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The role of enzymes in the adaptation of soybean of different philogenetic origin to growing conditions

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ABSTRACT

For the first time, the relation between the variety of enzyme forms and different growing conditions of soybeans is studied. Data on the obtained electrophoretic spectra of catalase, peroxidase, acid phosphatase, esterase and amylase of wild and cultivated soybean seeds were analyzed in detail and systematized. It was found that wild soybean has a high adaptive capacity and is characterized by high specific activity and a small number of studied enzyme forms. Low specific activity of soybean enzymes is compensated by a larger number of their forms, which increases the resistance of soybean in different growing conditions. Adaptive changes in specific activity and the number of enzyme forms under the influence of growing conditions cause changes in biometric and biochemical parameters of soybean of different phylogenetic origin. It is shown that the specific activity and multiple forms of catalases, peroxidases, acid phosphatases, esterases and amylases of soybean seeds can be used as markers of its adaptation to different growing conditions.

Keywords: *Glycinemax*, *Glycinesoja*, catalase, peroxidase,acid phosphatase, amylase,esterase, specific activity, plural enzymesforms,adaptation.

INTRODUCTION

The study of adaptation mechanisms of various organisms to the environment is a priority area of modern biological research. The general laws of the biochemical adaptation strategy of animals are described in a number of papers [1-3], but for plants, especially cultural ones, such generalizing studies are not available. The leading role in the maintenance of intracellular homeostasis and adaptation to stressors is played by enzymes [4].

Plural enzyme forms, including genetically determined isozymes, are of particular importance for the regulation of organism activity [5-8]. From the standpoint of genetics and selection, the importance of studying the polymorphism of enzyme systems lies in the fact that isozymes are effective genetic markers [9-13]. Using the information on isozymes, breeders are currently solving many theoretical and practical problems of selection [14-16]. The responsiveness of enzyme systems to changes in environmental conditions is repeatedly noted in the literature [1; 17-22].

In recent years, particular attention is paid to the study of the soybean genome [23-25], and polymorphism of proteins including enzymes [26-30]. Expanding and generalizing the factual material about the plural forms of soybean enzymes may contribute to studying the adaptation mechanisms of this exceptionally valuable plant species. Soybean (*Glycine max (L.) Merrill*) is a unique alternative source of protein and a cost-effective industrial crop. The Far East is a major soybean producer in Russia, but only 20% of the country's demand is supplied by the domestic

soybean, while its global production is rapidly increasing. The Amur region produces 60% of all soybeans in Russia. Acreage expansion is attributed not only to an increase in interest in soybean as a valuable high-protein, forage and food crop, but also to the favorable soil and climatic conditions for its cultivation [31-33].

There is also the northernmost geographical distribution of wild soybean (*Glycine soja Siebold & Zucc*) which has a high adaptive capacity [34] and is the source of numerous genes that are not found in the genome of cultivated soybean [35].

Studying the introduction of soybean in our country, the breeders focus on the agronomic characteristics. At the same time, little attention is given to the study of biologically active compounds of soybean [36] and enzymes that are involved in plant adaptation to environmental conditions [28].

The enzyme system including catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) is an indicator of the stability of the cultivar. Their role in the protection of plants (antipathogenic actions against viruses and bacteria, cold stress, various environmental pollutants) is well known. Amylases (EC 3.2.1.), esterases (EC 3.1.1) and acid phosphatase (EC 3.1.3.11) are the most important enzymes of major metabolic pathways of living systems. However, the study on the specific activity and the multiple enzyme forms of wild soybeans during the adaptation process is not paid enough attention.

The objective of the paper is to evaluate the possibility of using enzymes as markers of adaptation of soybean of different phylogenetic origin to growing conditions.

