусом мозаїки сої (ВМС), негативно впливає на продуктивність сої, структуру врожаю та якість насіння. Основними показниками якості насіння є вміст запасних протеїнів – гліциніну та β-конгліциніну, жиру, вуглеводів, флавоноїдів, активність інгібітора трипсину, лектинів, ліпоксигенази. Метою досліджень було вивчення впливу інфікування рослин вірусом мозаїки сої на біохімічний склад насіння сортів сої, створених класичними методами селекції (‘Кано’, ‘Кубань’, ‘Кордоба’) та сортів трансгенної сої (‘Грімо’ і ‘Монро’). Ідентифікацію ВМС здійснювали за допомогою твердофазного імуноензимного аналізу (DAS-ELISA) та двоетапної ЗТ-ПЛР. Електрофорез протеїнів проводили в 15% ПААГ, що містив 0,1% SDS, при pH 8,3 за методом Леммлі. Виявлено підвищення активності інгібіторів трипсину та лектинів і зниження вмісту флавоноїдів у насінні інфікованих рослин досліджуваних сортів порівняно з насінням здорових рослин. За інфікування ВСМ у насінні всіх досліджених сортів спостерігалося зниження вмісту гліциніну та співвідношення гліцинін/β-конгліцинін, за винятком насіння сорту ‘Монро’, в якому вміст гліциніну та β-конгліциніну був підвищений або на рівні насіння здорових рослин. Можна виділити сорт ‘Кано’ з високим вмістом флавоноїдів, сорти ‘Кубань’ і ‘Грімо’ з підвищеним або збереженим на рівні насіння здорових рослин вмістом вуглеводів, жиру та протеїну за інфікування ВСМ. Виявлено відмінності у відносному вмісті компонентів β-конгліциніну і гліциніну в ураженому насінні залежно від сорту сої. Отримані результати можуть бути використані для розробки методів добору сортів сої з високою якістю насіння та стійкістю до вірусної інфекції.

Ключові слова: вірус мозаїки сої, якість насіння, гліцинін, β-конгліцинін, флавоноїди.

## References

1. Debaeke P , Forslund A, Guyomard H, Schmitt B, Tibi A. Could domestic soybean production avoid Europe’s protein imports in 2050? *OCL*. 2022; 29: 38.
2. Rotundo JL, Marshall R, McCormick R, Truong SK, Styles D, Gerde JA, Gonzalez-Escobar E, Carmo-Silva E, Janes-Bassett V, Logue J, Annicchiarico P, de Visser C, Dind A, Dodd IC, Dye L, Long SP, Lopes MS, Pannecoucque J, Reckling M, Rushton J, Schmid N, Shield I, Signor M, Messina CD, Rufino MC. European soybean to benefit people and the environment. *Sci Rep.* 2024; 14(1): 7612.
3. Kim IS. Current Perspectives on the Beneficial Effects of Soybean Isoflavones and Their Metabolites for Humans. *Antioxidants (Basel).* 2021; 10(7): 1064.
4. Kang GY, Choi SW, Chae WG, Chung JI. Accumulation of triple recessive alleles for three antinutritional proteins in soybean with black seed coat and green cotyledon. *J Plant Biotechnol*. 2020; 47(2): 118-123.
5. Ramaroson ML, Koutouan C, Helesbeux JJ, Le Clerc V, Hamama L, Geoffriau E, Briard M. Role of Phenylpropanoids and Flavonoids in Plant Resistance to Pests and Diseases. *Molecules.* 2022; 27(23): 8371.
6. Zhang S, Du H, Ma Y, Li H, Kan G, Yu D. Linkage and association study discovered loci and candidate genes for glycinin and β-conglycinin in soybean (*Glycine max* L. Merr.). *Theor Appl Genet*. 2021; 134(4): 1201-1215.
7. Boehm JD Jr, Nguyen V, Tashiro RM, Anderson D, Shi C, Wu X, Woodrow L, Yu K, Cui Y, Li Z. Genetic mapping and validation of

