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ИЗМЕНЕНИЕ БИОХИМИЧЕСКИХ ПОКАЗАТЕЛЕЙ СОИ В ЗАВИСИМОСТИ ОТ УСЛОВИЙ ВЫРАЩИВАНИЯ

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Представлены результаты анализа изменений биохимических показателей сои в зависимости от условий выращивания. Объектом исследования служили образцы сои (*Glycine max* (L.) Merr) сорта Лидия и дикой сои (*Glycine soja* Sieb. & Zucc.) формы КА-1344, выращенные на естественных почвах (контрольной и с повышенным содержанием тяжелых металлов). Анализ проводили в листьях, стеблях, корнях, цветках и семенах культурной и дикой сои в фазу первого тройчатого листа, цветения и плодоношения. Содержание малонового диальдегида и удельной активности кислой фосфатазы определяли спектрофотометрическим методом, активность пероксидазы – колориметрическим, содержание цинка, меди и свинца – атомно-абсорбционным. Электрофоретические спектры кислой фосфатазы выявляли методом электрофореза на колонках 7,5%-го полиакриламидного геля. Выявление на геле зон с ферментативной активностью проводили соответствующими гистохимическими методами. Установлено, что выращивание сои на почве с повышенным содержанием цинка, меди и свинца приводит к их накоплению в ее органах. Наибольшее содержание исследуемых металлов установлено в корнях. Выращивание сои на почве с повышенным содержанием цинка, меди и свинца привело к увеличению удельной активности пероксидазы в ее органах. При этом содержание малонового диальдегида у культурной сои достоверно увеличивалось лишь в стеблях в фазу первого тройчатого листа и в корнях в фазу цветения, у дикой сои – в листьях, стеблях и корнях в фазу первого тройчатого листа и в стеблях в фазу цветения. Выявлено, что максимальной удельной активностью кислой фосфатазы обладают цветки. Культурная соя в условиях повышенного содержания цинка, меди и свинца в почве характеризовалась увеличением удельной активности кислой фосфатазы и появлением новых множественных форм. Для дикой сои в целом отмечено снижение удельной активности кислой фосфатазы и увеличение числа множественных форм фермента.

Ключевые слова: соя, *Glycine max*, *Glycine soja*, адаптация, тяжелые металлы, малоновый диальдегид, пероксидаза, кислая фосфатаза

CHANGES IN THE BIOCHEMICAL PARAMETERS OF SOYBEANS DEPENDING ON THE GROWING CONDITIONS

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The results of the analysis of changes in biochemical parameters of soybeans depending on growing conditions are presented. The object of the study was samples of soybean (*Glycine max*

(L.) Merr) of the Lydia variety and wild soybean (*Glycine soja* Sieb. & Zucc.) forms of KA-1344 grown on natural soils (control and with a high content of heavy metals). The analysis was carried out in leaves, stems, roots, flowers and seeds of cultivated and wild soybeans in the phase of the first triple leaf, flowering and fruiting. The content of malonic dialdehyde and the specific activity of acid phosphatase were determined by spectrophotometric method, the activity of peroxidase – by colorimetric method, the content of zinc, copper and lead – by atomic absorption. Electrophoretic spectra of acid phosphatase were detected by electrophoresis on columns of 7.5% polyacrylamide gel. Identification of zones with enzymatic activity on the gel was carried out by appropriate histochemical methods. It has been established that the cultivation of soybeans on soil with a high content of zinc, copper and lead leads to their accumulation in the organs of soybeans. The highest content of the studied metals is found in the roots. Growing soybeans on soil with a high content of zinc, copper and lead led to an increase in the specific activity of peroxidase in its organs. At the same time, the content of malonic dialdehyde in cultivated soybeans significantly increased only in stems during the phase of the first triple leaf and in roots during the flowering phase, and in wild soybeans in leaves, stems and roots during the phase of the first triple leaf and in stems during the flowering phase. It has been revealed that the flowers have the maximum specific activity of acid phosphatase. Cultivated soybeans, under conditions of increased zinc, copper and lead content in the soil, were characterized by an increase in the specific activity of acid phosphatase and the appearance of new multiple forms. For wild soybeans, in general, there was a decrease in the specific activity of acid phosphatase and an increase in the number of multiple forms of the enzyme.