MATERIALS AND METHODS

Material and cultivation conditions

The material for the study were 33 accessions of soybean (*Glycine max (L.) Merrill, 1917*) of various ecological and geographical origin (from the Amur, Volgograd, Voronezh, Omsk, Ryazan, Saratov regions, the Khabarovsk and Krasnodar Territories of Russia, as well as from Belarus, Bulgaria Canada, China, Poland, USA, Ukraine, Czech Republic and Sweden) obtained from the Federal Research Center «The N.I. Vavilov All-Russian Institute of Plant Genetic Resourses» (St. Petersburg). The collection was grown in 2000, 2001 and 2002 at the experimental field of Far Eastern State Agrarian University (southern agro-climatic zone). Forms of wild soybean (*Glycine soja Siebold & Zucc., 1845*) obtained from the three agro-climatic zones of the Amur region were cultivated in the same period in Sadovy settlement, Tambovskyarea, Amur region (southern agro-climatic zone)at the experimental field of All-Russian Research Institute of Soybean.

Meteorological conditions during the study differed from the mean annual temperature and precipitation (Fig. 1).

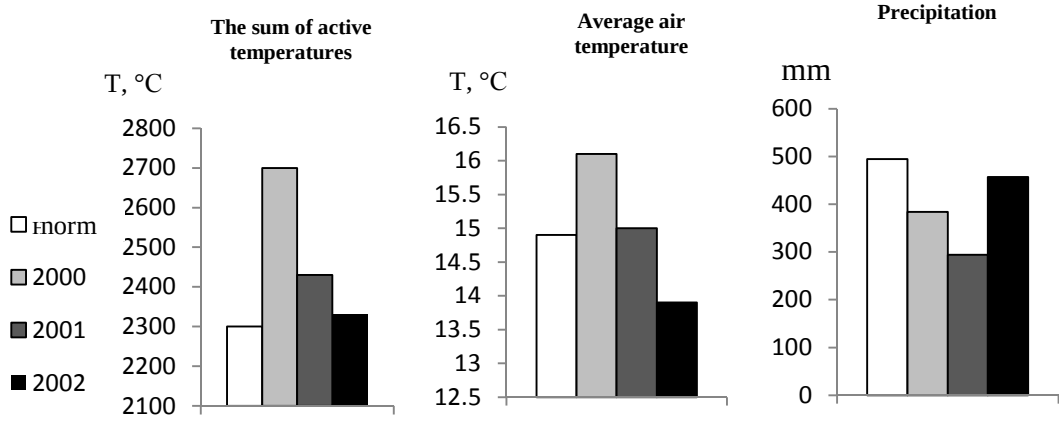

Fig. 1. The sum of active temperatures, average air temperature and precipitation in 2000-2002

In 2000, it was hot during the soybean development period. The air temperature was above the norm by 1.2 °C. The sum of active temperatures was above the long-term average annual data by 465 °C. Lack of moisture marks the first half of summer and September. And only during the third decade of July and August, a significant amount of precipitation is recorded, which improved the condition of soybeans during the pod formation. The temperature conditions in 2001 matched the long-term averages for the cultivation of soybeans in the Amur region. The sum of

active temperatures was slightly above the norm. Precipitation had a significant impact on the growth and development of soybean that year. In June-September, there was 60% of the norm.
The vegetation period of 2002 was more favorable for the growth and development of soybean. Only in the first half of the growing season the temperature was below the long-term average. There were minor rainfalls during the pod formation and seed ripening period.

Thus, it is shown that the limiting factor during the study was temperature.

Research methods
Field experiments were performed in the breeding nursery according to the method of studying the collection samples. The selection of samples for analyzes was performed according to State methods of crop variety testing.

We investigated the biometric (the number and weight of seeds per plant, weight of 1000 seeds) and biochemical indices of soybean of different phylogenetic origin (the amount of protein, oil and higher fatty acids in seed oils, specific activity and electrophoretic spectra of enzymes).

