AGRICULTURAL BIOLOGY,
2016, V. 51, № 1, pp. 89-99
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Plant tolerance — biochemical and physiological traits

UDC 633.16:581.19:57.042:546.815

doi: 10.15389/agrobiology.2016.1.89rus
doi: 10.15389/agrobiology.2016.1.89eng

STUDY OF ISOZYME POLYMORPHISM IN SPRING BARLEY (*Hordeum vulgare* L.) CONTRASTING IN TOLERANCE TO LEAD

A.V. DIKAREV, V.G. DIKAREV, S.A. GERAS'KIN, N.S. DIKAREVA

All-Russian Research Institute of Agricultural Radiology and Agroecology, Federal Agency of Scientific Organizations, 109 km, Kievskoe sh., Obninsk, Kaluzhskaya Province, 249032 Russia, e-mail dikarev.vlad@yandex.ru
Received June 6, 2015

Abstract

Lead is one of the most hazardous heavy metals (HM), for which the mechanisms of plant resistance is not completely clear. One of the mechanisms for implementing toxic effect of lead is the generation of reactive oxygen species (ROS), which normally perform important regulatory function, but cause multiple violations of vital activity of cells at increased concentrations. ROS level adjustment is carried out by antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (PER). The effectiveness of most enzymes depends upon the existence of multiple isoforms, ensuring the most optimal operation under changing environment conditions. As a working hypothesis, it has been suggested that the level of plant tolerance to the HM can be determined by differences in composition of izoforms of antioxidant enzymes differently contributeing to neutralization of the ROS excess. To test this hypothesis the study of isoenzymatic spectra of superoxide dismutase and peroxidase enzyme systems and key enzyme of protein metabolism, the glutamate dehydrogenase (GDH), was carried out using embryos of germinating seeds of the spring barley (*Hordeum vulgare* L.) cultivars. A totlal of 12 cultivars of spring barley contrasting in resistance to Pb^{2+} (6 tolerant cultivars and 6 sensitive cultivars) were studied. To analyze the composition of isoenzyme the germinating embryos of viable seeds were used with root length of 1 mm after 1 day germination. Homogenized embryos of seeds were subjected to electrophoresis using 7.5 % polyacrylamide gel (PAGE) units with an alkaline buffer system (pH 8.9). The analysis showed the presence of following isoenzymes from enzyme systems of superoxide dismutase, peroxidase and glutamate dehydrogenase: an obligate SODII, PERI and GDHI as well as rare SODI, SODIII + SODIV (always occurring together), SODV, PERII and GDHII. The frequencies of rare alleles were calculated and the highest frequencies were found for alleles SODI and SODIII + SODIV being the same in sensitive and resistant cultivars. A reliability of differences in the frequencies of rare allelic forms in the sensitive and resistant cultivars was assessed using φ-criterion of the angular Fisher transformation. The calculated value of Fisher's φ-criterion was proved to exceed the critical level. Thus the studied rare enzyme isoforms could be regarded as biochemical markers of sensitivity or tolerance to lead in barley plants. As our research ascertained, SODV and PERII are more common in lead sensitive cultivars, while GDHII is mostly found in tolerant ones. Frequency of SODIII and SODIV also reliably differed in the resistant and tolerant cultivars. The paper discusses the molecular genetic mechanisms of plant HM-resistance. It is first experimentally shown that spring barley plant polymorphism on resistance to lead is associated with biochemical polymorphism and correlates with a specific complex of superoxide dismutase, peroxidase and glutamate dehydrogenase isoenzymes. The data obtained can be used in selection programs aimed to producing lead-resistant barley cultivars.

Keywords: lead, barley, intraspecific polymorphism, contrast variants, isoenzymes.

The problem faced by modern agriculture under a decrease of usable territories and, consequently, the need to enhance production efficiency, is environment contamination with anthropogenic pollutants, in particular heavy metals (HM). Heavy metals are known to have an adverse effect on the growth and development of plants and animals [1-3]. Therefore, it is crucial to create crops with a high resistance to the toxic effect of HMs making it possible to have a sufficient amount of high-quality products.

Earlier [4], we determined the lead concentrations having a real toxic ef-

fect on barley seeds and investigated the intraspecific polymorphism by the resistance to lead in spring barley distinguishing 12 varieties with a contrast response (six resistant and six sensitive ones). A question arises on the relation between the polymorphism by morphological indicators as described in our previous research and the intraspecific variability on deeper levels of biological organization. For example, the genetic nature of the intraspecific polymorphism in radiation resistant hexaploid wheat plants has long been demonstrated [5]. It is reasonable to assume that contrast reactions of barley varieties to lead are related to their genetic peculiarities.