Key words: soya, *Glycine max, Glycine soja,* adaptation, heavy metals, malonic dialdehyde, peroxidase, acid phosphatase

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INTRODUCTION

Currently, one of the global environmental problems of our time is pollution of the environment with heavy metals [1]. To solve this problem different measures are taken, including monitoring studies of the state of various objects of the environment. Significant interest is represented by research of composition and intensity of influence of the chemical pollution of environment on the state of plants growing in different ecological and geographical conditions [2].

The influx of heavy metals in plants is determined by a complex of factors, the most important of which are soil properties and dynamics of soil processes, the concentration of elements in the environment and their physiological significance for plants, the physiological characteristics of plants, etc. For each plant a characteristic pattern of distribution of elements in organs and tissues is formed[1,2].

Excessive levels of chemical elements have depressing and even toxic effects on plants. There is a statement that there are no toxic metals, there are toxic concentrations. Consequently, trace elements and heavy metals are concepts referring to the same elements, characterizing their concentration in different

[1]Janadeleh H., Kardani M., Salemi M. Study of heavy metals effects on plants // Third International Symposium on Environmental and Water Resources, Engineering. Tehran, Iran, 2–3 June 2015.

[2]Loginova A.S., Otradnova M.I., Shilova N.A., Rogacheva S.M. Assessment of the safety of plant crops with soil contamination by heavy metals // Technogenic and natural safety: Proceedings of the IV All-Russian scientific and practical conference (Saratov, 19-21 April 2017). Saratov: LLC "Amirit", 2017. Pp. 69-72.

environments [3]. The effect of heavy metals on a plant may differ at various stages of its development. Oxidative stress caused by the action of heavy metals is one of the main ones adversely affecting crops [4].

The enzyme system can serve as an indicator of plant resistance. A well-studied enzyme is peroxidase (ACP 1.11.1.X), which plays an important role in the plant immune defense system [5]. Acid phosphatase (ACP 3.1.3.2) is of considerable interest since it is the most important enzyme of the main metabolic pathways of living systems and has not been sufficiently studied for plants [6, 7].

The aim of the study was to investigate the change in biochemical parameters in the organs of cultivated and wild soybean during ontogenesis depending on the growing conditions.

MATERIAL AND METHODS

The material for the study was samples of soybean variety Lydia and wild soybean form KA-1344. We previously analyzed soils in soybean growing areas [8]. For this study, natural soils in the background point (control) and with increased content of heavy metals (experiment) were selected (see Table 1).

Soybean samples were grown in a greenhouse at 18-28 ºC. Before planting in selected soil, seeds were surface sterilized with 70% ethyl alcohol solution, washed with distilled water, then planted in darkened vessels with soil. The analysis was performed in leaves, stems, and roots at the stage of the first triple leaf, in leaves, stems, roots, and flowers at the flowering stage, and in seeds at the fruiting stage.

For biochemical analysis, extracts of soluble soybean proteins were prepared[3]. Protein in the extracts was determined by the Lowry method [9], the specific activity of acid phosphatase by spectrophotometric method with p-nitrophenyl phosphate as a substrate. Specific activity was expressed in units per 1 mg of protein. Multiple forms of the enzyme were detected by electrophoresis in 7.5% PAAG, followed by staining of the zones with appropriate histochemical methods (see footnote 3). The standard criterion for characterizing multiple forms of enzymes is their relative electrophoretic mobility (Rf). Previously, 13 forms of acid phosphatase with Rf from 0.04 to 0.75 were identified [10].

The content of malondialdehyde (MDA) and specific activity of peroxidase were used as markers of oxidative stress. Specific peroxidase activity was determined by Boyarkin's photometric method according to modification by Mokronosov on photoelectric concentration colorimeter KFK-2 (Russia) by the change in optical density (see footnote 3). Benzidine hydrochloride (Interkhim, Russia) was used as a substrate. MDA content was determined by reaction with thiobarbituric acid, which at high temperature and acidic pH proceeds with the formation of a colored trimethine complex[4]. The contents of zinc, copper, and lead were determined by atomic absorption method on spectrophotometer KVANT.Z (Russia).

