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Occurrence of multiple forms of enzymes of cultivated soybean (*Glycine max (L.) Merrill, 1917*) and wild-growing soybean (*Glycine soja Siebold & Zucc., 1845*)

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ABSTRACT

A detailed analysis of wild-growing soybean (Glycine soja Siebold & Zucc., 1845) and 8 cultivars of soybean (Glycine max (L.) Merrill, 1917), which differ in earliness and which are recognized in the Amur region of the Russian Federation, was made using the methods of biochemistry and bioinformatics. We systematized the data on revealed electrophoretic spectra of catalases, peroxidases, acid phosphatases, esterases and amylases of soybean seeds of different phylogenetic origin. It is shown that wild-growing soybean has a high adaptive capacity; it is characterized by a small number of enzyme forms (catalases, peroxidases, acid phosphatases, esterases and amylases). A stable number of forms of the enzymes under study or their increase over the years of cultivation of varieties demonstrate the high adaptive potential of varietal soybean. Electrophoretic spectra of the enzymes of seeds can be used as the markers of soybean varieties adaptation to different growing conditions.

Keywords: soybean, polymorphism, enzymes, electrophoretic spectra bioinformatics.

The study of biochemical adaptation of living organisms to the ever-changing environment is at the heart of modern biological studies and requires knowledge of the basics of adaptive responses. Enzymes play the leading role in the maintenance of intracellular homeostasis and adaptation to stressors [1-3]. Common patterns of biochemical adaptation strategy of animals are thoroughly studied [4-6], however there are no generalizations of such magnitude for plants, especially cultivated ones.

An important role in the study of the underlying processes of regulation of life belongs to multiple forms of enzymes and in particular to genetically determined isoenzymes [1; 7]. Nowadays crop breeders solve many theoretical and applied problems of selection using information about isoenzymes [8].

Practical significance of the study of polymorphism of enzyme systems is in the fact that isoenzymes are effective genetic markers [9-12]. Responsiveness of enzyme systems to changes in environmental conditions was repeatedly described in literature [13-16]. Without any doubt, it is important to study the biochemical basis of metabolism of crops valuable for man.

Soybean is one of the most important crops in the world as a unique alternative source of protein and cost-effective technical crop. For the past 75 years the area of this crop in the world increased from 11 to 98 million hectares [17]. Soybean products are widely used recently even in medicine [18-20].

That is why genome and polymorphism of soybean enzymes are studied by a large group of researchers [21-30]. Additionally, soybeans are already subject to molecular genetic experiments with the gene transfer [31; 32].

Two groups of enzymes were selected as genetic markers for our studies: oxidoreductases (catalase (EC 1.11.1.6), peroxydases (EC 1.11.1.7)) and hydrolases (amylases (EC 3.2.1), esterases (EC 3.1.1) and acid phosphatase (EC 3.1.3.11)).

Obviously, the expansion and generalization of actual material on multiple forms of soybean enzymes may become one of the tools for the study of adaptation and agricultural biotechnology.

MATERIALS AND METHODS

In order to study the problems of biochemical adaptation it is necessary to have a directory of electrophoretic spectra of enzymes of wild-growing and cultivated soybeans, and to study the effect of the environment on the activity of enzymes and the identification of the best soybean cultivars adapted to growing conditions.

Therefore, in the early stages of the study, we set out to explore the electrophoretic spectra of enzymes of seeds of a number of cultivated and wild-growing soybean cultivars.

Far East, and mainly Amur region, produces 2/3 of the soybeans in Russia [17], so cultivars growing in the region were used in the research.

Research materials. The seeds of soybean cultivars (*Glycine max (L.) Merrill*, 1917) differing in earliness and origin were the material for the study. We used the early ripening cultivars of soybeans Zakat, Smena, Sonata (selection by All-Russian Soy Research Institute, Blagoveshchensk, Russia), Souer-4 (selection by Research Institute of Agriculture of the Southeast, Saratov, Russia) and mid ripening ones - VNIIS-1, October 70, Harmony (selection by All-Russian Soy Research Institute, Blagoveshchensk, Russia) and Luch Nadezhdy (selection by Far Eastern State Agrarian University, Blagoveshchensk, Russia), located in Amur region of Russia.

The forms of wild-growing soybean (*Glycine soja Siebold & Zucc*., 1845): CA -1344, CA -1388, CB -104, CB -49, CZ -6316, CZ -6359 were obtained from three agro-climatic zones of Amur region (CA - Arkharinsky region, south zone, CB - Belogorsky region, central zone, CZ - Zeyskiy region, northern area).

Seeds of cultivated soybean were obtained from two State sorting sites of Amur region (Tambovka and Mazanovo settlements). Wild-growing soybeans were grown in culture in the experimental field of the Institute of soybeans in Sadovy settlement of Tambovsky area of Amur region (southern agro climatic zone) for three years.

Research methods. Field experiments and selection of samples for analyzes were performed according to State methods of crop variety testing [33].

In order to determine multiple forms of enzymes protein extracts were prepared from the matter under study. Electrophoretic spectra of the enzymes studied were detected by electrophoresis on 7.5% polyacrylamide gel columns [34] on the BioRaid instrument (USA). Reagents by Reanal (Hungary), Sigma (USA), Fluka (Belgium), Panreac (Spain), and Merck (Germany) were used. Determination of enzymatic activity zones in gel was carried out using appropriate histochemical methods [35; 36]. Since the standard criterion for the characterization of multiple forms of enzymes is their relative electrophoretic mobility (Rf), the difference in the quality of soybean cultivars was evaluated by identified enzyme forms according to their Rf [35]. The numbering of forms is given from the most mobile (to anode) forms to the low-mobile ones. Each enzyme form was given an abbreviation in accordance with Rf values (C1-C8 for catalase, P1-P18 for peroxidase, AP1-AP13 for acid phosphatase, E1-E14 for esterase, and A1-A10 for amylase). Additional rare forms were designated with "*".