Protein and oil content, as well as their qualitative composition were determined by chromatography using Nir-42 scanner in the laboratory of All-Russian Research Institute of Soybean. For enzymatic analysis protein extracts were prepared from the test material (500 mg), in which protein was determined according to Lowry [37]. Enzyme activity was determined by the appropriate standard methods: catalase by the gasometric method [38], peroxidase according to Boyarkin [39], esterase activity according to Van Aspern[40], acid phosphatase and amylase by the photocolorimetry method [38]. Reagents by Reanal (Hungary), Sigma (USA), Fluka (Belgium), Panreac (Spain), and Merck (Germany). The specific enzyme activity was calculated as units per mg protein. Plural enzyme forms were detected by electrophoresis on the 7.5% columns of polyacrylamide gel on the Bio-Red instrument (USA). Determination of enzymatic activity zones (enzyme forms) in gel was carried out using appropriate histochemical methods [41; 42]. Since the standard criterion for the characterization of pluralenzyme forms is their relative electrophoretic mobility (Rf), the difference in the quality of soybean cultivars was evaluated by identified enzyme forms according to their Rf. The numbering of forms is given from the high-mobile (to anode) forms to the low-mobile forms. 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, A1-A10 for amylase). Additional rare forms were designated with «*» [43]. Areas of activity identified on enzymogramms (plural forms) were divided into 3 groups by the rate of occurrence (0-19% – low occurrence; 20-49% –average occurrence;>50%– a high occurrence of forms).

Biochemical studies were conducted in six analytical replicates. Statistic processing and calculation of correlation coefficients were carried out according to the methods of N.A. Plokhinsky.

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

RESULTS AND DISCUSSION

We have previously shown that the biochemical analysis of seeds of soybean cultivars zoned in the Amur region reveals changing the enzymatic activity of soybean seeds, depending on weather conditions, growing seasons and ecological conditions in the cultivation area. This is an indication of adaptive responses to different climatic conditions which, in its turn, leads to quantitative changes in antioxidants (carotene, ascorbic acid), minor constituents, oils, unsaturated higher fatty acids (oleic, linolenic acids), as well as yielding capacity [44]. Also, we have systematized data on electrophoretic spectra (multiple forms), catalase, peroxidase, acid phosphatase, esterase and amylase of soybean seeds of different phylogenetic origin, which differ in cultivars and lines [45].

Under natural conditions, morphological and biochemical parameters of wild soybean are largely influenced by environmental factors. Peroxidase and catalase enzymes are important anti-oxidants. We found increased activity of these enzymes in the seeds of wild soybean in comparison with the cultivated one, which is apparently due to the higher adaptive potential of wild soybean. Study of the activity of catalase and peroxidase in wild soybean seeds showed that the peroxidase activity increased, while the catalase activity decreased (Table. 1),which corresponds with the previously set data proving the inverse relationship in the activity of these enzymes in soybean seeds [46].

Table 1.Biochemical composition and morphological parameters of wild (A) and cultivated (B) soybean seeds grown in 2000-2002.

Feature	2000		2001		2002	
	A	B	A	B	A	B
Catalase (u/mgproteinx 10^{-3})	229	152	166	135	104	91
Peroxidase (u/mgprotein)	33.8	11	466	300	877	260
Acid phosphatase (u/mgproteinx 10^{-6})	246	174	146	188	159	255
Esterase (u/mgproteinx 10^{-3})	238	290	125	120	84	250
Amylase (u/mgprotein)	9.8	4.2	2.3	4.6	2.1	0.9
Protein, %	48.9	39.3	47.4	41.2	50.4	40.2
Oil,%	10.3	18.2	11.1	18.9	9.5	20.3
Oleic acid,%	-	12.4	16.4	13.5	14.6	12.4
Linolenicacid,%	-	10.2	16.5	9.0	7.1	7.7
Number of seeds per plant	608	48	1069	49	793	40
Weight of seeds per plant, g	19.8	6.5	33.5	5.9	22.6	5.9
Weight of 1000 seeds, g	32.7	132	29.8	120	29.5	147