The development of methods for marking biological properties in plant and the productivity traits remains the main practical application of the biochemical and molecular genetics as a theoretical basis of modern breeding. Initially, the morphological traits (shape, color, pubescence, etc.) were mostly used for genetic marking as they are more easy to detect and more available to the researcher [6, 7]. However, the instability of these traits, their dependence on the cultivation conditions, the polygenic nature and a high probability of subjective interpretation led to the fact that a key role in genetic marking belonged first to protein but then to molecular-genetic markers [8].

The main difficulty in marking biological traits in plants is due to the fact that the majority of such traits is related to many life functions, metabolic and morphological processes which are under complex genetic control, vary phenotypically in a wide range, and therefore, are not available for a classical genetic analysis. Complex properties may often be subdivided into simpler ones, thus showing a hierarchic structure. In this, some of the complex elements are monomorphic (or, in other words, are common for all species), others are polymorphic and specific for varieties and biotypes [9]. Such subdivision makes it possible to reveal the genetic nature of a complex trait, and to identify the availability and localization of locuses and simple genetic systems in the genome that correspond to a particular trait [5].

Many enzymes are known to have several isoforms of specific structure and conformations. These differences often led to different activity [10, 11]. Biotypes and cultivars may have only some of the isoforms with various portion and prevalence of each one. It contributes to intraspecific variation formed on the biochemical level. The isozyme polymorphism is generally neutral [12] and related to the role of enzyme in cell functions. There are a number of reports about association of isoforms dissimilar in the activity with environment stress resistance, which can be used to differentiate plants on their response to adverse environmental factors. Thus, an increased expression of the superoxide dismutase (SOD) genes involved in the LpFe-SOD and LpCu/Zn-SOD isoforms' control and higher activity of these isomers in the plant roots were found in two rye grass (*Lolium perenne* L.) varieties after plant exposure to aluminum [13]. However, the expression differed substantially between the varieties, and thus, according to the authors, influenced stress resistance. The work [14] dedicated to the oxidative stress in the root cells of field beans (*Vicia faba* L.) grown in lead-contaminated soil also reports that various SOD isoforms differ in capability to detoxicate reactive oxygen species produced in such conditions at higher rates. However, SOD isoforms can respond to environment pollutants in different ways. It is reported [15] that copper increased activity of all SOD isoforms in *Arabidopsis thaliana* leaves, while cadmium induced an increase in the activity of only Fe-SOD and Mn-SOD, while the Cu-SOD activity reduced compared to control.

We have analyzed the isozyme patterns of barley plants contrasting in the response to lead contamination, with special attention to glutamate dehydro-

genase, superoxide dismutase and peroxydase as related to plant resistance under environmental stress, and shown the tolerance identified at a morphological level to be pre-determined by biochemical peculiarities of the varieties. In this, we have identified the biochemical traits of barley plants associated with resistance or sensitivity to lead.

The purpose of our research was to identify the enzyme isoforms more frequently found in the varieties resistant or sensitive to lead.

Technique. A total of 12 varieties of two-rowed spring barley (*Hordeum vulgare* L.) contrasting in resistance to Pb^{2+} were studied. To analyze the isoenzyme composition, the embryos of viable seeds with root length of 1 mm after 1 day germination were sampled. For germination the roll method was used [16]. A total of 15 samples (embryo extracts) of each variety were analyzed for each of the enzymes studied. The experiment was repeated with 1-year (n = 180 per enzyme of each variety) and 3-year (n = 540 per enzyme of each variety) harvest samples, with a total number of 1,620.

The isoforms of glutamate dehydrogenase (GDH, K.F. 1.4.1.2), superoxide dismutase (SOD, K.F. 1.15.1.1), and peroxydase (PER, K.F. 1.11.1.7) were analyzed with Tris glycine electrode running buffer solution (pH 8.3) [17, 18] in 7.5 % polyacrylamide gel plates (pH 8.9) [19]. The germinating seeds were homogenized in glass mortar with 50 μl of extraction solution of 1 % Triton X-100 and 0.2 % β-mercaptoethanol at 1:1 (v/v) and equal volume of 50 % saccharose. The separation was performed using a vertical electrophoresis P9DS apparatus (Owl, USA) at ~350 V and ~50 mA for 3 hours with 1 ml of 0.1 % bromphenol blue as an internal reference dye. Then the gel was cut into three parts and prepared for histochemical assay as described [20]. Photos of the stained gels were analyzed to evaluate the frequency of each enzyme activity.