Identified forms of catalases were named as follows: forms with Rf=0.48 were named C1; with Rf=0.42 - C2; with Rf=0.37 - C3; with Rf=0.30 - C4; with Rf=0.23 - C5; with Rf=0.17 - C6; with Rf=0.13 - C7; and with Rf=0.07 - C8. Identified forms of peroxidases were named as follows: forms with Rf=0.83 - P1; with Rf=0.75 - P2; with Rf=0.62 - P3; with Rf=0.58 - P4; with Rf=0.55 - P5; with Rf=0.49 - P6; with Rf=0.45 - P7; with Rf=0,42 - P8; with Rf=0.39 - P9; with Rf=0.34 - P10; with Rf=0.29 - P11; with Rf=0.25 - P12; with Rf=0.22 - P13; with Rf=0.16 - P14; with Rf=0.13 - P15; with Rf=0,10 - P16; with Rf=0.07 - P17; and with Rf=0.02 – P 18.

For acid phosphatases forms with Rf=0.75 were named as AP1; with Rf=0.63 - AP2; with Rf=0,58 - AP3; with Rf=0.51 - AP4; with Rf=0.46 - AP5; with Rf=0.42 - AP6; with Rf=0,35 - AP7; with Rf=0.30 - AP8; with Rf=0.24 - AP9; with Rf=0,20 - AP10; with Rf=0.16 - AP11; with Rf=0.12 - AP12; and with Rf=0.04 - AP13.

For esterases activity zones with Rf=0,90 were named E1; with Rf=0.78 - E2; with Rf=0.72 - E3; with Rf=0.62 - E4; with Rf=0.56 - E5; with Rf=0,50 - E6; with Rf=0.44 - E7; with Rf=0.37 - E8; with Rf=0,30 - E9; with Rf=0,24 - E10; with Rf=0.18 - E11; with Rf=0.13 - E12; with Rf=0.07 - E13; and with Rf=0,03 - E14.

For amylases the identified zones of activity with Rf=0.52 were named A1; with Rf=0.46 - A2; with Rf=0.41 - A3; with Rf=0.36 - A4; with Rf=0.32 - A5; with Rf=0.25 - A6; with Rf=0.21 - A7; with Rf=0.15 - A8; with Rf=0.11 - A9; and with Rf=0.05 - A10.

Areas of activity identified on enzymogramms (forms of enzymes) were divided in 3 groups by the rate of occurrence (0-19% - low occurrence; 20-49% - average occurrence; > 50% - high occurrence of forms).

The search for homologues of typical specimens of the studied enzymes in the bases of nucleotide sequences was performed using BLAST server (http://blast.ncbi.nlm.nih.gov) as in [37].

Studies were conducted with the use of the equipment of the Interdepartmental Center of Analytical Control of the State of Environment of Far Eastern Federal University.

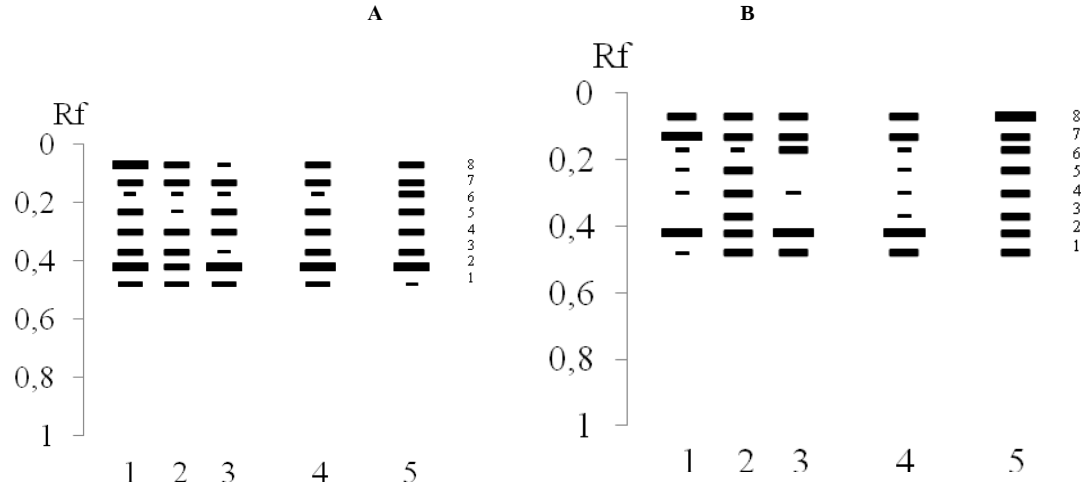
The sequences of enzymes *Glycine max* were used as the typical specimens: from catalases - Catalase-1/2 (IDP29756); peroxidases - Glutathioneperoxidase (IDC6T3W7), Peroxidase (IDO22443), Ascorbateperoxidase (IDQ43758); phosphatases - Acidphosphatase (IDO49855), Purpleacidphosphatase (IDQ09131), Phytase (IDQ6YGT9); esterases - Pectinesterase (IDI1J7T2); amylases - Alpha-amylase (IDF9W2W3), Beta-amylase (IDP10538).

RESULTS AND DISCUSSION

Catalases

The studies found that catalase is an enzyme with a small number of forms (Fig. 1). 8 forms of the enzyme were found in cultivated and wild-growing soybean seeds over the years of study. Electrophoretic spectra of catalases of soybean seeds of cultivars studied are relatively stable and consist of 6-8 forms (Fig. 1A, B).

6 catalase forms were identified for all cultivar of soybean: C6, C7 and C8 with low electrophoretic mobility, and C1, C2 and C4 with average mobility. Electrophoretic spectra of cultivars October-70, Sonata, Zakat, Smena, Luch Nadezhdy and Souer-4 were similar. All 8 forms of catalases were identified for these cultivars. C3 form was not identified for the seeds of VNIIS-1 cultivar. A total of 6 forms of catalases were revealed For Harmony cultivar.
The study of the occurrence rate of multiple forms of cultivated soybean catalases showed that C2 form has a high occurrence rate and it is typical for all cultivars except Luch Nadezhdy cultivar which is distinguished by high occurrence of C8 form with low electrophoretic mobility. Mid ripening cultivars had a modest occurrence rate of forms C4-C6 with average electrophoretic mobility.