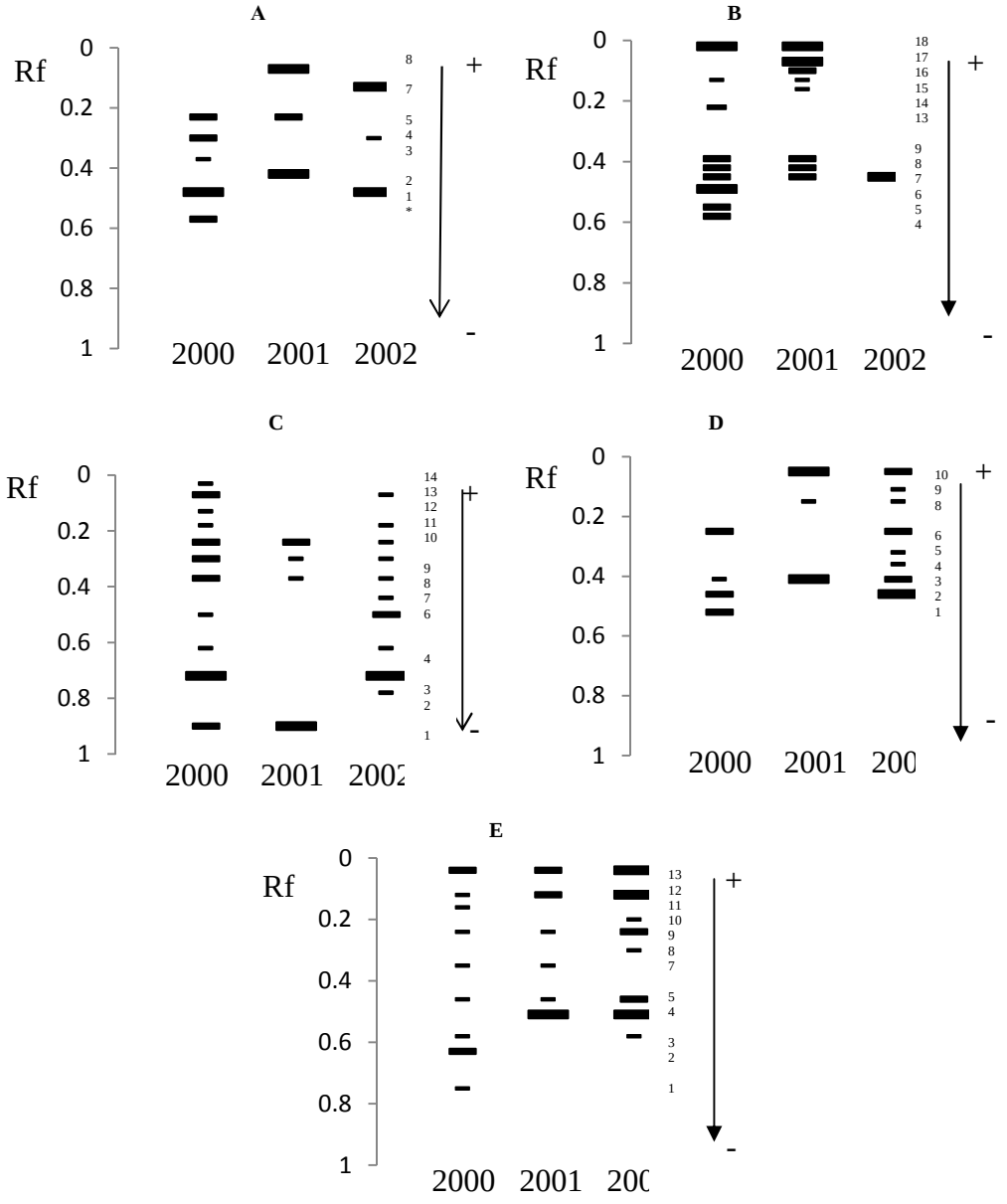

Fig. 2.Enzymogrammchartsof catalases (A), peroxidases (B), esterases (C), amylases (D) and acid phosphatases (E), found in wild soybeanseeds obtained in 2000, 2001 and 2002. The arrow shows the direction of electrophoresis from the anode to the cathode (0-19% – low occurrence; 0-49% –average occurrence; >50%– a high occurrence of forms)