The obtained data were compared for the groups of resistant and sensitive varieties using criterion φ of Fisher's angle-transformation [21] as recommended [5].

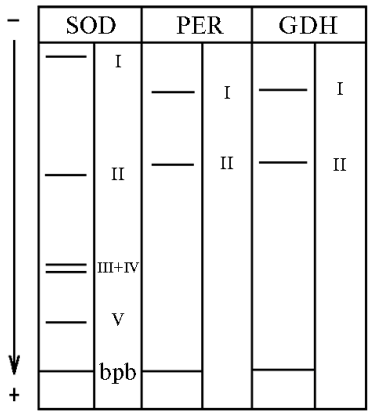
Results. Based on morphological parameters, 12 varieties contrasting in lead resistance (i.e. 6 resistant and 6 sensitive ones) (Table 1) were selected out of 100 barley varieties of various geographic origin harvested in 2009 [4]. Additionally, adequacy of the grouping was confirmed on seed samples harvested in 2008 and 2010.

1. The varieties of two-rowed spring barley (*Hordeum vulgare* L.) of various origin contrasting in resistance to lead used in the study

Name and origin	Variety	VIR catalog number
Resistant		
Vyatsky (Kirov Region)	*nutans*	k-30848
Teo (Great Britain)	*nutans*	k-29871
Zarya (Kirov Region)	*nutans*	k-4731
Donum (Czechia)	*nutans*	k-30863
Symphony (Kharkov Region)	*medicum*	k-30996
Pongo (Sweden)	*nutans*	k-30946
Sensitive		
Medicum 336 (Samara Region)	*medicum*	k-30962
Myt (Ukraine)	*medicum*	k-30993
Jelen (Yugoslavia)	*nutans*	k-30955
NSGL 1 (Yugoslavia)	*nudum*	k-30956
Zavetny (Rostov Region)	*medicum*	k-30959
Rubezh (Belarus)	*nutans*	k-29446

Note. VIR — Federal Research Center the N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg.

Obtained electrophoregrams showed the distribution of the isoforms as illustrated in the scheme (Fig.). The found alleles are numbered depending on the band distance to the reference dye position.



The general distribution of superoxide dismutase (SOD), **peroxydase** (PER) **and glutamate dehydrogenase** (GDH) **in the 12 studied varieties of two-rowed spring barley (*Hordeum vulgare* L.) of various origin contrasting in the resistance to lead in a model test**: I, II, III, IV, V — allele variants, bpb — bromphenol blue (reference dye).

The SODII, PERI, and GDHI isozymes were obligatory and found in all the investigated variants, while the SODI, SODIII+IV (always are linked), SODV, PERII, and GDHII were optional and found occasionally. The superoxide dismutase had mostly three alleles (SODII, SODIII, and SODIV), peroxydase had an allele (PERI), and glutamate dehydrogenase had one allele (GDHI), too. Moreover, SODI, SODV, PERII, and GDHII had rare bands. The SODIII and SODIV alleles also were rare unlike SODII with the 100 % presence in all the barley varieties studied.

After analyzing isozyme patterns of 12 barley varieties with a contrast lead tolerance for the 3-year harvest, we assessed the frequency of non-regular allelic isozymes (SODI, SODV, PERII, and GDHII) using φ-criterion of Fisher's angle-transformation (Table 2). Also, we determined the frequency the SODIII+IV isozymes which always were present or absent together as a couple of activity bands located close to each other. Here, we did not consider the SODII allele, as it was consistently present both in tolerant and sensitive varieties, and in the various varieties inside these groups. Similarly, the PERI and GDHI forms were not under consideration.

2. The occurrence of the superoxide dismutase (SOD), **peroxydase** (PER) **and glutamate dehydrogenase** (GDH) **isoforms in the 12 investigated varieties of two-rowed spring barley (*Hordeum vulgare* L.) of various origin contrasting in resistance to lead in the model experiment**

Isozyme	Frequency, %		Criterion φ of Fisher's angular transformation
	in resistant varieties	in sensitive varieties	
SODI	45.11	41.97	1.21
SODIII+IV	47.23	71.69	7.79
SODV	4.04	11.85	4.35
PERII	12.86	18.41	1.85
GDHII	20.37	9.91	3.41

Mostly, the Fisher's criterion φ exceeded the critical $\varphi_{cr.}^{0.01} = 2.31$, except PERII with the differences between the resistant and sensitive varieties significant at $\varphi_{cr.}^{0.05} = 1.64$, and SODI with no significant differences. Thus, the identified rare isozymes can be considered as the biochemical markers of barley resistance or sensitivity to lead. As our research shows, SODV and PERII are more often found in lead-sensitive varieties, while GDHII is more characteristic of resistant ones (see Table 2). The occurrence of SODIII and SODIV was also significantly different in resistant and sensitive varieties.