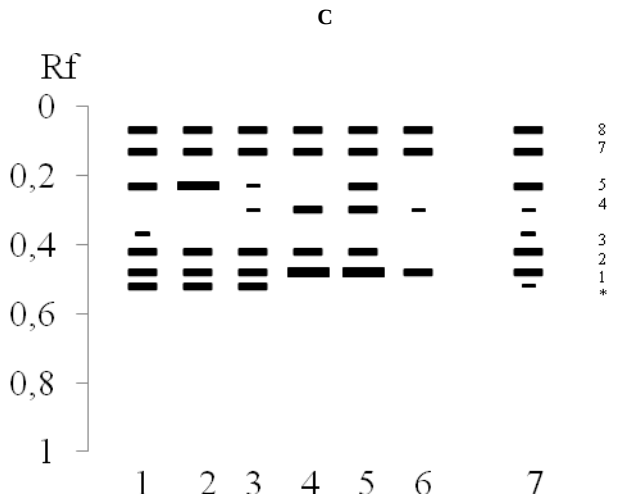


Fig. 1. Enzymogramm charts of the occurrence of catalase forms in early ripening cultivars of soybean seeds (A): 1 – Sonata, 2 – Zakat, 3 – Smena, 4 – on average for cultivars selected by Russian soy research Institute, 5 - Souer-4; mid ripening cultivars (B): 1 – VNIIS-1, 2 - October-70, 3 – Harmony, 4 - on average for cultivars selected by Russian soy research Institute, 5 – Luch Nadezhdy and lines of wild-growing (C) soybean: 1 - CA-1344, 2 - CA-1388, 3 – CB-104, 4 - CB-49, 5 - CZ-6316, 6 - CZ-6359, 7 – on average for the lines.

The analysis of enzymogramm charts of catalases of wild-growing soybean revealed that catalase forms C1, C7 and C8 are the most stable and are found in all the samples studied (Fig. 1C). Forms with average electrophoretic mobility are minor and vary in the lines of wild-growing soybean from one (for line CZ-6359) to three (for lines CA-1344, CB-104 and CZ-6316). The largest number of multiple forms of catalases of wild-growing soybean (7) is identified for lines CA-1344 and CB-104, and the smallest number (4) - for line CZ-6359.

One should note the presence of catalase form with Rf=0.52, named C* in lines CA-1344, CA-1388 and CB-104, which was absent in cultivated soybean seeds. In addition to that, C6 form common to all cultivars of soybean was not found in the seeds of wild-growing soybean.

The highest rate of occurrence of multiple forms of catalases of wild-growing soybean was recorded for form C5 in line CA-1388 and for form C1 in lines CB-49 and CZ-6316. C3 form was registered only in line CA-1344 and it has a very low rate of occurrence. Forms of catalases with low electrophoretic mobility C7 and C8 have an average rate of occurrence in all the samples studied. Form C2 also has an average rate of occurrence except for line CZ-6359, where it was not found. It was revealed that highly mobile form C* has an average rate of occurrence.

In summary, it was found that forms C1, C2, C7 and C8 are typical for most soybean cultivars and lines studied.

Peroxydases

Electrophoretic spectra of peroxidases of cultivated soybean vary widely (Fig. 2). During the research 18 forms of the enzyme were found in the seeds, but not a single cultivar containing all forms of peroxidases was identified. The maximum number of forms (17) was registered for VNIIS-1 cultivar (P5 form is missing) and for Sonata cultivar (P1 form is missing) (Fig. 2A, B). The lowest number of forms (7) was registered for Luch Nadezhdy cultivar. Forms P16-P18 with low electrophoretic mobility and P9-P10 with average electrophoretic mobility were found for all cultivars. The highly mobile form P1 was found only in two soybean cultivars – VNIIS-1 and October-70, and P5 form – in Sonata and Harmony cultivars.

The analysis of the rate of occurrence of multiple forms of peroxidases of soybean seeds showed no forms with a high rate of occurrence. It was found that early ripening and mid ripening cultivars are characterized by a low rate of occurrence of forms with high electrophoretic mobility of the enzyme.

