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Chemical Composition of Seeds in Soybean *Glycine soja* (Fabaceae) of Amur Oblast

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Abstract—The wild soybean *Glycine soja* Sieb. et Zucc. is an ancestor of the cultivated soybean *Glycine max* (L.) Merr. and a source of many valuable genes missing in the *G. max* genome, including genes that determine stress resistance to adverse environmental factors. Biochemical parameters (protein, oil, ascorbic acid, carotene, higher fatty acids, and specific activities and multiple forms of enzymes of the oxidoreductase and hydrolase classes) were studied in five *G. soja* accessions from the collection of the All-Russian Institute of Soybean (KA-1413, KA-342, KBl-29, KBl-24, and Kel-72). The accessions provide unique natural gene banks. Wild seeds were collected in three districts (Arkharinskii, Blagoveshchensk, and Belogorskii) of Amur Oblast. Based on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), ribonuclease (RNase), acid phosphatase, esterase, and amylase (AML) activities and biochemical parameters of seeds, the *G. soja* accession KA-1413 was found to have higher contents of protein, oleic acid, and linolenic acid; a lower polyphenol oxidase specific activity; and higher activities of SODs, esterases, and RNases. The accession KA-1413 was therefore recommended to use as a source of dominant genes in breeding to increase the adaptive potential of new soybean varieties. A higher heterogeneity of multiple forms was observed for SOD, AML, RNase, and esterase, which can provide markers of adaptation to environmental conditions.

Keywords: *Glycine soja*, ascorbic acid, carotene, higher fatty acids, oxidoreductases, hydrolases, specific activity, multiple forms of enzymes

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INTRODUCTION

The soybean *Glycine max* (L.) Merr. is an important crop species that provides for food security of the global population [1, 2]. Its wild ancestor *Glycine soja* Siebold et Zucc. is an annual self-pollinating herb with a twinning stem (Fig. 1). A *G. soja* plant is totally covered with downward brown hairs and has trifoliate compound leaves [3]. Morphological traits of *G. soja* have been studied comprehensively. Ala [4] has observed that the shape and size of leaf blades, seed color, and other characters vary among *G. soja* accessions. In nature, *G. soja* grows on sunny slopes, roadsides, along rivers, in sparse forests, and in areas affected by human activities (in abandoned fields, cultivated areas, and around villages). Destruction of natural *G. soja* habitats as a result of land clearing for agricultural or industrial purposes has reduced the resources of wild germplasm [5].

The primary genetic center of origin of *G. soja* includes Northeast China, Taiwan, Japan, Korea, and the Russian Far East (the northern boundary of the range in Amur Oblast). High genetic diversity of Far Eastern *G. soja* accessions is determined by contrast climatic conditions among other factors and makes it possible to use *G. soja* in breeding programs to improve the adaptive potential of new varieties [6]. Natural and anthropogenic *G. soja* populations contain unique and valuable genes that have been lost during domestication, and their studies make it possible to create unique natural gene banks of *G. soja* as the nearest *G. max* relative [2, 7–9].

Creating high-yielding soybean varieties that adapt well to adverse environmental conditions is a main problem of breeding [10]. Genetic diversity of legumes has decreased as a result of breeding and artificial selection for commercially valuable traits. Consequently, new soybean varieties obtained mostly by hybridization have traits that differ genetically from those of their wild ancestors [11]. Compared with *G. soja*, *G. max* has lost approximately 50% of its genetic diversity [12]. A narrow norm of reaction of modern soybean varieties is among the main factors responsible for exhaustion of gene pool reserves that can be used to breed for commercially valuable traits [7].