Табл. 1. Характеристика почв для исследований
Table 1. Characteristics of soils for research

Soil	Humus, %	pH	Content, mg/kg				
			Water-soluble forms of phosphate ions converted to P_2O_5	Water-soluble forms of potassium ions	Labile forms		
					zinc	lead	copper
Control	4,2 ± 0,6	5,1 ± 0,2	9,2 ± 1,4	3,1 ± 0,5	<1,0	<0,5	<1,0
Experiment	4,6 ± 0,7	5,0 ± 0,2	15,7 ± 2,4	7,3 ± 1,2	20,2 ± 6,1	0,9 ± 0,3	2,4 ± 0,7

[3]Ivachenko L.E., Kashina V.A., Mascaltsova V.I., Razantsvey V.I., Stasiuk E.M., Trofimtsova I.A. Methods for studying the polymorphism of soybean. Blagoveshchensk: Publishing house of BSPU, 2008. 138 p.

[4]Rogozhin V.V., Rogozhina T.V. Practicum on physiology and biochemistry of plants: a textbook. SPb.: GIORD, 2013. 352 p.

Statistical processing of the obtained data was performed using Microsoft Excel software. Plant material was analyzed in two biological and three analytical replications (6 replications in total). The results were expressed as the mean (n = 6) ± standard deviation; the differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Currently, increased attention is paid to the accumulation and distribution of heavy metals in plant organs, as well as their effect on the main physiological processes and productivity. The typical distribution of metals in plant organs is as follows (in descending order): root > aboveground mass > generative organs [11]. The ability to accumulate metals varies not only between species, but also between varieties and genotypes. Accumulation of heavy metals in reproductive organs and seeds is less intense [12]. This is of great biological importance related to the preservation of reproductive ability and seed productivity.

During the conducted analysis, soybean organs were distributed according to their ability to accumulate zinc (in descending order): roots > seeds > leaves = flowers > stems (see Fig. 1, a). It is noted in the literature that, in general, zinc is distributed among different plant organs as follows: roots > leaves > stems > trunk (stem)5. An increase in zinc concentration in all organs of soybean plants under conditions of high content of heavy metals in soil was observed. The maximum was recorded in soybean roots, from 37.65 to 56.02 mg/kg.

The distribution of copper by soybean organs is as follows (in descending order): roots > flowers = leaves > seeds > stems (see Fig. 1, b). The content of copper in the samples grown on the experimental soil is higher than in the control. The maximum accumulation of copper was recorded in soybean roots, from 18.83 to 28.97 mg/kg.

Lead accumulation capacity (in descending order): roots > leaves = stems > flowers = seeds (see Fig. 1, c). Most researchers who have studied the distribution of lead in the organs of various plant species report the predominant accumulation of the element in roots [13]. We also noted this pattern: lead accumulation during cultivation on experimental soil occurs in all organs of soybean. The concentration of lead in the roots of soybean grown on experimental soil was higher by more than 2.5 times relative to the control. The minimum concentration of