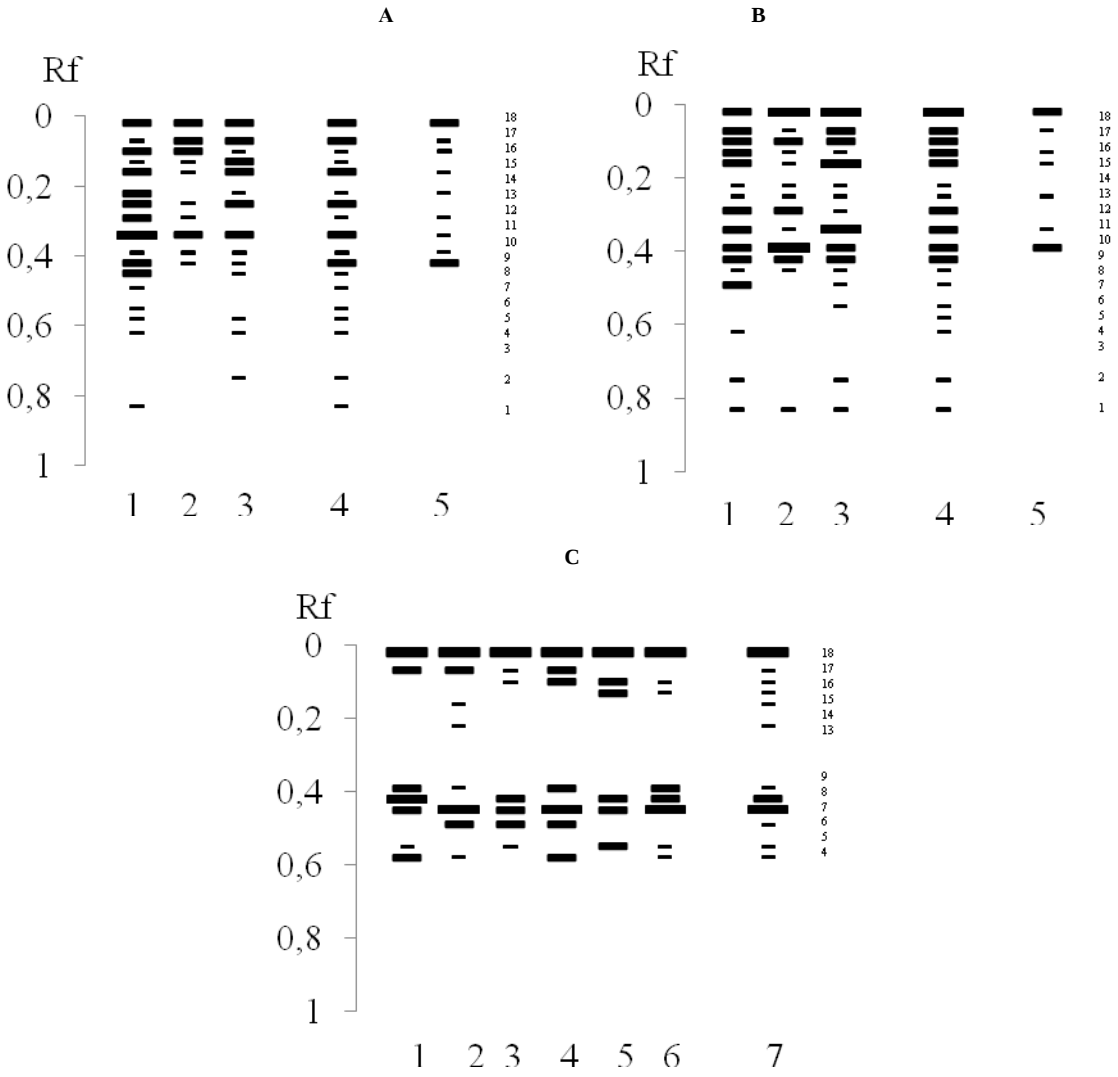


Fig. 2. Enzymogramm charts of the occurrence of peroxidase forms in early ripening cultivars of soybean seeds (A): 1 – Sonata, 2 – Zakat, 3 – Smena, 4 - on average for cultivars selected by soybean breeding Institute, 5 - Souer-4; mid ripening cultivars (B): 1 – VNIIS-1, 2 - October-70, 3 – Harmony, 4 - on average for cultivars selected by soybean breeding Institute, 5 – Luch Nadezhdy and lines of wild-growing (C) soybean: 1 - CA-1344, 2 - CA-1388, 3 - CB-104, 4 - CB-49, 5 - CZ-6316, 6 - CZ-6359, 7 - on average for the lines.

One should note the average rate of occurrence of forms P8-P10, P17-P18 in the seeds of the studied cultivars selected by Russian soy research Institute of Russian Academy of Agricultural Sciences. Souer-4 and Luch Nadezhdy soybean cultivars are characterized by the absence of forms with high electrophoretic mobility and a low rate of occurrence of other forms of peroxidase, which is associated with low activity of the enzyme in these cultivars and shows the inverse relationship of activity of peroxidases and catalases in soybean seeds [27].

Studies of wild-growing soybean seeds showed 12 peroxidase forms (Fig. 2C). Peroxidase forms P7 and P18 were detected in all lines of wild-growing soybean. However, forms of peroxidases with high electrophoretic mobility (P1-P3) and average electrophoretic mobility (P10-P12) are not detected. The highest number of peroxidase forms was found in lines CA-1388 and CZ-6359 (8 each), and the smallest - in line CZ-6316 (6). As shown on peroxidase enzymogramm charts P17 form was detected in lines CA-1344, CA-1388, CB-104 and CB-49. However in the lines obtained in the northern agro-climatic zone, CZ-6316 and CZ-6359, a less mobile form P15 was registered.

Low-mobile form P18 has a relatively high rate of occurrence in all the studied lines. Form P7 has either a high or medium rate of occurrence in wild-growing soybeans. It should be noted that each line of wild-growing soybean has either a relatively high rate of occurrence of forms P18 and P7, or an average rate of occurrence of P7, which is compensated for by the presence of P8 form. Forms P13 and P14 were identified only in line CA-1388 and have a low rate of occurrence of peroxidases.

In summary, a high number of peroxidase forms were found in soybean seeds, mainly with low and average electrophoretic mobility.

Acid phosphatases

13 forms of acid phosphatases (Fig. 3A, B) were identified in cultivated soybean seeds. All forms of the enzyme were registered in the seeds of Souer-4 cultivar. AP13 and AP12 forms with low electrophoretic mobility and KF6 with average electrophoretic mobility were identified annually.

