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ECOLOGICAL  
MANAGEMENT  
OF GLUTATHIONE  
FUNCTIONS  
IN AGROBIOCENOSES



ACADEMY OF APPLIED SCIENCES ACADEMY OF MANAGEMENT  
AND ADMINISTRATION IN OPOLE

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**ECOLOGICAL MANAGEMENT OF GLUTATHIONE FUNCTIONS  
IN AGROBIOCENOSES**

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# ECOLOGICAL MANAGEMENT OF GLUTATHIONE FUNCTIONS IN AGROBIOCENOSSES

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## PREFACE

A rather important role for the viability of a plant cell is played by the control of metabolism and development processes, which is largely carried out due to thiol-disulfide exchange. SH-groups of cysteine residues are quite significant for the functioning of enzymes and processes that are the basis of responses to environmental factors and intracellular information transmission - cell signaling. The basic mechanism of the central role of thiol-mediated redox (redox) control in cellular metabolism is the ability of thiol groups to reversibly change their redox state with subsequent changes in conformational, catalytic, or regulatory functions of the protein. The basis of cellular redox homeostasis, with the help of which the redox state of thiol groups of proteins can be supported, is the ratio of reduced (GSH) and oxidized (GSSG) glutathione, which is present in most cells in a millimolar concentration [Meredith, Reed, 1982, Sies, 1999, Ogawa, 2005, Shanmugam et al., 2012, Janssen-Heininger et al., 2013, Nagy, 2013, Kalinina et al., 2014, Dmitriev et al., 2015].

Glutathione (GSH) is a biologically active substance, a tripeptide (L-gamma-glutamyl-L-cysteinyl-glycine M, 307 D), which is one of the universal regulators of biochemical and physiological homeostasis of any organism [Van der Meide et al., 1993, Zhu et al., 1999; Pietrini et al., 2003; Yadav, 2010, Noctor et al., 2011, Cuypers et al., 2012]. At the initial stage of synthesis, ATP-dependent formation of the dipeptide  $\gamma$ -glutamylcysteine synthetase occurs. There is also information that 60-70% of the pool of this enzyme is localized in chloroplasts, it was also found in the cytosol [Gryshko, Syshchikov, 2006]. It has been established that the thiol (sulfhydryl) group is the main functional part of the tripeptide and is easily subjected to both enzymatic and non-enzymatic oxidation, resulting in the formation of the disulfide (oxidized) form of glutathione (GSSG).

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Reduced glutathione (GSH) is a low-molecular-weight thiol, the biosynthesis and catabolism of which occurs via the glutamyl cycle. The oxidation reaction of reduced glutathione is catalyzed by enzymes with different specificities for hydrogen acceptors. The reverse process – reduction of oxidized glutathione – is catalyzed by glutathione reductase (GR). In the reduced state, the thiol group is able to supply a reducing equivalent ( $H^+ + e^-$ ) to other molecules, such as ROS, to neutralize them, or to protein cysteines to maintain their reduced forms. When an electron is supplied, glutathione itself becomes reactive and quite easily reacts with another reactive glutathione to form glutathione disulfide (GSSG). There is an opinion that one of the functions of glutathione in plants is the maintenance of ascorbic acid in a reduced form to improve its physiological functions [Kulinsky, Kolesnychenko, 1990, Meshchishen et al., 2005, Seth et al., 2012, Kolupav, Yastreb, 2015]. The combination of antioxidant properties and the ability to activate the transcription of genes, including some antioxidant enzymes, as well as to inhibit the redox pathways of apoptosis activation indicates a rather important contribution of reduced glutathione and glutaredoxin to the antioxidant defense system, which increases the resistance of cells against oxidative stress [Hayes, McLellan, 1999, Allen, Mieyal, 2012, Board, Menon, 2013, Lillig, Berndt, 2013].