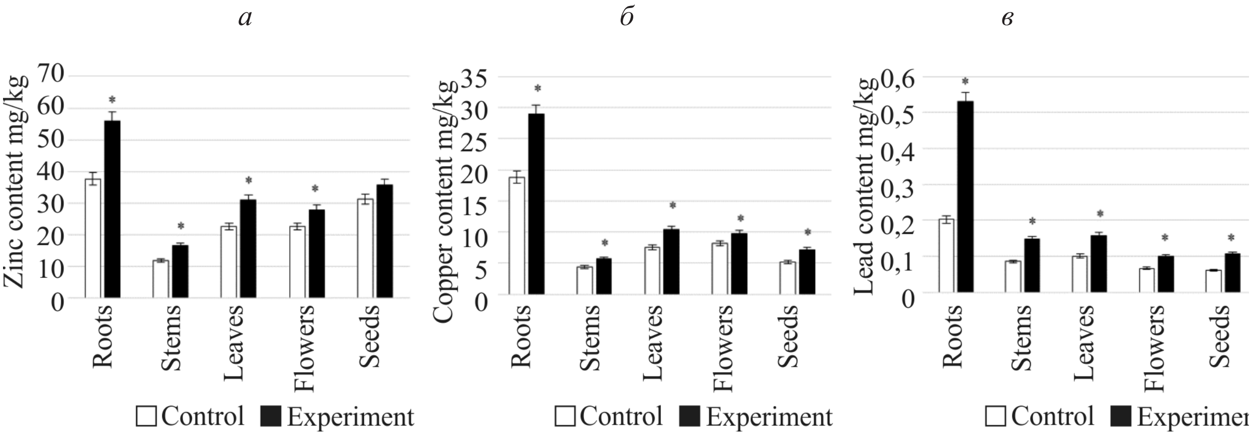

Рис. 1. Содержание цинка (*а*), меди (*б*) и свинца (*в*) в органах сои (среднее) в зависимости от агрофона. Здесь и в рис. 2–5 различия с контролем достоверны при $p \leq 0{,}05$

Fig. 1. The content of zinc (*a*), copper (*b*) and lead (*c*) in soybean organs (average) depending on the agricultural background

*Here and in Figs. 2-5 differences with control are significant at $p \leq 0.05$

[4] *Rogozhin V.V., Rogozhina T.V.* Practicum on physiology and biochemistry of plants: a textbook. SPb.: GIORD, 2013. 352 p.
[5] *Kabata-Pendias A.* Trace elements in soils and plants. L.: CRC Press, 2011. 505 p.

lead was established in the generative organs of soybean, which indicates the protective mechanisms that prevent the accumulation of heavy metals in the seeds of plants.

Accumulation of heavy metals leads to increased oxidative stress, markers of which are MDA content and peroxidase test. The analysis showed a significant increase in specific peroxidase activity in most organs of cultivated and wild soybean (see Fig. 2).

MDA content in cultivated soybean significantly increased in the stems at the first triplet leaf phase and in the roots at the flowering phase; in wild soybean, it increased in the leaves, stems, and roots at the first triplet leaf phase and in the stems at the flowering phase (see Fig. 3).

In the course of the studies, it was found that soybean flowers were characterized by maximum specific activity and high heterogeneity of acid phosphatase (see Fig. 4, A). When growing cultivated and wild soybean on control soil, six and five forms, respectively, were detected in the flowers. Growing soybean on experimental soil resulted in an increase in the specific activity of acid phosphatase in flowers of cultivated soybean and a decrease in wild soybean. At the same time, the number of forms of the enzyme increased for wild soybean (a new form of KF10 was noted) and remained stable for cultivated soybean (see Fig. 4, B). A significant increase in specific activity of acid phosphatase in leaves (1.7 and 1.4-fold) and roots of cultivated soybean (1.4 and 1.6-fold) during the first triple leaf phase and flowering, respectively, was observed. The number of forms of the enzyme also increased: a new form of KF5 (first triplet leaf) was detected in roots, KF10 in leaves, and KF9 in roots (flowering). Seeds were characterized by minimal activity. Cultivation of cultivated soybean on experimental soil led to an increase in specific activity and a number of multiple forms of acid phosphatase in seeds; a new form of the enzyme, KF4, was noted (see Fig. 4, B).

For wild soybean, a decrease in the specific activity of acid phosphatase in leaves, stems, and roots during the phase of the first triplet leaf was noted by 1.1; 2.5 and 1.8 times, respectively, and in flowers by 1.2 times. New forms of the enzyme in leaves (KF12), roots (KF6, KF10) and flowers (KF10) were revealed. An increase in the specific activity of the enzyme was observed in leaves and stems of wild soybean during the flowering phase by 1.5 and 2.6 times, respectively, and in seeds, which correlates with the appearance of a new form of the enzyme in leaves and seeds (KF5) (see Fig. 5).