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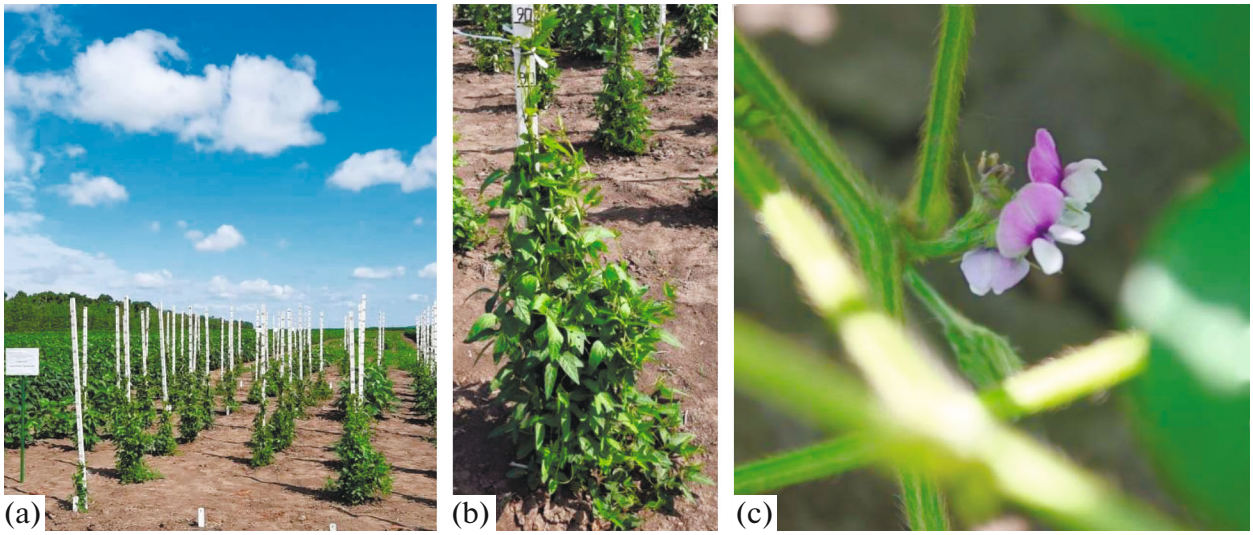

Fig. 1. *Glycine soja* Siebold et Zucc: (a) wild soybean plants in a crop rotation field of the All-Russian Research Institute of Soybean (Sadovoe village, Tambov District, Amur Oblast), (b) wild soybean accession KA-1413, and (c) wild soybean flowers.

Following many researchers, including Ala, Kalitskaya, and Sinegovskaya [13–17], wild soybean forms can serve as donors of early ripening, a high seed production, a high protein content, and resistance to certain diseases and are important to include in breeding programs in order to improve the adaptation potential in new soybean varieties. This makes it possible to utilize the *G. soja* potential to a fuller extent [2, 18, 19]. Wild soybean is a high-protein oil-yielding plant. The protein and oil contents in *G. soja* seeds range from 47.9 to 52.3% and from 9.3 to 12.0%, respectively [20, 21]. Protein and oil contents in seeds correlate inversely with each other [22]. A higher α-linolenic acid content in seed oil is characteristic of *G. soja* [23].

Based on the literature, *G. soja* is phylogenetically diversified, adapted to various environments, and resistant to a variety of abiotic and biotic stress factors [21, 24–26]. Hybridization of *G. max* with *G. soja* helps to develop new cultivated varieties with a higher stress resistance [27].

Plant resistance to environmental factors is an important component of the adaptive potential in grain legume and oil crop varieties and is determined mostly by the antioxidant system (AOS). Ascorbic acid (AA) and carotenoids attract the greatest interest among low-molecular-weight metabolites of the AOS. AA is synthesized in the cytosol and is involved in detoxification of hydrogen peroxide and inhibition of lipid peroxidation (LPO) [28, 29]. Carotenoids are components of antenna complexes in chloroplast reaction centers and act continuously to reduce the singlet oxygen content [30]. Hayrulina and Semenova [31] have found that *G. soja* seeds accumulate AA and tocopherol in water stress in greater amounts as compared with *G. max* seeds.

Soybean adaptation to growth conditions is determined at the biochemical level. Changes in enzyme activities are known to accompany changes in growth conditions [24]. Oxidoreductases provide universal indicators of the plant state [32]. The oxidoreductase group includes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7), and polyphenol oxidase (PPO, EC 1.10.3.1) [33, 34]. These enzymes act as antioxidants and play a role in detoxification of reactive oxygen species [35]. SOD catalyzes superoxide radical reduction to hydrogen peroxide. A higher SOD activity correlates with plant resistance to drought, pathogens, and other biotic and abiotic factors [26]. CAT eliminates excess hydrogen peroxide, but has low affinity for the substrate and is therefore efficient only at high H_2O_2 concentrations [36]. POD activity depends on the soil composition, temperature regimen, and effects of viral and bacterial pathogens and increases with increasing metabolism during intense spring growth and flowering [37]. PPO is a defense enzyme and plays an important role in degrading phenols and flavonoids in plants. PPO activity in the cell has been shown to increase in stress and to prevent propagation of reactive oxygen species [32].

Hydrolase enzymes are of interest because they are involved in the initiation and development of pathological processes in plant tissues. Enzymes of carbohydrate metabolism hydrolyze, synthesize, and modify carbohydrates and provide promising biomarkers [38]. It is important to note that esterases catalyze many reactions of ester hydrolysis and possess high catalytic activities [39]. Ribonuclease (RNase, EC 3.1) is a defense enzyme that has a broad substrate specificity and is capable of neutralizing the effects of a wide range of virus, bacterial, and other infections. RNA is

genetic material in the majority of plant viruses, and damage-induced extracellular RNases can therefore be assumed to play a role in antiviral defense in early infection.