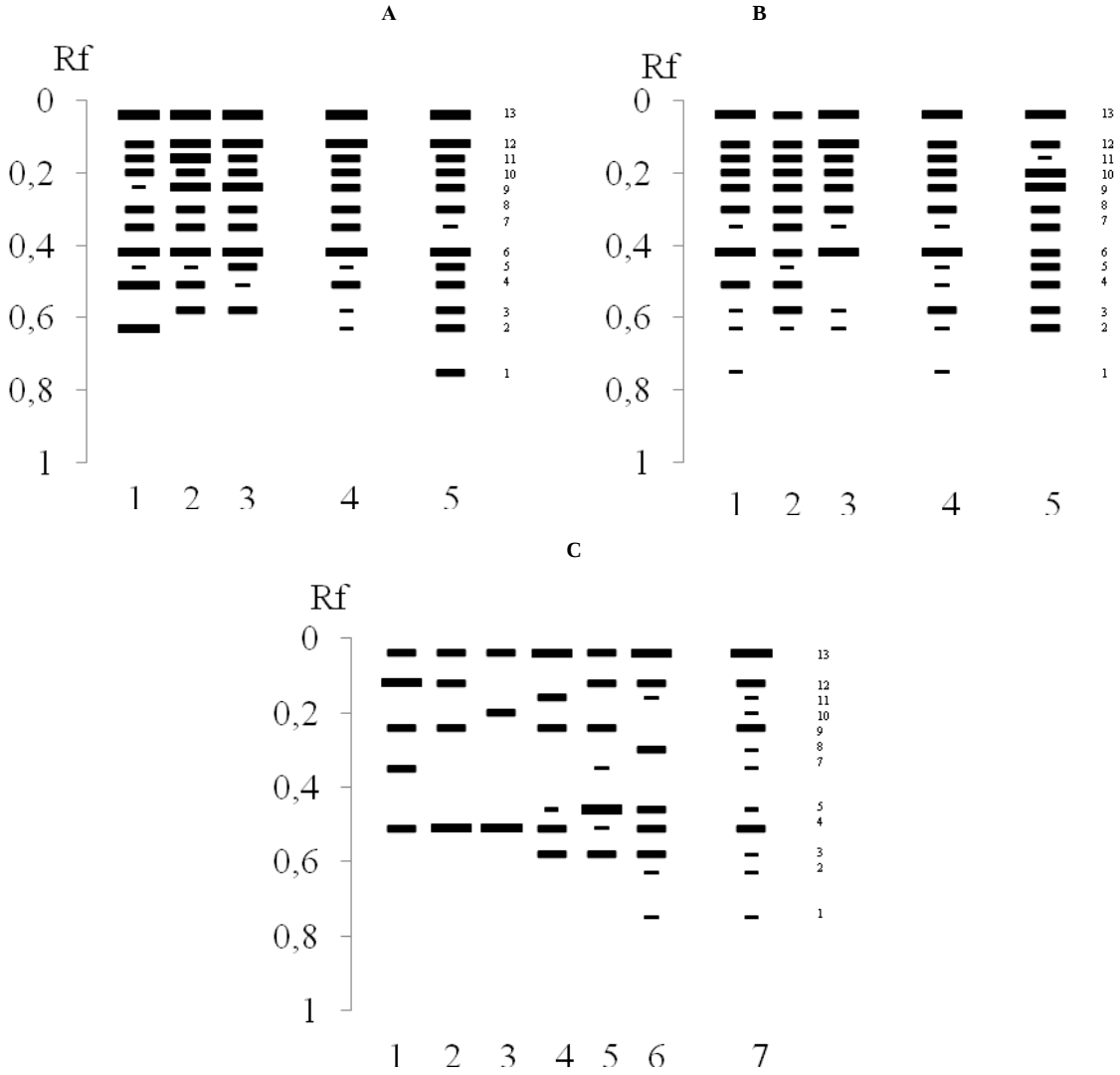


Fig. 3. Enzymogramm charts of the occurrence of acid phosphatase forms in early ripening cultivars of soybean seeds (A): 1 – Sonata, 2 – Zakat, 3 – Smena, 4 - on average for cultivars selected by Russian soy research Institute, 5 - Souer-4; mid ripening cultivars (B): 1 – VNIIS-1, 2 - October-70, 3 – Harmony, 4 - on average for cultivars selected by Russian soy research Institute, 5 – Luch Nadezhdy and lines of wild-growing (C) soybean: 1 - CA-1344, 2 - CA-1388, 3 - CB-104, 4 - CB-49, 5 - CZ-6316, 6 - CZ-6359, 7 - on average for the lines.

Intermediate forms AP7-AP11 with low electrophoretic mobility are also present in all cultivars tested, but with a lower rate of occurrence in different years. Forms of acid phosphatases AP1-AP5 with medium and high electrophoretic mobility are presented in negligible quantities. Cultivar differences were registered in the forms of the enzyme with low molecular mass. For example, C5 form was not identified in VNIIS-1 cultivar. Apart from AP1 form, AP3 form was not identified in the seeds of Sonata cultivar as well, AP2 – in Zakat and Smena cultivars, and AP5 – in Harmony cultivar. Forms AP13 and AP6 were characterized by a high rate of occurrence in all recognized cultivars under study.

Seeds of early ripening soybean varieties, except for Sonata cultivar, had increased content of AP12 form with low electrophoretic mobility. The average content of AP1 form with high electrophoretic mobility was found only in

seeds of Sauer-4 soybean cultivar, and very low content - in seeds of VNIIS-1 cultivar. It was found that early ripening varieties have a greater rate of occurrence of enzyme forms with high electrophoretic mobility as compared to mid ripening cultivars. Seeds of mid ripening cultivar Luch Nadezhdy differed significantly by multiple forms of acid phosphatases from the studied mid ripening varieties selected by Russian soy research Institute.

The study of enzymogramm charts of acid phosphatases of wild-growing soybean revealed 12 forms of the enzyme (Fig. 3C). AP 13 and AP4 were stable forms appearing in all the studied lines. The form with average electrophoretic mobility KF6, typical for all studied varieties of soybean was absent in wild-growing soybean. The largest number of forms of acid phosphatases (7, 9) was detected in the lines obtained from the northern agro-climatic zone (CZ-6316 and CZ-6359). Interestingly, no acid phosphatases with high electrophoretic mobility were found in the lines obtained from the southern agro-climatic zone (CA-1344, CA-1388). The lowest number of forms of the enzyme (3) was found in seeds of the line CB-104. Forms AP6-AP8 were not identified in lines CA-1388, CB-104, and CB-49.