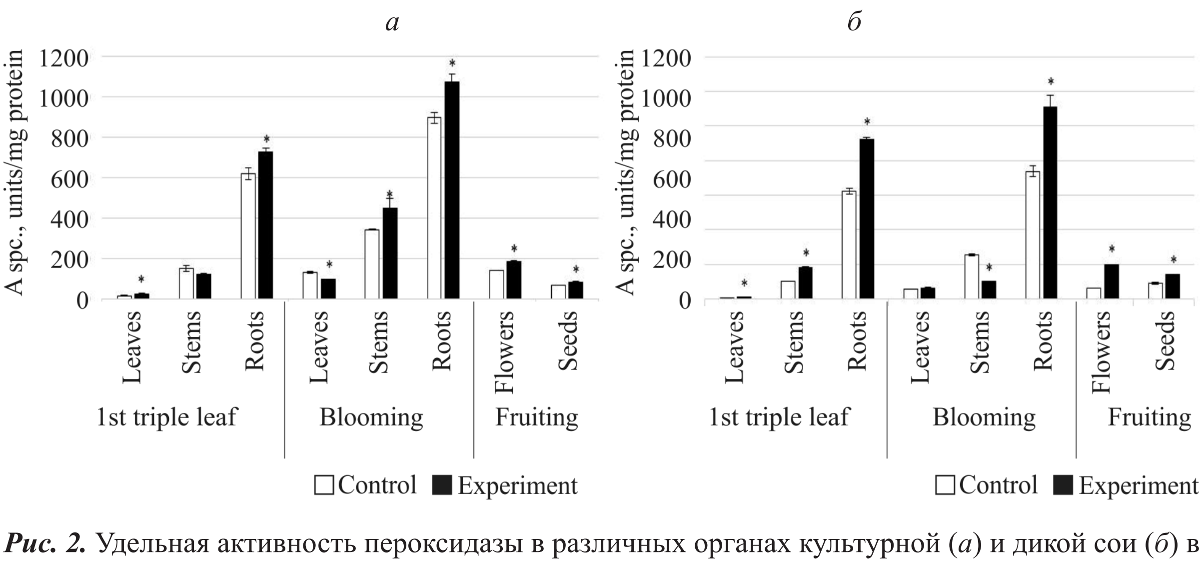

Рис. 2. Удельная активность пероксидазы в различных органах культурной (*а*) и дикой сои (*б*) в фазу первого тройчатого листа, цветения и плодоношения

Fig. 2. Specific activity of peroxidase in various organs of cultivated (*a*) and wild soybean (*b*) in the phase of the first trifoliate leaf, flowering and fruiting