Thus, the data from the Table 2 suggest that the lead resistance polymorphism we discovered when studying 100 varieties of spring barley [4] is associated with the biochemical polymorphism of antioxidant enzyme isoforms. Heavy metals, including lead, may cause oxidative stress in higher plants [22, 23]. Reactive oxygen species (ROSs) are the key source of plant cell severe damage [24-26] due to extremely high chemical reactivity. In our previous research [4], we demonstrated that lead has depressed considerably the barley seedling growth and caused morphological failures. These manifestations are probably due to an increased ROS production. Such ROSs as $O_2^{-\bullet}$, H_2O_2, and $OH^{\bullet}$ are constantly produced as a result of metabolic processes in chloroplasts,

mitochondria, and peroxisomes [27]. Normally, ROS production is controlled by the antioxidant systems [28]. Under stress, however, there is a risk of cell damage due to excess ROS production [29]. An increased ROSs level can lead to crucial cell failures because of DNA modification, and oxidization of proteins and lipids [30, 31]. At the same time, ROSs can cause an increased expression of genes encoding SOD and PER responsible for their neutralization, thus also influencing cell homeostasis.

This is a common scheme of how these enzymes work:

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2,$$

$$2H_2O_2 \xrightarrow{PER} 2H_2O + O_2,$$

$$\text{L-glutamate} + H_2O + NADP^+ \xleftrightarrow{GDH} \text{2-oxoglutarate} + NH_3 + NADPH + H^+.$$

SOD is one of the main enzymes responsible for stress protection under ROS generation [32]. Primarily, SOD must transform superoxide anion $O_2^{-\bullet}$ into hydrogen peroxide with subsequent production of water and molecular oxygen. Adverse environments lead to unequal development of oxidative stress in cell compartments which results in expression of SOD genes encoding isoforms to protect cell structures [33]. The presence of SOD isozymes in plants and their genetic control was first demonstrated in corn [34, 35]. In our study, a higher frequency of the rare SOD and PER isoforms was found in the lead-resistant barley varieties compared to sensitive ones. It may be assumed that the tolerance is related to a higher genetic diversity of antioxidant systems enabling the plants to endure stress better. Higher polymorphism is known to increase the capability of withstanding technogenic stresses [36]. At biochemical level this polymorphism appears as optional SOD isomers. The higher their frequency and diversity, the higher the level of polymorphism is. As to our data, the rare isoforms contribute to a higher plant tolerance.

The appearance and increased frequency of optional SOD isozymes in HM-resistant varieties compared to sensitive ones may be due to the ROS-inducing environments (e.g. arid or saline soils, metal deposits, etc.) at breeding. The available publications [37, 38] suggest that the responses of the plant antioxidant systems to HMs and natural factors are similar. Here, the technogenic load should also be taken into account. Three of six resistant varieties are from the industrial countries (Great Britain, Czech Republic, and Sweden) with an increased level of heavy metals in soils in many regions. It can be considered as an evidence of the above hypothesis. Indeed, the varieties grown at high natural level of arsenic differ in their tolerance [39]. In such conditions, adaptively more efficient isoforms could be fixed by selection with conservation of their alleles in the genome. At the same time, the breeding for economically important traits could also contribute to the revealed biochemical peculiarities. Thus, in the seeds rich in fats and exposed to atmospheric oxygen, an increased levels of organic peroxides and, as a result, free radicals can be produced. These could provoke development of additional antioxidants required to maintain homeostasis. Such peculiarities of the enzyme system can be fixed in gene pool [40] which was shown while studying the transcription of the gene encoding the Mn-SOD isoform. As it has turned out, mercury causes increased expression of this gene and, as a result, the isozyme activity rises, which is quite in line with our hypothesis. Another evidence for better withstanding to stresses in polymorphic samples can be found in the report of V. Rancelis et al. [36]. The authors showed that after treating bean plants with cobalt the occurrence of rare SOD variants grew. In this regard, we can also mention the increased frequency of optional antioxidant enzyme isoforms (SOD, PER, etc.) in chronically irradiated pine populations (*Pinus sylvestris* L.) after Chernobyl accident [41]. This example also indicates the uniformity of an organism's response to various stressors.