Isozyme systems and multiple forms of enzymes are widely used to solve various biological problems and, in particular, to study the population genetic diversity in *G. soja* [40–44]. Protein markers make it possible to assess the variation of particular loci in various genotypes without crossing plants; for example, electrophoretically detectable isozymes can be used as markers of the respective genes [43, 45]. New multiple forms of enzymes arise as a result of changes in abiotic and biotic environmental factors, providing evidence for a plant adaptive response [46, 47]. In view of the above, the following enzymes were assayed in *G. soja*: SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), PPO (EC 1.10.3.1), RNase (EC 3.1), acid phosphatase (EC 3.1.3.2), esterase (EC 3.1.1.X), and amylase (AML, EC 3.2.1.1).

Biochemical parameters are now considered as promising diagnostic criteria of plant resistance to growth conditions. Use of *G. soja* is expected to increase as data on the genome and genetic diversity of the species accumulate and breeding instruments are improved [48, 49]. The expectation implies that *G. soja* accessions are readily available for soybean studies and breeding. This renders it necessary to characterize their morphological and commercially valuable traits and to monitor their biochemical and genetic parameters so that the accessions can be stored as genetic material in gene banks. Comprehensive studies in *G. soja* will provide genetic and biochemical data essential for its cultivation, enables a breakthrough in breeding, ensure stable development of soybean industry, and allow efficient use of its genetic resources [11].

The objective of this work was to study the chemical composition of seeds in *G. soja* accessions from the collection of the All-Russian Institute of Soybean, meaning their prospective use in breeding.

MATERIALS AND METHODS

Seeds of five *G. soja* accessions were collected in three regions of Amur Oblast (Fig. 2). The accessions KA-1413 and KA-342 were from Arkharinskii District; KBl-29 and KBl-24 were from Blagoveshchensk District, and KBel-72 was from Belogorskii District, providing unique natural gene banks. Wild soybean plants were grown from the seeds in a crop rotation field of the All-Russian Research Institute of Soybean (Sadovoe village, Tambov District, Amur Oblast) in 2019.

Test plants were grown in meadow chernozem-like soil according to a soybean cultivation technique developed for the southern agricultural area of Amur Oblast [50]. Material was collected and examined in 2020. Malondialdehyde (MDA), carotene, and AA contents and enzyme activities in seeds of the *G. soja* accessions were measured at the Biotechnology Laboratory (All-Russian Institute of Soybean).

To extract proteins, seeds (500 mg) were homogenized with 0.15 M NaCl at 5°C for 15 min. The extract was centrifuged at 3000 rpm for 15 min. The pellet was discarded, and the supernatant was tested for protein and MDA contents and enzyme specific activities [34, 51, 52].

The protein content was measured by the Lowry assay. Measurements against a control sample were performed at 750 nm, using a CARY 50 spectrophotometer and cuvettes with a path length of 1 cm [53]. MDA was measured by the thiobarbituric acid reaction, which proceeds at a higher temperature (100°C) in an acid milieu (pH 2.5–3.5) to produce a colored trimethine complex. The optical density at 532 nm was measured in cuvettes with a path length of 1 cm in a CARY 50 spectrophotometer against a control sample, which contained the reaction mixture and a protein extract, but without thiobarbituric acid [51].

Protein, oil, and fatty acid (FA) contents were determined at the Laboratory of Agricultural Product Processing (All-Russian Institute of Soybean) by near-infrared spectroscopy, using a FOSS NIR Systems 5000 analyzer.

Carotene was assayed by Pleshkov's photocolorimetric method. Measurements were performed at 440 nm, using sodium dichromate as a standard (the corresponding carotene amount in 1 mL is 0.00416 mg). The carotene content was expressed in mg/100 g [54]. AA was assayed by Tillmans' titration method as described by Ermakov [55], which is a common biochemical method used in plant studies. The AA content was expressed in mg%.

SOD activity was measured in the assay based on SOD-mediated inhibition of nitroblue tetrazolium reduction. Measurements were performed at 560 nm against a dark control, using a CARY 50 spectrophotometer and cuvettes with a 1-cm path length. CAT activity was inferred from the rate of hydrogen peroxide decomposition to water and oxygen; spectrophotometric measurements were carried out at 240 nm against a control, using cuvettes with a 1-cm path length [34]. POD activity was measured colorimetrically according to Mokronosov's modification of Boyarkin's method. Measurements were performed in a KFK-2 instrument, using cuvettes with a path length of 2 cm. Activity was inferred from the rate of benzidine oxidation to benzidine blue in the presence of hydrogen peroxide. PPO activity was measured in a KFK-2 instrument as described by Ermakov. The method is based on measuring the optical density at a wavelength of 590 nm for 2 min for pyrocatechol oxidation products formed within a certain period of time (20 s); measurements were carried out using cuvettes with a 2-cm path length [52].