the loci controlling 7S α' and 11S A-type storage protein subunits in soybean [*Glycine max* (L.) Merr.]. *Theor Appl Genet.* 2018; 131(3): 659-671.
8. Zheng J, Zheng X, Zhao L, Yi J, Cai S. Effects and interaction mechanism of soybean 7S and 11S globulins on anthocyanin stability and antioxidant activity during *in vitro* simulated digestion. *Curr Res Food Sci.* 2021; 4: 543-550.
9. Sun H, Liu X, WangYZ, Liu JX , Feng J. Soybean glycinin- and β-conglycinin-induced intestinal immune responses in a murine model of allergy. *Food Agric Immunol.* 2012; 24(3): 357-369.
10. Elmore MG, Groves CL, Hajimorad MR, Stewart TP, Gaskill MA, Wise KA, Sikora E, Kleczewski NM, Smith DL, Mueller DS, Whitham SA. Detection and discovery of plant viruses in soybean by metagenomic sequencing. *Virol J.* 2022; 19(1): 149.
11. Widyasari K, Alazem M, Kim KH. Soybean Resistance to Soybean Mosaic Virus. *Plants (Basel)*. 2020; 9(2): 219.
12. Andayanie WR, Adinurani PG, Lukito M. Response of soybean lines to Soybean mosaic virus under drought stress. *J Trop Plant Pests Dis.* 2022; 22(1): 41-47.
13. Choi H, Jo Y, Chung H, Choi SY, Kim SM, Hong JS, Lee BC, Cho WK. Investigating Variability in Viral Presence and Abundance across Soybean Seed Development Stages Using Transcriptome Analysis. *Plants (Basel).* 2023; 12(18): 3257.
14. Mishchenko L, Dunich A, Mishchenko I, Molodchenkova O. Molecular and biological properties of soybean mosaic virus and its influence on the yield and quality of soybean under climate change conditions. *Agric Forest.* 2018; 64(4): 39-47.
15. Mishchenko L, Dunich A, Mishchenko I, Dashchenko A, Kandaurova K. Seed-borne and seedtransmitted viral diseases and their effect on yield of different soybean genotypes. *Agric Forest*. 2022; 68(4): 165-175.
16. Mishchenko LT, Dunich AA, Shcherbatenko IS. Phylogenetic analysis of Ukrainian seed-transmitted isolate of Soybean mosaic virus. *Biopolym Cell.* 2018; 34(3): 229-238.
17. Mishchenko I, Dashchenko A, Dunich A, Mishchenko L. Influence of abiotic and biotic factors on productivity of transgenic soybean and molecular properties of disease pathogen. *Agric Forest.* 2019; 65(4): 15-25.
18. Crowther JR. ELISA. Theory and practice. New York. Hamana: Press, 1995. 223 p.
19. Sherepitko DV, Budzanivska IG, Polischuk VP, Boyko AL. Partial sequencing and phylogenetic analysis of Soybean mosaic virus isolated in Ukraine. *Biopolym Cell.* 2011; 27(6): 472-479.
20. Kjeldahl J. A New Method for the Determination of Nitrogen in Organic Matter. *Zeitschrift für Analytische Chemie.* 1883; 2: 366-382.
21. Utility model patent No. 107671, Ukraine. The method of selection of soybean genotypes for the food sector / Adamovska VG, Molodchenkova OO, Kartuzova TV, Sichkar VI, Lavrova GD. The applicant and owner of the patent is the Plant Breeding & Genetics Institute- National Center of Seed and Cultivar Investigation; statement 24.06.2016.
22. Osterman LA. Methods for the study of proteins and nucleic acids: Electrophoresis and ultracentrifugation. M.: Nauka, 1981. 288 p. (In Russian).
23. Liu K. Soybean Trypsin Inhibitor Assay: Further Improvement of the Standard Method Approved and Reapproved by American Oil Chemists' Society and American Association of Cereal Chemists International. *J Am Oil Chem Soc.* 2019; 96: 635-645.
24. Lutsik MD, Panasyuk EN, Lutsik AD. Lectins. Lviv: Vishcha school, 1981. 155 p. (In Russian).
25. Mishchenko L, Nazarov T, Dunich A, Mishchenko I, Ryshchakova O, Motsnyi I, Dashchenko A, Bezkrovna L, Fanin Y, Molodchenkova O, Smertenko A. Impact of Wheat Streak Mosaic Virus on Peroxisome Proliferation, Redox Reactions, and Resistance Responses in Wheat. *Int J Mol Sci.* 2021; 22(19): 10218.
26. Zakaryan H, Arabyan E, Oo A, Zandi K. Flavonoids: promising natural compounds against viral infections. *Arch Virol.* 2017;162(9):2539-2551.
27. Breitenbach Barroso Coelho LC, Marcelino Dos Santos Silva P, Felix de Oliveira W, de Moura MC, Viana Pontual E, Soares Gomes F, Guedes Paiva PM, Napoleão TH, Dos Santos Correia MT. Lectins as antimicrobial agents. *J Appl Microbiol.* 2018; 125(5): 1238-1252.
28. Clemente M, Corigliano MG, Pariani SA, Sánchez-López EF, Sander VA, Ramos-Duarte VA. Plant serine protease inhibitors:

biotechnology application in agriculture and molecular farming. *Int J Mol Sci.* 2019; 20(6): 1345.

29. Onodera Y, Ono T, Nakasato K, Toda K. Homogeneity and microstructure of tofu depends on 11S/7S globulin ratio in soymilk and coagulant concentration. *Food Sci Technol Res.* 2009; 15(3): 265-274.
30. Dukariya G, Shah S, Singh G, Kumar A. Soybean and its products: nutritional and health benefits. *J Nut Sci Heal Diet.* 2020; 1(2): 22-29.
31. Ajibola CF, Aluko RE. Physicochemical and functional properties of 2S, 7S, and 11S enriched hemp seed protein factions. *Molecules.* 2022; 27(3): 1059.
32. Song B, Oehrle NW, Liu S, Krishnan HB. Development and characterization of a soybean experimental line lacking the α' subunit of β-conglycinin and G1, G2, and G4 glycinin. *J Agric Food Chem*. 2018; 66(2): 432-439.