H_2O_2 produced in cells due to SOD is toxic [42], and its concentration must be kept low. Hydrogen peroxide, similar to other ROSs, can also be induced by metal ions and pathogens. For example, it is reported [43, 44] that in sensitive plants the H_2O_2 level is substantially higher than in resistant ones. At the same time, at low concentrations H_2O_2 activates a number of protective mechanisms [45], including cell wall regeneration [43], binding and neutralization of pathogens and harmful ions [46], a response to hypersensitivity, and synthesis of proteins and phytoalexins [47]. That is why it may be assumed that in resistant varieties these compounds are produced in relatively small amounts and stimulate the protective functions enabling better capability to withstand stress caused by HMs and making the varieties more adapted to adverse environments. As for the sensitive varieties, they have an excess of H_2O_2, which on the contrary leads to weaker protection and damages. The above mechanisms of peroxide action in cells can explain how the plants of the investigated varieties respond to the presence of Pb^{2+}.

However, the obtained data should be discussed not only with regard to the effects of low-molecular compounds. As it was already mentioned, the antioxidant enzyme system protects plants from excessive ROS concentrations caused by environmental stress. The excess of peroxides is controlled by PER. The high frequency of PERII that we observed in sensitive varieties is probably related to higher sensitivity of plants to HM ions. Peroxidase is a highly labile enzyme that can respond to most homeostatic abnormalities. Thus, it was ascertained [48] that one of the peroxidase isozymes determines high resistance to virus infections in various red clover lines. This isozyme activates peroxidase cleavage, so the authors offered using this form as a general resistance marker in view of similarity in plant response to various stressors. Such data show that peroxidase markers make it possible to provide more comprehensive characterization of plant protection to various environments which can be taken into account in breeding.

The biological role of GDH is to catalyze the reductive amination of 2-oxoglutarate to glutamate and the reverse oxidative deamination [49] with involvement of reductive and oxidative NAD forms as coenzymes. Mostly due to this fact, the nitrogen compounds are taken up by plants and metabolized with synthesis of nitrogen-containing organic compounds and their destruction resulted in releasing nitrogen as ammonium salts. Thus, this enzyme is a link between the two fundamental processes characteristic of an autotrophic organism, i.e. nitrogen and carbon assimilation, and plays an important role in controlling plant development. GDH has a number of isoforms, in particular plants have light and heavy forms [50, 51] catalyzing amination and deamination, respectively. The differences in the level of these forms regulate the organism's balance between catabolism and anabolism. According to the Chatelier's principle, a catalist speeds up chemical reaction bidirectionally, that is the enzyme enhances amination and deamination with equal probability, and kinetic disbalance [52, 53] may depend on environmental conditions and stress exposure. Accumulation of various compounds (e.g. sugars, amino acids, ammonium ions) is related to the GDH function in cells, which may indirectly contribute to the development of resistance to adverse environments such as drought or salination, to biomass accumulation and higher tolerance to toxicants, for example, herbicides and perhaps also HMs [54, 55]. The substantial disbalance towards the formation of 2-oxoglutarate was reached due to plant genetic modifications.

In view of the significant electrophoretic mobility of GDHII as compared to GDHI, it can be assumed that the first of them corresponds to the lightest of the described isoforms, mainly catalyzing the amination. Conse-

quently, in such plants the anabolism is more active compared to those in which this isomer is absent. With regard to the data we obtained, this tendency is of a special interest, as excessive glutamate is mostly produced so a direct reaction of deamination must prevail. Since there were cases when an isozyme catalyzing primarily the glutamate synthesis was revealed, the identified isoform had to have an increased activity. As we have showed the GDHII to be more often found in the resistant varieties, it could be assumed that their tolerance is related to the higher activity of the synthesis processes contributing to intensive accumulation of biomass. Similar data were obtained by R. Ameziane et al. [56]. As a result, the plants with GDHII allele developed quicker and were more adapted to adverse environments. The results on genetic control of *Pinus sylvestris* L. tolerance to air pollution [57] was in line with our findings of the GDH two isoforms, of which the lighter one (GDHII) was found in resistant plants.

At the same time, there is evidence [58] that the catabolic-type GDH is activated not only by ammonium ions, but also by single- and double-charged metal cations, and the affinity with such cations may be higher than with NH_4^+. It is reasonable to assume that in sensitive varieties the relatively high concentration of the Pb^{2+} ions may cause a shift in the enzyme synthesis balance towards such GDH form, and it can be inherited. That is why such isozyme in sensitive varieties is capable of expressing even without HM exposure. From this, it becomes clear why a light GDH form responsible for anabolism, and, consequently, less sensitive to cations is found in sensitive barley varieties more rarely.