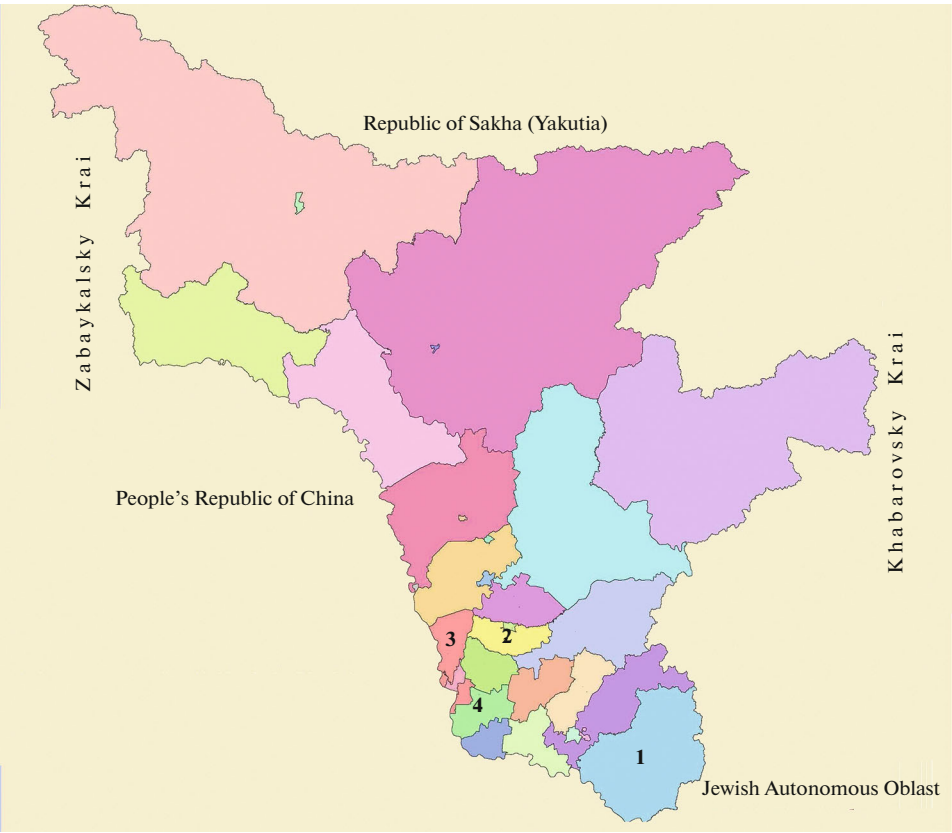

Fig. 2. Areas of origin of the *G. soja* Sieb. et Zucc. accessions examined in this work: 1, Arkharinskii District (KA-1413 and KA-342); 2, Belogorskii District (KBel-72); 3, Blagoveshchensk District (KBl-29 and KBl-24); 4, Tambovskii District.

To measure RNase specific activity, highly polymeric yeast RNA was used as a substrate. Spectrophotometry against a control was carried out at 260 nm, using cuvettes with a 1-cm path length. Acid phosphatase specific activity was assayed using *p*-nitrophenyl phosphate (disodium salt) as a substrate. Measurements at 415 nm were performed against a control in a CARY 50 sphectrophotometer, using cuvettes with a 1-cm path length. Esterase complex specific activity was measured by the Van Asperen method. Measurements at 550 nm were performed against a control in a CARY 50 spectrophotometer in cuvettes with a 1-cm path length. AML complex specific activity was assayed spectrophotometrically, by measuring the amount of starch noncleaved by AML after treating the sample with 0.3% I_2 in 3% KI (an aqueous solution). The optical density at 670 nm was measured against water in cuvettes with a 1-cm path length [56].

Electrophoretic patterns of the enzymes under study were obtained by PAGE in 8 or 10% gel in a Mini-PROTEAN Tetra vertical electrophoresis system (Bio-Rad) [57]. Enzyme forms were stained in gel by histochemical methods [56, 58–60]. Relative electrophoretic mobility (Rf) was used as a standard criterion to characterize multiple forms of enzymes. Forms were numbered in the order of decreasing electrophoretic mobility. Abbreviated designations were assigned to all forms according to their Rf [46, 61].