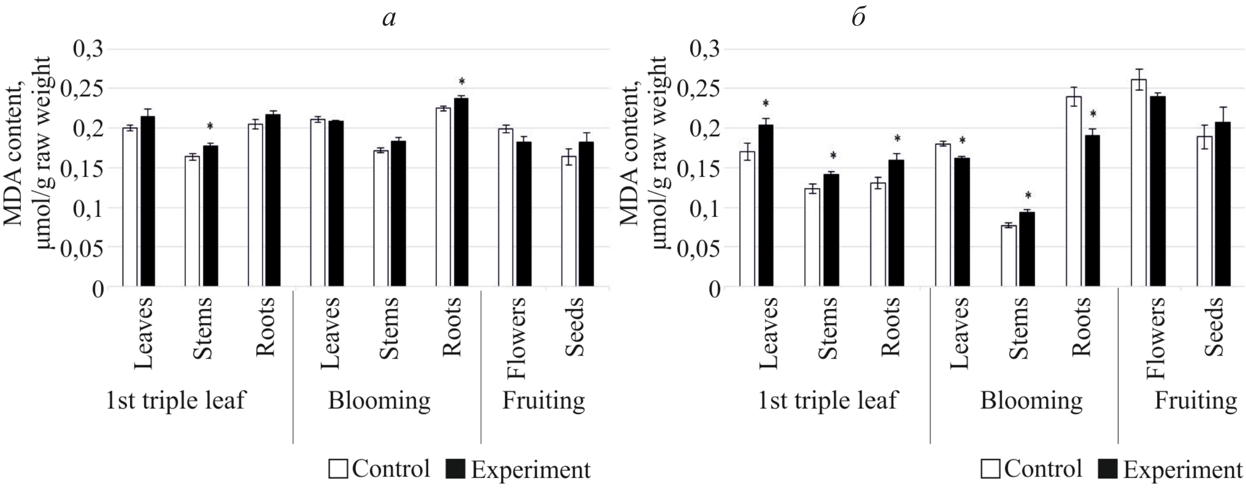

Рис. 3. Содержание МДА в различных органах культурной (*а*) и дикой сои (*б*) в фазу первого тройчатого листа, цветения и плодоношения

Fig. 3. The content of MDA in various organs of cultivated (*a*) and wild (*б*) soybeans in the phase of the first trifoliate leaf, flowering and fruiting

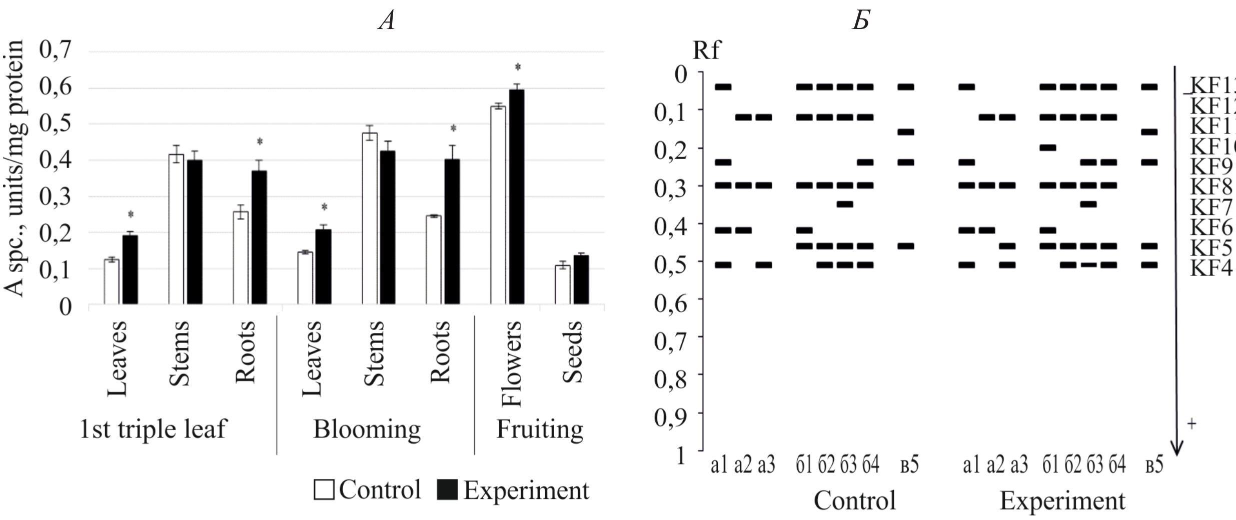

Рис. 4. Удельная активность (*А*) и схемы энзимограмм (*Б*) кислой фосфатазы в различных органах культурной сои в фазу первого тройчатого листа (а), цветения (б) и плодоношения (в):
1 – листья; 2 – стебли; 3 – корни; 4 – цветки; 5 – семена. Стрелка указывает направление электрофореза (от катода к аноду). Справа указана нумерация выявленных форм фермента

Fig. 4. Specific activity (*A*) and enzyme diagrams (*B*) of acid phosphatase in various organs of cultivated soybean in the phase of the first trifoliate leaf (a), flowering (b) and fruiting (c):
1 – leaves; 2 – stems; 3 – roots; 4 – flowers; 5 – seeds. The arrow indicates the direction of electrophoresis (from the cathode to the anode). On the right is the numbering of the identified forms of the enzyme