So, the data we obtained enable us to conclude that the lead resistance polymorphism of spring barley is related to biochemical polymorphism and correlates with a certain complex of superoxide dismutase, peroxydase and glutamate dehydrogenase isozymes. Evidently, there are specific alleles encoding the synthesis of isoforms of these enzymes and in some way determining the tolerance or the sensitivity. We have identified the isozymes of superoxide dismutase and peroxydase more frequent in lead-sensitive varieties, and ascertained the increased occurrence of the glutamate dehydrogenase isozyme in resistant ones. The differences between the groups, contrasting in resistance, with regard to isozyme frequency are confirmed statistically. Thus, it is offered to consider such isoforms of superoxide dismutase, peroxidase and glutamate dehydrogenase as biochemical markers to differentiate resistant and sensitive two-rowed spring barley varieties. The obtained data can be used in breeding lead-resistant barley.

REFERENCES

1. Gural'chuk Zh.Z. *Fiziologiya i biokhimiya kul'turnykh rastenii*, 1994, 26(2): 107-117.
2. Fenik S.I., Trofimyak T.B., Blyum Ya.B. *Uspekhi sovremennoi biologii*, 1995, 115(3): 261-276.
3. Sharma R., Dubey R.S. Lead toxicity in plants. *Braz. J. Plant Physiol.*, 2005, 17(1): 35-52.
4. Dikarev A.V., Dikarev V.G., Dikareva N.S., Geras'kin S.A. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2014, 5: 78-87 (doi: 10.15389/agrobiology.2014.5.78rus, doi: 10.15389/agrobiology.2014.5.78eng).
5. Sarapul'tsev B.I., Geras'kin S.A. *Geneticheskie osnovy radiorezistentnosti i evolyutsiya* [Evolution and the genetic bases for radioresistance]. Moscow, 1993.
6. Konarev V.G. *Belki rastenii kak geneticheskie markery* [Plant proteins as genetic markers]. Moscow, 1983.
7. Sozinov A.A. *Polimorfizm belkov i ego znachenie v genetike i selektsii* [Protein polymorphism and its role in genetics and breeding]. Moscow, 1985.
8. Chesnokov Yu.V. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2005, 1: 20-40.
9. Altukhov Yu.P. *Geneticheskie protsessy v populyatsiyakh* [Genetic processes in populations]. Moscow, 2003.
10. Timoshkina N.N., Vodolazhskii D.I., Usatov A.V. *Ekologicheskaya genetika*, 2010, 8(1): 12-24.
11. Staszak J., Grulke N.E., Marrett M.J., Prus-Glowacki W. Isozyme markers as-