The specificity of the structure of glutathione and the relatively high (1-10 mM) level of concentration inside the cell allows it to take a fairly active part in the work of the antioxidant system, in the reduction of disulfides, hydroperoxides, sulfenic acid, nitrosothiols, detoxification of aldehydes, xenobiotics, in the synthesis of steroids and eicosanoids, in the regulation of the homeostasis of some metals, ensuring the necessary level of reduced ascorbic acid, as well as influencing the processes of protein folding and the regulation of numerous intracellular signaling pathways, which are of quite important importance in the control of expression genes, cell cycle, apoptosis. The

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contribution of the glutathione-dependent system in response to the action of ROS is largely decisive in the fate of the cell [Circu, Aw, 2008, 2012, Kulinsky, Kolesnychenko. 2010].

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## CHAPTER 1. ECOLOGICAL MANAGEMENT OF ENVIRONMENTAL CHARACTERISTICS OF GLUTATHIONE CHARACTERISTICS OF LUTATHIONE

Glutathione was discovered by F. Hopkins in 1921. It is a crystalline powder with a melting point of 190-1920 C. From a solution of 0.5 N H<sub>2</sub>S<sub>04</sub> precipitates in the form of an insoluble copper mercaptide upon addition of C<sub>2</sub>O, and the chemical structure of this tripeptide was established in the mid-30s of the last century by C. Harrington and T. Mead [Kidd, 1997, Filippovych, 1999]. It is one of the most important endogenous antioxidants, a low-molecular intracellular thiol, which accounts for 90-95% of the total number of thiol compounds in the cell [Kosower, Kosower, 1978, Noctor et al., 1996]. The main low-molecular compound in the body of plants containing a thiol group is glutathione. The tripeptide glutathione ( $\gamma$ -glutamyl-cysteine-glycyl) is the main redox buffer in animal cells, being in two forms - reduced and oxidized. The functions of glutathione in plant cells are also extremely important, as in animal cells: glutathione directly takes an active part in protecting cells from oxidative stress, acting as a substrate for the work of antioxidant enzymes, regulating the plant cell cycle and morphogenesis processes both *in vivo* and *in vitro* [ Khaertdinova et al., 2013].

The specificity of the structure of reduced glutathione and the relatively high (1-10 mM) level of concentration in the middle of the cell allows it to take an active part in the work of the antioxidant system, in the reduction of disulfides, hydroperoxides, sulfenic acid, nitrosothiols, detoxification of aldehydes, xenobiotics, synthesis of steroids and eicosanoids, in regulation of the homeostasis of some metals, ensuring the necessary level of restored ascorbic acid, as well as influence the processes of protein feeding and the regulation of



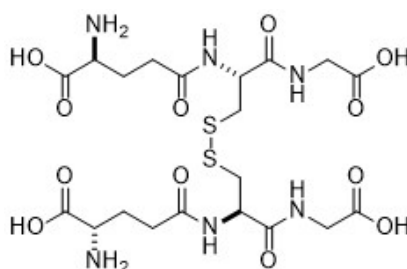
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numerous intracellular signaling pathways, which are of considerable importance in the control of gene expression, cell cycle and apoptosis [Townsend et al., 2003, Fernandes, Holmgren, 2004, Kulinsky, Kolesnychenko, 2009 , 2010, Galano, Alvarez-Idaboy, 2011, Circu, Aw, 2012, Deponete, 2013]. Both the compartmentalization and the concentration of glutathione in different organelles change at different stages of the cell cycle [Markovic et al., 2007].

The thiol tripeptide glutathione is a water-soluble antioxidant and redox buffer of plants, which is involved in the processes of regulation of the cell cycle and development, transport and storage of sulfur, in response to stress and detoxification of heavy metals [Maughan, Foyer, 2006].

Glutathione protects thiol groups of proteins, inactivates radical particles, destroys peroxide compounds, reacts with ROS. It is an important substrate for the synthesis of phytochelatin, which are important in the detoxification of heavy metals in the plant cell [Yadav, 2010].