The seeds of wild soybean and cultivated soybeans, obtained under the conditions of 2000, when the growing season was marked by high temperatures and the seed ripening period was marked by a large amount of precipitation, catalase activity was stable and high, which correlates with the maximum number of forms (five and eight respectively) of this enzyme (Figure 1A, 2A). For the cultivars of cultivated soybean, six enzyme forms were identified with high (C2 and C3) and average rate of occurrence (C1, C4, C5, C6). In the seeds of wild soybean, four catalase forms were identified with high (C1) and average rate of occurrence (C1*, C4 and C5), among which that year we found a more mobile additional rare form C1*.That year peroxidase activity of soybean seeds was characterized by a low number of forms with average rate of occurrence (P6-P8), which corresponds to a low specific activity of the enzyme (Fig. 3B, Table 1). The seeds of wild soybeancontained6 forms of peroxidases with average rate of occurrence (P4, P5, P7-P9) and 2 forms with high rate of occurrence (P6 and P18), having a minimum specific activity of the enzyme (Fig. 2B, Table1).

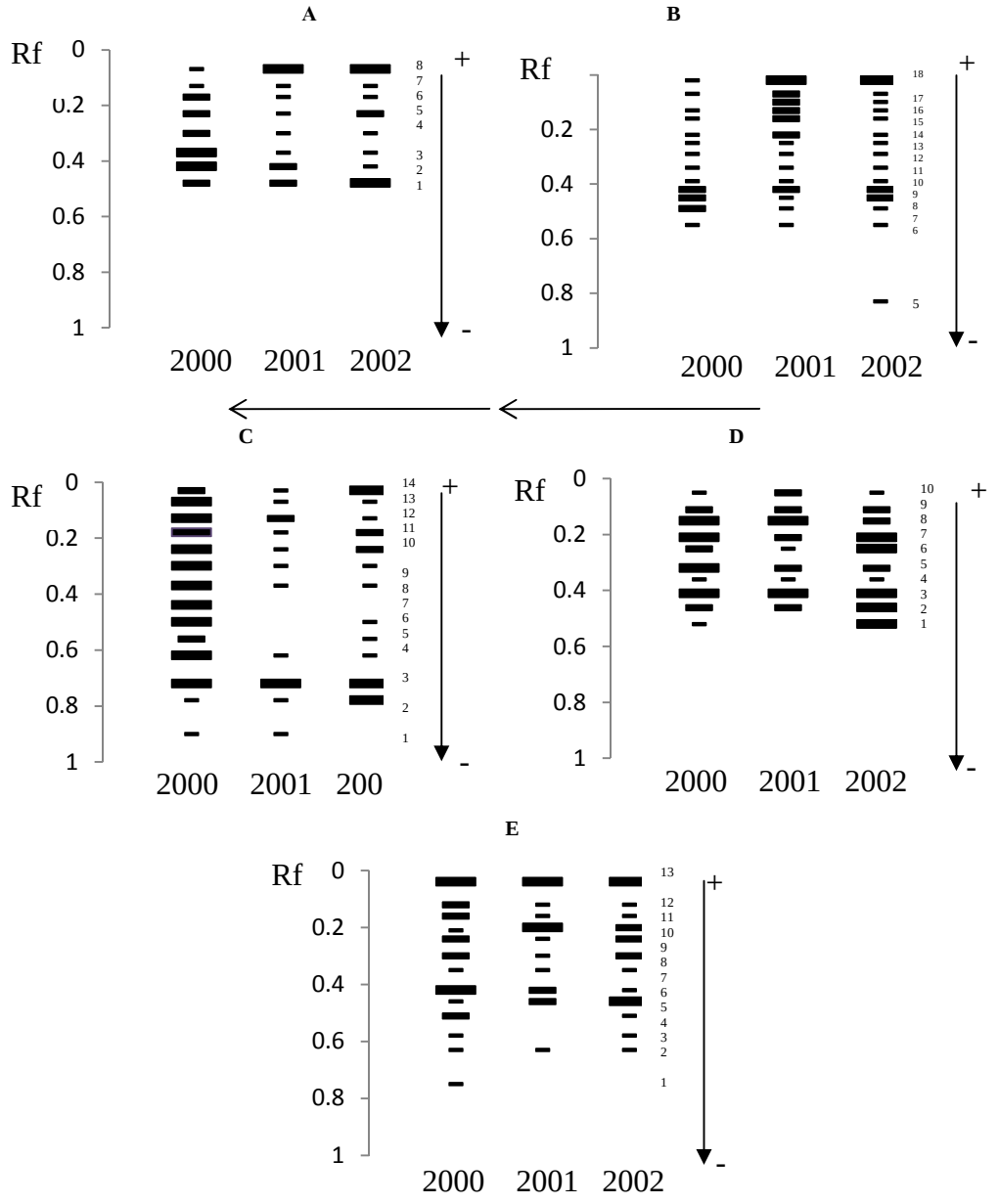

Fig. 3.Enzymogrammcharts of catalases (A), peroxidases (B), esterases (C), amylases (D) and acid phosphatases (E), found in soybean seeds of various ecological and geographical origin obtained in 2000, 2001 and 2002. The arrow shows the direction of electrophoresis from the anode to the cathode (0-19% – low occurrence; 0-49% –average occurrence; >50%– a high occurrence of forms).

In 2001, soybean seeds obtained in a dry summer, with a lack of moisture at the beginning of the growing season and during the pod formation period, had a greater specific activity of peroxidase and, as a consequence, the number of enzyme forms increased to 8 in the seeds of wild soybean and to 14 in cultivated ones. The specific activity of catalase decreased, with 6 forms of average to high rate of occurrence in the seeds of wild soybean(P7-P9 and P16-