sociated with O_3 tolerance indicate shift in genetic structure of ponderosa and Jeffrey pine in Sequoia National Park, California. *Environ. Pollut.*, 2007, 149: 366-375 (doi: 10.1016/j.envpol.2007.05.026).
12. Altukhov Yu.P., Salmenkova E.A. *Genetika*, 2002, 38(9): 1173-1195.
13. Cartes P., McManus M., Wulf-Zotelle C. Differential superoxide dismutase expression in ryegrass cultivars in response to short term aluminum stress. *Plant Soil*, 2012, 350: 353-363 (doi: 10.1007/s11104-011-0919-3).
14. Wang C., Tian Y., Wang X., Geng J., Jiang J., Yu H., Wang C. Lead-contaminated soil induced oxidative stress, defense response and its indicative biomarkers in roots of *Vicia faba* seedlings. *Ecotoxicology*, 2010, 19: 1130-1139 (doi: 10.1007/s10646-010-0496-x).
15. Drazkiewicz M., Skorzynska-Polit E., Krupa Z. The redox state and activity of superoxide dismutase classes in *Arabidopsis thaliana* under cadmium or copper stress. *Chemosphere*, 2007, 67: 188-193 (doi: 10.1016/j.chemosphere.2006.08.032).
16. *GOST 12038-84. Semena sel'skokhozyaistvennykh kul'tur. Metody opredeleniya vskhozhesti* /Pod redaktsiei T.I. Vasilenko [State Standard 12038-84 (SU). Crop seeds. Estimation of germination rate. T.I. Vasilenko (ed.)]. Moscow, 2010.
17. Maurer G. *Disk-elektroforez* [Disk electorphoresis]. Moscow, 1971.
18. Osterman L.A. *Metody issledovaniya belkov i nukleinovykh kislot. Elektroforez i ul'tratsentrifugirovanie* [Study of proteins and nucleic acids — methods: electrophoresis and ultracentrifugation]. Moscow, 1981.
19. Pleshkov B.P. *Praktikum po biokhimii rastenii* [Practical work on plant biochemistry]. Moscow, 1976.
20. Manchenko G.P. *Handbook of detection of enzymes on electrophoretic gels*. NY, 1994.
21. Zykova N.Yu. *Metody matematicheskoi obrabotki dannykh* [Data mathematical processing]. Voronezh, 2008.
22. Boening D.W. Ecological effects, transport and fate of mercury: a general review. *Chemosphere*, 2000, 40: 1335-1351 (doi: 10.1016/S0045-6535(99)00283-0).
23. Wang Y., Greger M. Clonal differences in mercury tolerance, accumulation and distribution in willow. *J. Environ. Qual.*, 2004, 33: 1779-1785 (doi: 10.2134/jeq2004.1779).
24. Zhou Z.S., Huang S.Q., Guo K., Mehta S.K., Zhang P.C., Yang Z.M. Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. *J. Inorg. Biochem.*, 2007, 101: 1-9 (doi: 10.1016/j.jinorgbio.2006.05.011).
25. Cho U.H., Park J.O. Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.*, 2000, 156: 1-9 (doi: 10.1016/S0168-9452(00)00227-2).
26. Cargnelutti D., Tabaldi L.A., Spanevello R.M. Mercury toxicity induces oxidative stress in growing cucumber seedlings. *Chemosphere*, 2006, 65: 999-1006 (doi: 10.1016/j.chemosphere.2006.03.037).
27. Chen J., Shiyab S., Han F.X. Bioaccumulation and physiological effects of mercury in *Pteris vittata* and *Nephrolepis exaltata*. *Ecotoxicology*, 2009, 18: 110-121 (doi: 10.1007/s10646-008-0264-3).
28. Apel K., Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 2004, 55: 373-399 (doi: 10.1146/annurev.arplant.55.031903.141701).
29. del Rió L.A., Sandalio L.M., Corpas F.J., Palma J.M., Barroso J.B. Reactive oxygen species and reactive nitrogen species in peroxisomes, production, scavenging, and role in cell signaling. *Plant Physiol.*, 2006, 141: 330-335 (doi: 10.1104/pp.106.078204).
30. Polesskaya O.G. *Rastitel'naya kletka i aktivnye formy kisloroda* [Plant cell and reactive oxygen species]. Moscow, 2007.
31. Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. Reactive oxygen gene network of plants. *Trends Plant Sci.*, 2004, 9: 490-498 (doi: 10.1016/j.tplants.2004.08.009).
32. Fried R. Enzymatic and non-enzymatic assay of superoxide-dismutase. *Biochemie*, 1975, 57: 657-660 (doi: 10.1016/S0300-9084(75)80147-7).
33. Inze D., Montagu M. Oxidative stress in plants. *Curr. Opin. Biotechnol.*, 1995, 6: 153-158 (doi: 10.1016/0958-1669(95)80024-7).
34. Scandalios J.G. Oxygen stress and superoxide dismutases. *Plant Physiol.*, 1993, 101: 7-12.
35. Alscher R.G., Erturk N., Heath L.S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.*, 2002, 53: 1331-1341 (doi: 10.1093/jexbot/53.372.1331).
36. Rancelis V., Cesniene T., Kleizaite V. Influence of cobalt uptake by *Vicia faba* seeds on chlorophyll morphosis induction, SOD polymorphism and DNA methylation. *Environ. Toxicol.*, 2010, 10: 5-15 (doi: 10.1002/tox.20609).
37. Taylor N.L. Targets of stress-induced oxidative damage in plant mitochondria and their impact on cell carbon/nitrogen metabolism. *J. Exp. Bot.*, 2004, 55(394): 1-10 (doi: 10.1093/jxb/erh001).
38. Vacca R.A. Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat shock-induced programmed cell death in tobacco. *Plant Physiol.*, 2004, 134(3): 1100-1112 (doi: 10.1104/pp.103.035956).
39. Mallick S., Sinam G., Sinha S. Study on arsenate tolerant and sensitive cultivars of *Zea mays* L.: differential detoxification mechanism and effect on nutrient status. *Ecotoxicology and Environmental Safety*, 2011, 74: 1316-1324 (doi: 10.1016/j.ecoenv.2011.02.012).
40. Elbaz A., Wei Y., Meng Q. Mercury-induced oxidative stress and impact on antioxidant