All biochemical assays were carried out using two biological and three analytical replicates [62]. Correlation analyses were performed for quantitative and qualitative characters. Experimental data were analyzed statistically. Results were expressed as mean ± standard deviation ($n = 6$); differences were considered significant at $p \le 0.5$.

RESULTS AND DISCUSSION

MDA. The MDA content is known to characterize activity of oxidation processes and to reflect the adaptive potential in plants [63]. In our sample, higher MDA concentrations were observed in KA-342 and KA-1413 seeds from the Arkharinskii district (0.8 and 0.86 μmol/g dry weight, respectively) and KBl-29 seeds (0.93 μmol/g dry weight) (Fig. 3). A lower MDA content was detected in the accession KBel-72 (0.50 μmol/g dry weight) from the Belogorskii district.

Biochemical parameters. The protein content is known to negatively correlate with the oil content in wild soybean, as has been observed in our and other works [21]. In this study of the chemical composition of seeds in several *G. soja* accessions, a higher protein

content (47.4%) accompanied by a lower oil content (9.6%) was observed in the accession KA-1413, suggesting a higher intensity of metabolic processes (Table 1). We assumed therefore that the oil content is lower because oil is consumed in ATP production.

The contents of unsaturated higher fatty acids and, in particular, oleic and linolenic acids, varied greatly. The oleic acid content was maximal (30.0%) in KA-1413 seeds and minimal (20.2%) in KBl-24 seeds. The linolenic acid content in seeds of the accession KA-1413 was 2% higher than in the other *G. soja* accessions, the finding being related to the protective mechanisms that involve linolenic acid [64]. Note that the contents of saturated carboxylic acids (stearic and palmitic) were rather low in seeds of *G. soja* KA-1413 (3.51 and 9.10%, respectively), while their maximum contents (3.77 and 9.41%, respectively) were detected in KBl-24 seeds.

Quality of soybean oil depends on the content of unsaturated higher carboxylic acids. A higher oleic acid content and a lower linolenic acid content provide for better oil quality [64, 65]. Higher contents of linolenic, oleic, and linoleic acids were observed in seeds of the *G. soja* accession KA-1413; the finding matched a higher protein content and agreed with literature data [66]. Our study showed that the oleic acid content increased to a greater extent, while the linolenic acid content, to a lower extent in *G. soja* KA-1413 seeds. We therefore think that quality of oil produced from cultivated soybean will improve if KA-1413 is introduced in breeding programs.

Vitamin C and carotene act as low-molecular-weight antioxidants and inhibit the production of reactive oxygen species. The highest carotene content (0.18 mg/100 g) was observed in seeds of the *G. soja* accession KBl-24; and the highest AA content (52.92 mg/dL), in KA-342 seeds in our analysis (Fig. 4). The substances prevent the damaging effect of reactive oxygen species on seeds and provide for better seed adaptation to growth conditions. It should be noted that the carotene content was higher in *G. soja* seeds from Blagoveshchensk and Belgorodskii Districts and that the AA content was higher in the southernmost part of Amur Oblast (Arkharinskii District).

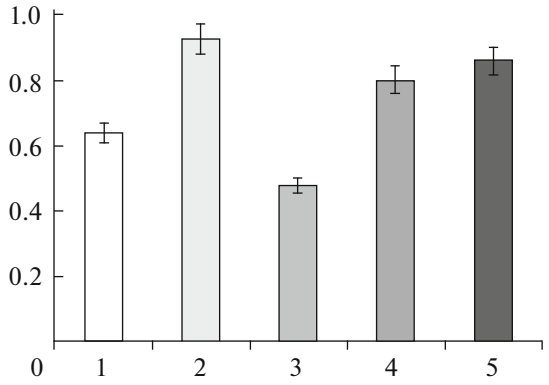

Fig. 3. MDA content in wild soybean seeds (ordinate, μmol/g fresh weight). Accessions (abscissa): 1, KBl-24; 2, KBl-29; 3, KBel-72; 4, KA-342; 5, KA-1413.

Oxidoreductase activity. Oxidoreductases are among the most informative parameters of cell metabolism. SOD specific activity was much the same in seeds of all accessions examined, varying from 150 to 180 units/mg protein. PPO activity in seeds was lower (1.0–2.19 units/mg protein) in all *G. soja* accessions and especially low in KA-1413 (0.2 units/mg protein) (Fig. 5a). This is possibly associated with insignificant oxidation processes and high activities of other antioxidant enzymes. For example, POD specific activity in seeds was very high (361–801 units/mg protein) in all of the *G. soja* accessions. POD and CAT are known to act as antagonistic enzymes in soybean seeds and seedlings [46]. An increase in POD specific activity is accompanied by a decrease in SOD specific activity. Lower CAT activity in seeds of the *G. soja* accessions KA-342 and KA-1413 was apparently compensated for by an increase in the number of POD forms (up to three forms) (Fig. 6).