If AP12 form is absent in seeds, then AP11 or AP10 are found which probably contribute to the adaptive capacity of wild-growing soybean

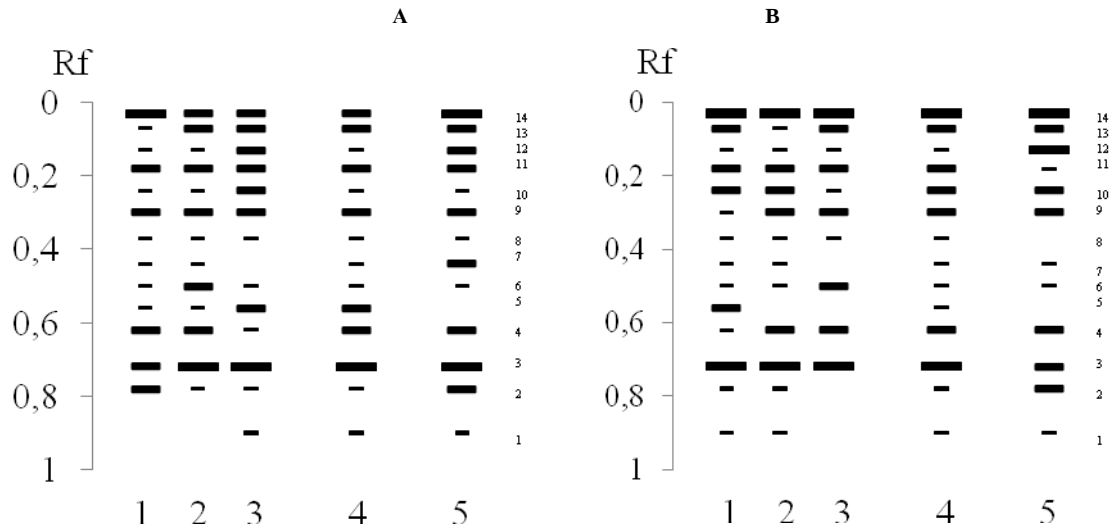
In each line of wild-growing soybeans there is only one form with a high rate of occurrence. In line CA-1344 it is AP12, in lines CA-1388 and CB-104 - AP4, and in line CB-49 - AP13. Forms AP1, AP2 and AP8 were not revealed, except in line CZ-6359, where a few were found. Form AP10 was present only in line CB-104 and it had an average rate of occurrence. Form AP9 had an average rate of occurrence in lines CA-1344, CA-1388, CB-49 and CZ-6316, and was absent in the rest of them.

In summary, forms of acid phosphatases with high and average electrophoretic mobility are mostly present in in soybean seeds.

Esterases

14 forms of esterases were identified in soybean seeds, differing in the rate of occurrence between the cultivars (Fig. 4A, B). From 10 forms (for Harmony cultivar) to 13 forms of the enzyme (for VNIIS-1 October-70 and Smena cultivars) were identified. 12 forms of esterases were found in each of the remaining cultivars. Form of the enzyme with low (E9, E12, E14), average (E7) and high (E3, E4) electrophoretic mobility were identified in all the studied varieties. Notably,

E14 form was found in all cultivars except Smena for all the years of research. Esterase form E3 was registered in soybean seeds of all varieties, while forms E5-E8 are not present in soybean seeds in some years of the study. It should be noted that the form of the enzyme with high electrophoretic mobility (E1) was identified only in soybean varieties October-70 and Luch Nadezhdy.



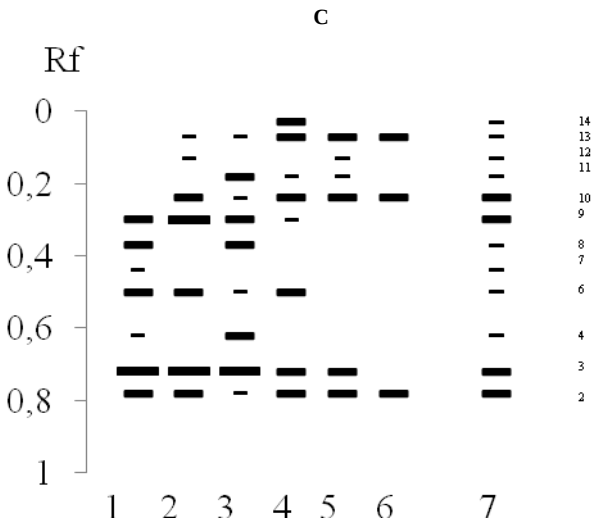


Fig. 4. Enzymogramm charts of the occurrence of esterase forms in early ripening cultivars of soybean seeds (A): 1 – Sonata, 2 – Zakat, 3 – Smena, 4 - on average for cultivars selected by Russian soy research Institute, 5 - Souer-4; mid ripening cultivars (B): 1 – VNIIS-1, 2 - October-70, 3 – Harmony, 4 - on average for cultivars selected by Russian soy research Institute, 5 – Luch Nadezhdy and lines of wild-growing (C) soybean: 1 - CA-1344, 2 - CA-1388, 3 - CB-104, 4 - CB-49, 5 - CZ-6316, 6 - CZ-6359, 7 - on average for the lines.

Forms E14 and E3 had a high rate of occurrence in mid ripening cultivars, excluding Luch Nadezhdy cultivar that was distinguished by high presence of E12 form (Fig. 4B).

It is necessary to mention the low occurrence rate of this form in early and mid ripening varieties selected by Russian soy research Institute. Forms E5-E8 with average electrophoretic mobility had a low rate of occurrence in all the studied mid ripening cultivars. It was observed that form E5 was not present in cultivars selected by Russian soy research Institute, and E8 – in Luch Nadezhdy cultivar. Forms E1 and E2 with low electrophoretic mobility had a low rate of occurrence both in mid and early ripening cultivars selected by Russian soy research Institute, while E2 form had an average rate of occurrence in varieties Souer-4 and Luch Nadezhdy.

The analysis of enzymogramm charts of esterase of wild-growing soybean revealed 12 forms of the enzyme (Fig. 4C). E2 and E3 were the two highly stable forms of esterases. However, only 3 forms of the enzyme E13, E10 and E2 were found in the line CZ-6359, where the stable form E2 is not present. No forms with low electrophoretic mobility were detected in line CA-1344, but an increased number of esterase forms with average electrophoretic mobility were registered. No esterase forms with average electrophoretic mobility were identified in wild-growing soybean obtained from the northern agro-climatic zone, which probably affected the overall decline in the number of forms of the enzyme, and, consequently, on the adaptive potential of lines CZ-6316 and CZ-6359. The greatest number of forms of esterases was found in the lines of wild-growing soybean obtained from the central agro-climatic zone CB-104 and CB-49 (8 and 9 forms, respectively). It is important to note the absence of forms of E1 and E5 which are typical for soybean.