CONCLUSION

It was found that the cultivation of soybean on soil with high content of zinc, copper and lead leads to their accumulation in soybean organs. The highest content of the studied metals was found in the roots. When growing cultivated and wild soybean on the soil with increased content of heavy metals, an increase in specific activity of peroxidase in most organs of cultivated and wild soybean was observed. The MDA content in cultivated soybean increased significantly only in the stems at the phase of the first triple leaf and in the roots at the flowering phase; in

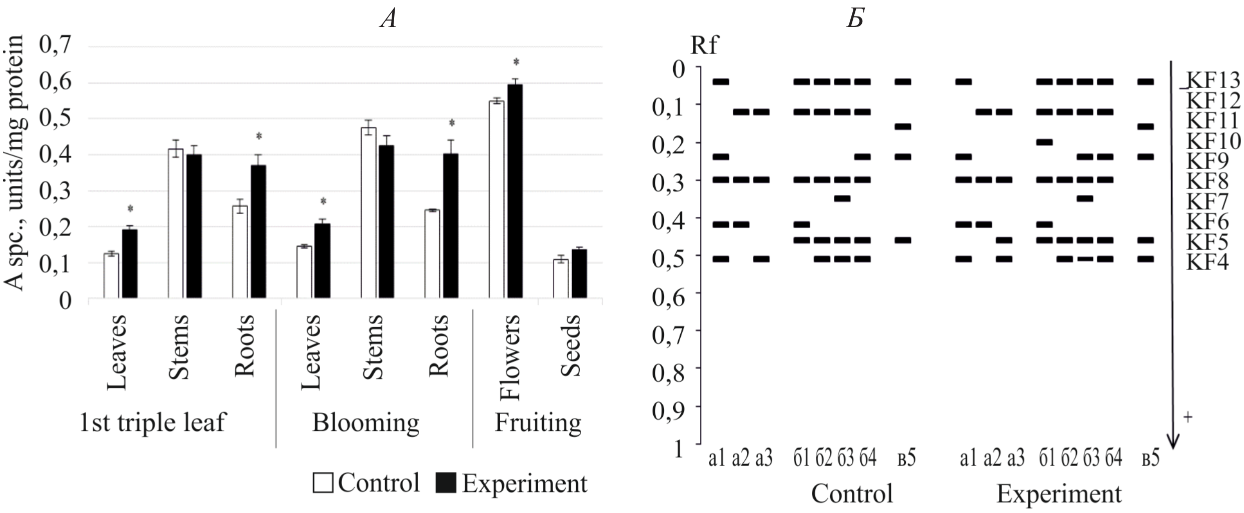

Рис. 5. Удельная активность (*А*) и схемы энзимограмм (*Б*) кислой фосфатазы в различных органах дикой сои в фазу первого тройчатого листа (а), цветения (б) и плодоношения (в):
1 – листья, 2 – стебли, 3 – корни, 4 – цветки, 5 – семена. Стрелка указывает направление электрофореза (от катода к аноду). Справа указана нумерация выявленных форм фермента.

Fig. 5. Specific activity (*A*) and schemes of enzymograms (*B*) of acid phosphatase in various organs of wild soybean in the phase of the first trifoliate leaf (a), flowering (b) and fruiting (c):
1 – leaves, 2 – stems, 3 – roots, 4 – flowers, 5 – seeds. The arrow indicates the direction of electrophoresis (from the cathode to the anode). On the right is the numbering of the identified forms of the enzyme.

wild soybean, it increased significantly in the leaves, stems, and roots at the phase of the first triple leaf and in the stems at the flowering phase. Analysis of the specific activity of acid phosphatase showed that the greatest activity of the enzyme was in the flowers, which is most likely due to the participation of acid phosphatase in the mobilization of phosphate, which is required in the processes occurring at the flowering phase. In cultivated soybean plants with increased content of zinc, copper and lead in the soil, the specific activity of acid phosphatase increased and new multiple forms appeared: in leaves - KF10, in roots - KF5 and KF9 and in seeds - KF4. For wild soybean, a decrease in the specific activity of acid phosphatase and an increase in the number of multiple forms of the enzyme in leaves - KF5 and KF12, roots - KF6 and KF10, flowers - KF10, seeds - KF5 were generally observed.

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