Oxidoreductase zymograms of *G. soja* seeds showed six SOD forms, two CAT forms, three POD forms, and one PPO form. It should be noted that the numbers of SOD and PPO multiple forms were the

Table 1. Biochemical parameters (%) of seeds in the *G. soja* accessions

Parameter	*Glycine soja* accession				
	KBl-24	KBl-29	KBel-72	KA-342	KA-1413
Protein	44.18	44.93	45.29	44.82	47.37
Oil	10.39	9.97	10.08	10.12	9.60
Stearic acid	3.77	3.74	3.67	3.67	3.51
Palmitic acid	9.41	9.27	8.97	9.08	9.10
Oleic acid	20.20	23.55	27.41	26.41	30.02
Linoleic acid	50.96	50.80	50.75	50.80	51.09
Linolenic acid	11.48	11.93	11.75	11.89	13.74

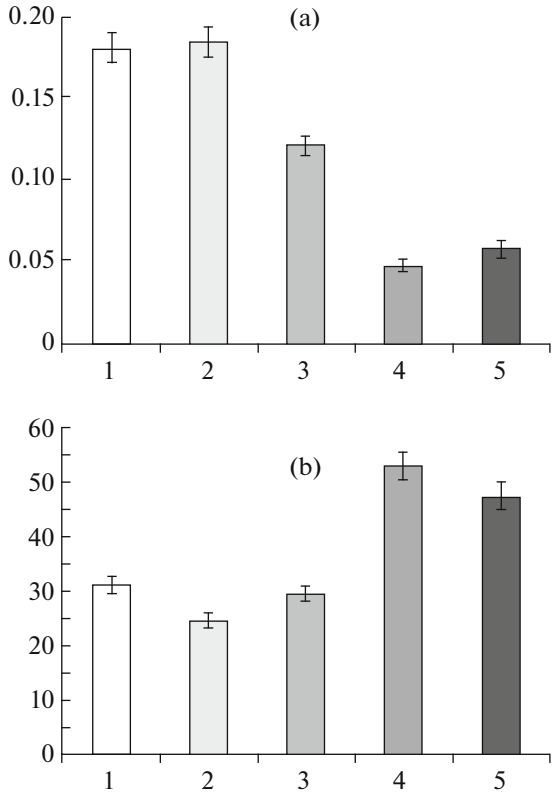

Fig. 4. (a) Carotene and (b) AA contents in *G. soja* Sieb. et Zucc. seeds. Accessions (abscissa): 1, KBl-24; 2, KBl-29; 3, KBel-72; 4, KA-342; 5, KA-1413. Ordinate: (a) carotene content, mg/100 g and (b) AA content, mg/dL.

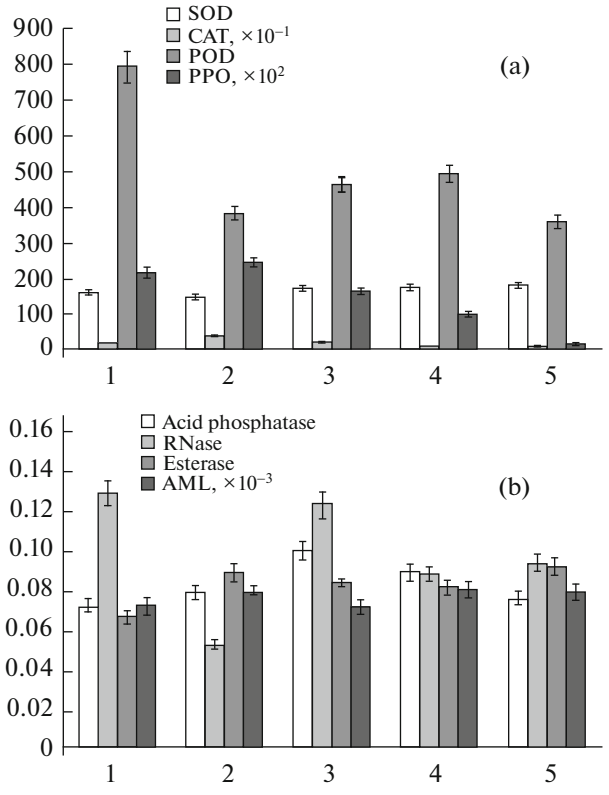

Fig. 5. Specific activities of (a) oxidoreductases and (b) hydrolases in wild soybean seeds. Accessions (abscissa): 1, KBl-24; 2, KBl-29; 3, KBel-72; 4, KA-342; 5, KA-1413. Ordinate: (a) oxidoreductase and (b) hydrolase specific activities (A_{sp}, units/mg protein).

same in seeds of all *G. soja* accessions under study. The number of CAT multiple forms was also the same (one form in each accession), but the forms differed in electrophoretic mobility (Rf). Three POD forms were observed in seeds of the accessions KBel-72, KA-342, and KA-1413. Lower activities of antioxidant enzymes agree well with higher contents of carotene and AA in soybean seeds.