The form with high electrophoretic mobility E1 was not detected in any of the test samples, and the form E2 was registered in all lines. However, it has a low or average rate of occurrence. E3 form has a high rate of occurrence in lines CA-1344, CA-1388 and CB-104, as well as E9 form - in line CA-1388. Interestingly, E4 form is found in lines CA-1344 and CB-104, E7 form - in line CA-1344, E8 form - in lines CA-1344 and CB-104, E12 form - in lines CA-1388 and CZ-6316, and E14 form - in CB-49. All of these forms have either average of low rate of occurrence. E10 and E13 forms were present in all lines of wild-growing soybean with a low rate of occurrence, with the exception of the line CA-1344.

In summary, forms E3, E9 and E10 are typical for all examined soybean varieties and lines. However, a large number of forms with low electrophoretic mobility were identified in cultivated soybean.

Amylases

Ten amylase forms were revealed in 8 cultivated soybean varieties under study (Fig. 5A, B). All forms of the enzyme were identified in soybean seeds of VNIIS-1, October-70 and Souer-4 cultivars. A1 form was identified only in three cultivars VNIIS-1, October 70, and Souer-4. Eight forms of amylases were registered in Luch

Nadezhdy cultivar. Besides A1 form, A6 form with average electrophoretic mobility was not identified in this cultivar as well.

A10 form with low electrophoretic mobility has a high rate of occurrence in early ripening cultivars, and A3 form with high electrophoretic mobility has a high rate of occurrence in mid ripening cultivars

High rate of occurrence of enzyme forms the with low electrophoretic mobility and the presence of one form (A3 or A2) with high electrophoretic mobility should be noted for soybean varieties Souer-4 and Luch Nadezhdy. A1 form is found only in mid ripening cultivars VNIIS-1, October-70 and Harmony. Amylase forms A4-A7 with average electrophoretic mobility were characterized by average or low rate of occurrence.

Now it can be seen that throughout the years of research of cultivars a minimal number of amylase forms were revealed for Luch Nadezhdy cultivar, and the maximum - for cultivars VNIIS-1 and Sonata.

In the study of enzymogramm charts of amylases of wild-growing soybean 9 forms of the enzyme were identified (Fig. 5C). It is shown that no forms with high electrophoretic mobility were identified for amylases. The least common form A1 is found in lines CB-104 and CZ-6316.