P18), and 7 forms in cultivated ones (P8, P13-P18). The number of catalase forms decreased to 3 with average and high rate of occurrence (C1, C2, C8 in cultivated one and C2, C5, C8 in wild one).

In the more favorable weather conditions in 2002, there was a minimum catalase activity in the obtained soybean seeds of different phylogenetic origin. The seeds of wild soybean revealed only two forms of catalases (C2 and C8), while the cultivated one had three forms (C1, C5 and C8) with average and high rate of occurrence. The specific activity of peroxidases in the seeds of wild soybean was maximum that year, but only one form of the enzyme with a high rate of occurrence (P7)was found. The seeds of some cultivated soybean cultivars and accessions (Ke 73042, Lambert (USA), 76-11 (Canada), No. 4888 (Poland), Omsk 4 (Russia)) had a low specific activity of peroxidase, indicating their good tastiness and ability to be used in the selection process. This fact is reflected in the general peroxidase specific activity of cultivated soybean seeds in 2002 year, which was lower than that of wild soybean(Table 1). Also, in cultivated soybean we noted a high number of plural forms of peroxidase (15), including one with a high (P18) and two (P7, P8) with an average rate of occurrence.

The results obtained for the antioxidant enzyme activity suggest that the increased temperature enhances the metabolic processes in soybeans and clears the plant from the harmful hydrogen peroxide.

The rate of biochemical processes in soybean seeds depends on the level of hydrolytic enzyme activity which is characterized by broad substrate specificity. We have previously studied the electrophoretic spectra of ribonuclease, amylase and esterase in the collection of seeds of 15 soybean cultivars of different ecological and geographical origin, grown in different agro-climatic zones (Amur and Moscow regions). It is shown that soybean cultivars grown in the conditions of the Amur region have a high enzyme heterogeneity of hydrolase complex [47].

The activity of acid phosphatase, esterase and amylase of soybeans also varied throughout the years of study, as well as oxidoreductases (Table 1, Fig. 1, 2).