Hydrolytic activity. Production of compounds that suppress hydrolytic activity of microorganisms is an important component of the plant defense response to pathogens. Because RNA acts as genetic material in the majority of plant viruses, extracellular damage-induced RNases can be assumed to play a role in antivirus defense. This supports the hypothesis that plant extracellular RNases are involved in virus resistance [67, 68]. Higher RNase specific activity in seeds was observed in all but one accession, KBl-29, suggesting higher virus resistance (Fig. 5b). RNase zymograms of *G. soja* seeds showed the same pattern with three forms for all of the accessions examined (Fig. 7). Acid phosphatase specific activity in seeds was low (0.071–0.099 units/mg protein) in all accessions (Fig. 5b), and a single stable acid phosphatase form (AP7, Rf = 0.35) was detected accordingly (Fig. 7).

Esterase specific activity in seeds varied from 0.066 units/mg protein in KBl-29 to 0.091 units/mg protein in KBl-24. The highest esterase activity was observed in KBel-72 seeds, which additionally displayed relatively high RNase specific activity. The spectrum of multiple forms of esterases was stable in seeds of all *G. soja* accessions and included three forms of the enzyme with the same electrophoretic mobilities. Higher esterase activity is possibly due to an increase in metabolism caused by ester hydrolysis. AML specific activities in seeds of the *G. soja* accessions were found to be higher (71–80 units/mg protein) than in *G. max* seeds (55 units/mg protein on average), suggesting more intense starch hydrolysis. AML multiple form spectra of *G. soja* seeds differed in the number of enzyme forms. A single form of the enzyme was detected in seeds collected in Arkharinskii District, in agreement with the same AMP specific activity. The highest number (three) of AML forms was observed in seeds of the accessions KBl-29 and KBel-72.

Our analysis of the results of enzymatic activity assays showed that the accessions from Arkharinskii District are highly similar in hydrolases, SOD, and CAT; differ in POD and PPO; and slightly differ in in

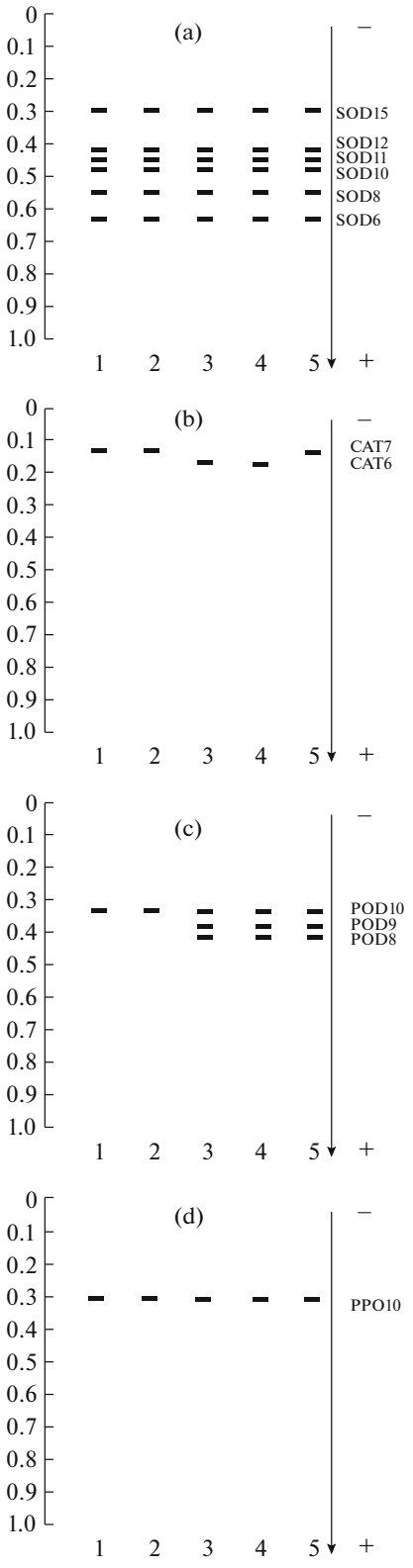

Fig. 6. Schematic zymograms of oxidoreductases of wild soybean seeds: (a) SOD, (b) CAT, (c) POD, and (d) PPO. Accessions (abscissa): 1, KBl-24; 2, KBl-29; 3, KBel-72; 4, KA-342; 5, KA-1413. Ordinate, relative electrophoretic mobility (Rf). The electrophoresis direction from the cathode to the anode is shown with arrows.