enzymes in *Chlamidomonas reinhardtii*. *Ecotoxicology*, 2010, 10: 8-18.
41. Volkova P.Yu., Geras'kin S.A. *Ekologicheskaya genetika*, 2013, 9(3): 48-62.
42. Dat J., Vandenabeele S., Vranova E. Dual action of the active oxygen species during plant stress responses. *Cell. Mol. Life Sci.*, 2000, 57: 779-795 (doi: 10.1007/s000180050041).
43. Lanubile A., Bernardi J., Marocco A. Differential activation of defense genes and enzymes in maize genotypes with contrasting levels of resistance to *Fusarium verticillioides*. *Environ. Exp. Bot.*, 2012, 78: 39-46 (doi: 10.1016/j.envexpbot.2011.12.006).
44. Lamb C., Dixon R.A. The oxidative burst in plant disease resistance. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.*, 1997, 48: 251-275 (doi: 10.1146/annurev.arplant.48.1.251).
45. Bolwell G.P., Butt V.S., Davies D.R., Zimmerlin A. The origin of the oxidative burst in plants. *Free Radic. Res.*, 1995, 23: 517-523 (doi: 10.3109/10715769509065273).
46. Lane B.G. Oxalate, germin, and the extracellular matrix of higher plants. *FASEB J.*, 1994, 8: 294-301.
47. Hammond-Kosack K.E., Jones J.D.G. Resistance gene-dependent plant defense responses. *Plant Cell.*, 1996, 8: 1773-1791 (doi: 10.2307/3870229).
48. Sheen S.J., Diachun S. Peroxidases of red clover resistant and susceptible to bean yellow mosaic virus. *Acta Phytopathol. Acad. Sci. Hung.*, 1978, 13(2): 21-28.
49. Grabowska A., Nowicki M., Kwinta J. Glutamate dehydrogenase of the germinating triticale seeds: gene expression, activity distribution and kinetic characteristics. *Acta Physiologiae Plantarum*, 2011, 33: 1981-1990 (doi: 10.1007/s11738-011-0801-1).
50. Loulakakis K.A., Roubelakis-Angelakis K.A. The seven NAD(H)-glutamate dehydrogenase isoenzymes exhibit similar anabolic and catabolic activities. *Physiol. Plant.*, 1996, 96: 29-35 (doi: 10.1111/j.1399-3054.1996.tb00179.x).
51. Purnell M.P., Scopelites D.S., Roubelakis-Angelakis K.A. Modulation of higher-plant NAD(H) dependent glutamate dehydrogenase activity in transgenic tobacco via alteration of beta-subunit level. *Planta*, 2005, 222: 167-180 (doi: 10.1007/s00425-005-1510-z).
52. Dikson M., Uebb E. *Fermenty. V 3 tomakh* [Enzymes. V. 1, V. 2, V. 3]. Moscow, 1982.
53. Metsler D. *Biokhimiya. Khimicheskie reaktsii v zhivoi kletke. V 3 tomakh* [Biochemistry. The chemical reactions in living cell. V. 1, V. 2, V. 3]. Moscow, 1980.
54. Mungur R., Glass A.D.M., Goodenow D.B., Lightfoot D.A. Metabolite fingerprinting in transgenic *Nicotiana tabacum* altered by the *Escherichia coli* glutamate dehydrogenase gene. *J. Biomed. Biotechnol.*, 2005, 2: 198-214 (doi: 10.1155/JBB.2005.198).
55. Mungur R., Wood A.J., Lightfoot D.A. Water potential is maintained during water deficit in *Nicotiana tabacum* expressing the *Escherichia coli* glutamate dehydrogenase gene. *Plant Growth Regulation*, 2006, 50(2): 231-238 (doi: 10.1007/s10725-006-9140-4).
56. Ameziane R., Bernhardt K., Lightfoot D.A. Expression of the bacterial *gdhA* gene encoding a glutamate dehydrogenase in tobacco and corn increased tolerance to the phosphinothricin herbicide. In: *Nitrogen in a sustainable ecosystem: from the cell to the plant.* M.A. Martins-Loucao, S.H. Lips (eds.). Leiden, 2000.
57. Geburek T., Scholz F., Knabe W. Genetic studies by isozyme gene loci on tolerance and sensitivity in air polluted *Pinus sylvestris* field trial. *Silvae genetica*, 1987, 36(2): 49-53.
58. Wooton J.C. Re-assessment of ammonium-ion affinities of NADP-specific glutamate dehydrogenases. *Biochem J.*, 1983, 209: 527-531.