The soybean seeds of different phylogenetic origin obtained in hot summer (2000) showed a high specific activity of esterase and an increased number of enzyme forms (Table 1, Fig. 2C, 3C). This indicates an increase in the metabolic processes under stress. The seeds of cultivated soybean show the maximum number of esterase forms (10) with a high rate of occurrence (E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13). This year is particularly interesting in that the seeds of cultivated soybean had additional forms (E4*, E6*-E9*) with an average electrophoretic mobility, which increased the number of esterase forms in the seeds of some cultivars up to 17. A small number of esterase forms were found in the cultivars with the parentage containing wild soybeans. In 2001, in drought conditions, we obtained soybean seeds having a average specific activity of the esterase, which had only two enzyme forms with a high and average rate of occurrence. Wild soybean revealed forms E2 and E10, and cultivated soybeans E3 and E12. We should note the absence of forms with a average electrophoretic mobility (E5-E7), which appear at high and low temperatures. This observation is confirmed by the fact that in 2002, when the mean annual temperature decreased by 1°C, soybean seeds of different phylogenetic origin had E5-E7forms, with E6 form in the seeds of wild soybean having an average rate of occurrence. It should be noted that this year showed some forms with high rate of occurrence: E3 in wild soybean seeds, E2, E3, E14incultivated soybean seeds. These facts relate to the valuesof the specific activity of esterase: it is minimal in wild soybean seeds, and high in cultivated soybean seeds.

The analysis of enzimogrammcharts of amylase in soybean seeds of different phylogenetic origin revealed significant differences in the spectrum of pluralenzyme forms during the studied period (Fig. 2D, 3D). The year 2000 was marked by a temperature increase of 1.3 °C, and high values of specific activity of the enzyme (Table 1). Moreover, in wild soybean seeds it was more than 2-fold higher than in cultivated soybean seeds. Interestingly, the seeds of wild soybean had only three forms (A1, A2, A6) with an average rate of occurrence and no forms of amylase with low electrophoretic mobility (A7-A10). However, the seeds of cultivated soybean contained all known amylase forms of soybeans. Four of them (A3, A5, A7, A8) had a high rate of occurrence. In 2001, which was characterized by a significant decrease in precipitation (by 200 mm) compared to the long-term averages, the specific activity of amylase was different: maximum in cultivated soybean seeds, and low in wild soybean seeds. The seeds of wild soybean revealed forms A3 and A10 with a high rate of occurrence and form A8 with a low one. Cultivated soybean seeds had two forms with high rate of occurrence (A3 and A8) and five forms with average one (A2, A5, A7, A9, A10). We should note the identity of the revealed forms of amylase in the phylogeny, which may have formed in the harsh climatic growing conditions. In 2002, the samples of soybean seeds of different phylogenetic origin had low (in wild soybean) and minimum (in cultivated soybeans) specific activity of amylase. However, the analysis of enzymogrammcharts of this enzyme allowed to find out an increase in plural forms (to 8 in wild soybean seeds, and 10 in cultivated ones), which apparently enhances glucose metabolism at low temperatures. It should be noted that the seeds of wild soybean had one form (A2) with high rate of occurrence and three forms

(A3, A6, A10) with average one, while the cultivated ones had five forms (A1, A2, A3, A6, A7) with high rate of occurrenceand three forms (A5, A8, A9) with average one.

In 2000, soybean seeds are characterized by high heterogeneity of acid phosphatases, particularly in the seeds of cultivated soybean, which had forms AP4, AP6, AP8, AP9, AP11, AP12 and AP13 with high and average rate of occurrence(Fig. 3E) and average specific activity of this enzyme (Table. 1, Fig. 2E, 3E). Wild soybean seeds which hadhigh specific activity of acid phosphatase showed only two forms with average rate of occurrence(AP3, AP13). Lack of precipitation in 2001 caused a decrease in enzymatic activity: the values of the specific activity of acid phosphatase were average, the number of pluralenzyme forms decreased. The seeds of wild soybean had one form with high rate of occurrence(AP4) and two forms with average one (AP12, AP13), while the seeds of cultivated soybean had two forms with high rate of occurrence(AP10, AP13) and two forms with average one (AP5, AP6). In the conditions of the growing season of 2002, the seeds of cultivated and wild soybean had five forms of acid phosphatases with high and average rate of occurrence: AP5, AP8, AP9, AP10, AP13 in the cultivated one; AP4, AP5, AP9, AP12, AP13 in the wild one. That year's specific activity of the enzyme differed significantly: for wild soybean it was average, whereas for cultivated one it was maximum.