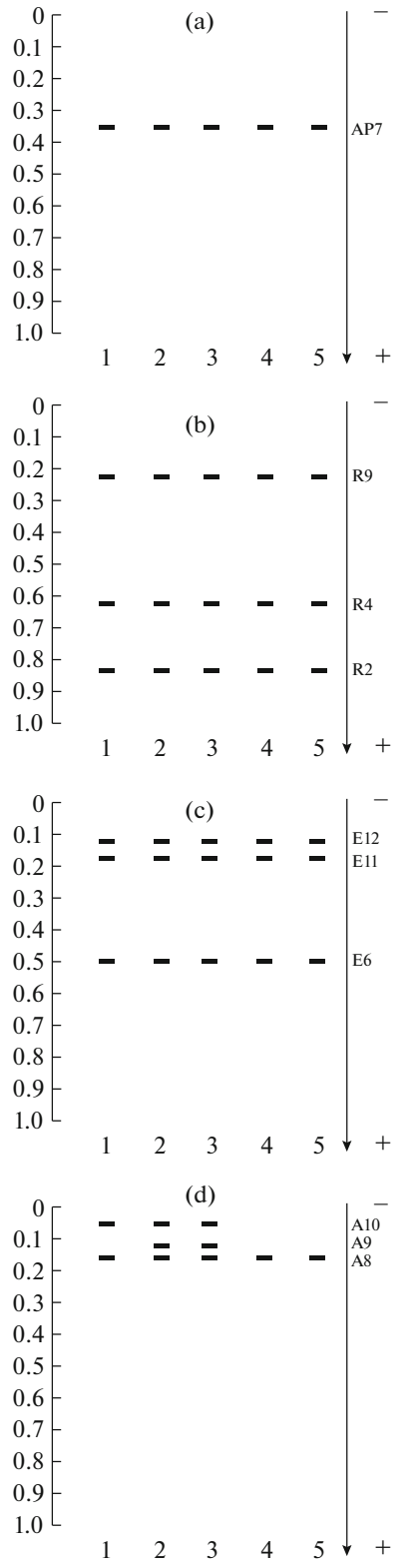

Fig. 7. Schematic zymograms of hydrolases of wild soybean seeds: (a) acid phosphatase, (b) RNase, (c) esterase, and (d) AML. Accessions (abscissa): 1, KBl-24; 2, KBl-29; 3, KBel-72; 4, KA-342; 5, KA-1413. Ordinate, relative electrophoretic mobility (Rf). The electrophoresis direction from the cathode to the anode is shown with arrows.

electrophoretic patterns (with the exception of CAT). The electrophoretic patterns of enzymes were much the same in seeds from different regions (with the exception of CAT, POD, and AML). It should be noted that SOD, POD, RNase, and esterase showed higher heterogeneity in *G. soja* seeds and can be used as adaptation markers.

Thus, the *G. soja* accession KA-1413 can be recommended as a gene source for breeding programs on evidence of our data on enzymatic activities and biochemical composition of seeds in several *G. soja* accessions. Its use will help to improve the adaptive potential in new soybean varieties. A balance of phosphate, lipid, carbohydrate, and nucleic acid metabolism supports the idea.

CONCLUSIONS

Wild soybean *G. soja* Sieb. et Zucc. accessions with higher contents of biochemical components; lower PPO activity; and higher values of SOD, esterase, and RNase activities are advisable to select in order to use them as sources of resistance genes in breeding aimed at improving the adaptive potential in new varieties of the cultivated soybean *G. max* (L.) Merr. The accession KA-1413 was found to meet the above requirements (protein, 47.37%; oleic acid, 30.02%; linolenic acid, 13.74%; linoleic acid, 51.09%; A_{sp}(PPO), 2.00 ± 0.02 units/mg protein; A_{sp}(SOD), 182 ± 14 units/mg protein; A_{sp}(RNase), 0.093 ± 0.011 units/mg protein; A_{sp}(E), 0.091 ± 0.008 units/mg protein). SOD, POD, RNase, and esterase display a higher heterogeneity in *G. soja* accessions and can be used as markers of adaptation to environmental conditions.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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