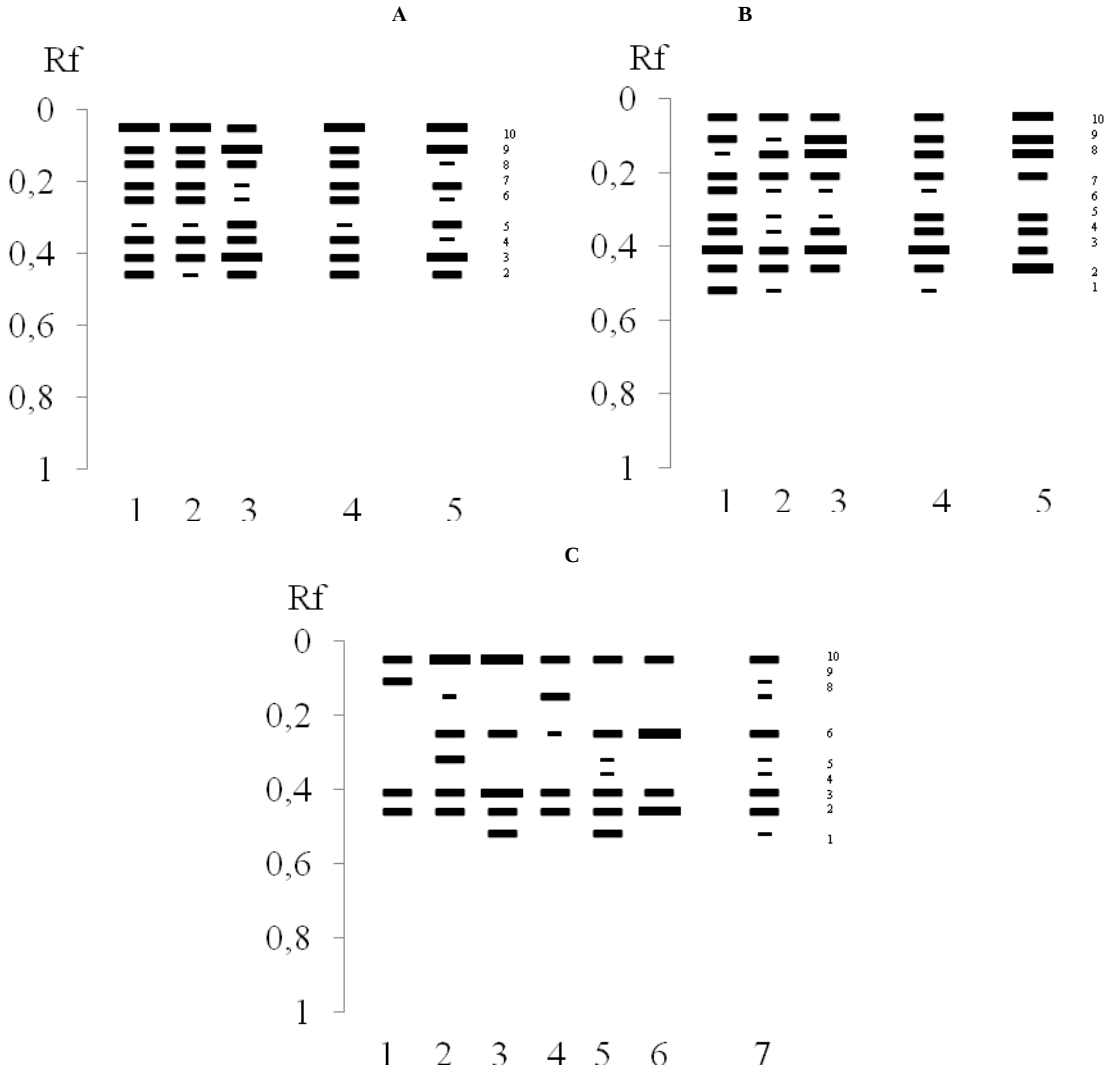


Fig. 5. Enzymogramm charts of the occurrence of amylase forms in early ripening cultivars of soybean seeds (A): 1 – Sonata, 2 – Zakat, 3 – Smena, 4 - on average for cultivars selected by Russian soy research Institute, 5 - Souer-4; mid ripening cultivars (B): 1 – VNIIS-1, 2 - October-70, 3 – Harmony, 4 - on average for cultivars selected by Russian soy research Institute, 5 – Luch Nadezhdy and lines of wild-growing (C) soybean: 1 - CA-1344, 2 - CA-1388, 3 - CB-104, 4 - CB-49, 5 - CZ-6316, 6 - CZ-6359, 7 - on average for the lines.

Forms with average electrophoretic mobility A3 and A2, as well as low mobile forms A10 and A6 are the stable ones. However, forms of amylases from A4 to A8, including A6 form, were absent in line CA-1344. The largest number of forms of amylases (7) was registered in line CZ-6316, and the lowest (4) - in lines CA-1344 and CZ-6359.

A10 form had a high rate of occurrence in lines CA-1388 and CB-104, A3 form - in line CB-104, and A2 form - in line CZ-6359. Form A6 had a high rate of occurrence only in line CZ-6359. It's worth noting the absence of A7 form. Interestingly, forms A4, A5 and A8 and A9 were not present in most cases, except line CZ-6316, where A4 and A5 had a low rate of occurrence; line CA-1344, where the A9 form had an average rate of occurrence; and lines CA-1388 and CB-49, where A8 form had a low rate of occurrence. It was found that forms A2, A3 and A10 had the highest rate of occurrence in all the tested samples of wild-growing soybean.

In summary, the research has shown that early ripening varieties Smena, Sonata and Zakat selected by Russian soy research Institute and cultivar Souer-4 had a higher occurrence of the studied enzyme forms except for peroxidases. A large number of enzyme forms of early ripening cultivars of soybean leads to the increase of metabolic processes that probably contributes to early ripening of seeds. It is revealed that Souer-4 and Luch Nadezhdy cultivars were different in composition and occurrence of forms of the studied enzymes from other studied varieties selected by Russian soy research Institute, which, apparently, are very close genetically.

The analysis Enzymogramm charts of C, P, AP, E and A of wild-growing soybean revealed that line CZ-6316 has an increased adaptive capacity, because it has high heterogeneity of the studied enzymes, while a low number of multiple forms of peroxidases indicates improved taste of the line. In connection with the above, we can recommend this line of wild-growing soybean to be introduced into the culture of dominant genes in the selection process.

Bioinformatics research

To check the number of isoforms of the studied catalases, peroxidases, acid phosphatases, esterases and amylases we conducted a computer search of nucleotides that may be responsible for the expression of these enzymes in the soybean genome (Table. 1).

Table 1Homologues of enzymes adaptation in soy genome

	Total number of homologues	Homology (%) / E value	Genes identified	Tentative genes
Catalase	7	86-100/0.0	7	-
Peroxidases:				
Glutathione peroxidase	18	36-100/7e^{-145}-0.003	3	15
Peroxidase	182	48-100/0.0-0.64	21	161
Ascorbateperoxidase	43	38-100/0.0-6.4	21	13
Phosphatases:				
Acid phosphatase	22	24-100/0.0-7.9	2	20
Phytase	45	22-100/0.0-0.001	2	43
Purple acid phosphatase	37	22-100/0.0-0.043	4	32
Pectinesterase	49	25-100/ 0.0-7.4	-	49
Amylases:				
Alpha-amylase	19	26-99/0.0-5.3	1	18
Beta-amylase	24	36-100/0.0-6e^{-34}	7	17

In silico data generally correlate with the results of biochemistry. The low number of isoforms of esterases and acid phosphatases may be explained by the fact that the gene information has not been marked up yet in the sequence database used in the research.

CONCLUSION

In conclusion, it was found that seeds of cultivated soybean selected by Russian soy research Institute have similar electrophoretic spectra of the studied enzymes. Forms with the high occurrence rate of the studied hydrolases were most commonly found in the seeds of the studied soybean cultivars. And only two forms of oxidoreductases with a high rate of occurrence were found: P18 and C12. Forms AP13, P18 and P7 were identified in all the samples of wild-growing soybean seeds, which may characterize the stability of the soybean genome.

No common forms of peroxidases were identified in cultivated soybean seed of other selections studied indicating the good taste of these soybean varieties. However, forms C8 or CK2 are frequently encountered which apparently are responsible for molecular mechanisms of adaptation, which is important for adaptive selection. Hydrolases of

these soybean seeds are quite varied, and they are represented mostly by forms with high and average rates of occurrence, which is important for enhancement of biochemical processes in soybeans.

A previously identified regularity between peroxidases and catalases was revealed in early and mid ripening cultivars: many forms of peroxidases with high and average rate of occurrence were revealed in mid ripening cultivars, and catalases were mainly represented by medium and rare occurring forms. An inverse relationship is determined in seeds of early ripening cultivars. Hydrolase form A10 with a high occurrence rate was identified in early ripening varieties of soybean selected by Russian soy research Institute; A3 and E14 were identified in mid ripening varieties. Early ripening cultivars are characterized by a large number of forms of acid phosphatases with high and average rates of occurrence. However, the heterogeneity of acid phosphatases in mid ripening cultivars selected by Russian soy research Institute is higher than that in early ripening cultivars.

In our view, characterization of soybean varieties by multiple forms of enzymes will allow to create a catalog of soybean varieties, indicating the forms of enzymes that can be used in selection to improve the methods of creating adaptive soybean varieties.

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