Thus, soybean seeds obtained in 2000, when it was hot during the growing season, are marked by high specific activity of the studied hydrolases in wild soybean seeds (Table 1) and an increased number of their forms in the seeds of cultivated soybean. The smallest number of hydrolase forms was found in 2001. In 2001-2002 there wereminor differences in the specific activity of amylase and acid phosphatase.

The analysis of enzymogrammcharts of soybean seeds of different phylogenetic origin revealed a high occurrence of forms of catalases, peroxidases, esterases, amylases and acid phosphatases depending on the weather conditions for growing soybeans(Table. 2).

Table 2.The occurrence of forms of catalases, peroxidases, esterases, amylases and acid phosphatases depending on the weather conditions for growing soybeans

Enzymes		**1**	**2**	**3**	**4**
Catalases	W	C1	C2; C8	C1; C7	
	C	C2; C3	C8	C8	C1
Peroxidases	W	P6; P18	P17; P18		P7
	C		P18	P18	
Esterases	W	E3	E1	E3	
	C	E4; E6-E13		E2; E12	E3
Amylases	W		A3; A10	A2	
	C	A5		A1; A6	A2; A3; A7; A8
Acid phosphatases	W		AP4	AP4; AP12	AP13
	C	AP6	AP10	AP5	AP13

Note: 1– high temperature and low precipitation; 2 – normal temperature and drought; 3 – low temperature and normal precipitation; 4– occur in all conditions; W–wild soybean; C – cultivated soybeans.

For wild soybean only two enzymes were revealed whose forms (P7 and AP13) are found in all growing conditions. Interestingly, in wild soybeans forms C1 and E3 occur at high and low temperatures and forms A3 and A2 occur in drought and low temperatures, while in cultivated soybean they are found under all cultivation conditions.

Analyzing the results of the biochemical and morphometric parameters (Table 1), it should be noted that the weather conditions during the growing season (temperature increase by 1.3 °C or the lack of moisture by 60%) cause changes in the biochemical composition of seeds and, as a result, in the yield structure. For cultivated soybean, high temperatures during the growing season increase linolenic acid content and seed weight per plant, but reduce protein and oil content. For cultivated soybean moisture deficit increased the content of oleic acid and protein, as well as the number of seeds per plant, and reduced the weight of 1000 seeds and the content of linolenic acid. Comparing the results of studies on wild soybean according to agronomic and morphological characteristics we have found that in 2002 the seeds accumulated the maximum amount of protein and minimum of oil. The maximum number of seeds having a high weight per plant, oleic and linoleic acid content in the seeds of wild soybean were significantly higher in 2001, which is apparently related to dry weather conditions.

CONCLUSION

As a result of three years of research on electrophoretic spectra of enzymes of soybean seeds of different phylogenetic origin, we identified 8 forms of catalases, 14 forms of peroxidases and esterases, 10 forms of amylases, 13 forms of acid phosphatases. In general, the results of research for the first time allowed us to characterize the

electrophoretic spectra of soybean enzymes and determine the effect of climatic growing conditions on their variability.

For the first time it is found that the activity of catalases, peroxidases, acid phosphatases, esterases and amylases may be an indicator of soybean adaptive capabilities. Stable specific activity and number of enzyme forms or their increase in various growing conditions indicate a high adaptive capacity of soybeans. It was found that wild soybean, having a high adaptive capacity, is characterized by high specific activity and a small number of observed enzyme forms. Low specific activity of the enzymes of cultivated soybean is compensated by an increase in the number of forms, which increases the resistance of soybean in different growing conditions. Thus, it is shown that the adaptive capacity of wild soybeanis determined by the high specific activity of enzymes in the seeds, while in cultivated soybeans it is determined by the increased number of enzyme forms.

It was found that different weather conditions of growing soybean seeds of different phylogenetic origin are marked by significant changes in the activity and the number of forms of the enzymes, which affected the biochemical composition of seeds.

Thus, for the first time we carried out a comprehensive approach to the study of the role of enzymes in the process of adaptation of soybean of different phylogenetic origin and found that the electrophoretic spectra of seed enzymes can be used as markers of soybean cultivars adapting to different growing conditions.

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