Glutathione, being a peptide in the classical sense of this concept, is still not a protein tripeptide, that is, it is not formed by matrix synthesis or post-translational modification; its molecular mass is 307 g/mol. Synthesis of glutathione de novo is carried out exclusively in the cytosol in two steps catalyzed by glutamate cysteine ligase and glutathione synthetase [Meister, Tate, 1976]. The main pool of glutathione synthetase, as well as  $\gamma$ -glutamylcysteine synthetase, is located in chloroplasts [Herschbach et al., 1998]. The activity of glutathione synthetase is completely inhibited by p-chloromercury benzoate and divalent mercury, which indicates the presence of a thiol group in the active center [Murata, Kimura, 1990].



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The peculiarity of the first stage of synthesis is the formation of a peptide bond by joining not the  $\alpha$ -, but the  $\gamma$ -carboxyl group of glutamic acid to the amino group of cysteine. Due to such an unusual structure of the molecule, the hydrolysis of this peptide bond is carried out by a single membrane enzyme,  $\gamma$ -glutamyltranspeptidase, located only on the outer surfaces of certain types of cells. And as a result of this, glutathione is resistant to intracellular degradation and is metabolized extracellularly only in tissues where  $\gamma$ -glutamyltranspeptidase is present [Meister, Anderson, 1983].

At physiological pH values, this tripeptide has two negatively charged carboxyl groups and a positively charged amino group. Due to the presence of a sulfhydryl group in the molecule, reduced glutathione can act as an electron donor, i.e. exhibit the functions of a reducing agent. The one-electron reaction of reduced glutathione with free radicals leads to the formation of the thiol radical GS $\cdot$ . Dimerization of GS $\cdot$  with another GS $\cdot$  radical gives glutathione disulfide GSSG [Kosower, Kosower, 1978].

Due to the presence of two carboxyl groups, one amino group and one thiol group, glutathione is well soluble in aqueous solutions and polar solvents. The  $\gamma$ -glutamyl peptide bond between glutamic acid and cysteine protects the tripeptide from degradation by intracellular proteases. One of the currently known enzymes that hydrolyze this bond is  $\gamma$ -glutamyltranspeptidase, which is located on the outer side of the cytoplasmic membrane. The terminal carboxyl residue of glycine protects the released glutathione molecule from being cut by intracellular  $\gamma$ -glutamylcyclotransferase. Thus, reduced glutathione is protected from intracellular degradation and is metabolized mainly in the intracellular space [Sies, 1999].

At the initial stage of synthesis, the ATP-dependent formation of the dipeptide  $\gamma$ -glutamylcysteine from glutamine and cysteine occurs and is catalyzed by  $\gamma$ -glutamylcysteine synthetase. This enzyme, isolated from plant cells, has a

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molecular weight of 60 kDa. When dithiotrietol is added to the incubation medium, the enzyme is inactivated with its decomposition into subunits with a molecular weight of about 34 kDa. Thus, a change in the cysteine content can affect the level of  $\gamma$ -glutamylcysteine formation and glutathione synthesis. The acceptor specificity of amino acids for this enzyme is wide enough, and therefore it can interact with some analogs of glutamine, such as  $\beta$ -aminoglutarate and  $\alpha$ -aminomethylglutarate [Rueggsegger, Brunold, 1992]. The reaction mechanism includes the formation of enzyme-bound  $\gamma$ -glutamyl phosphate and its interaction with the amino group of cysteine [Meister, 1981]. Features of the structure of this tripeptide determine its main biological functions. It directly or indirectly takes an active part in many important biological processes, which include protein and DNA synthesis, transport of compounds, regulation of enzyme activity, metabolism and protection of cells against environmental stresses [Rennenberg, 1982, 1995].

Synthesis of glutathione in plant cells occurs in chloroplasts and cytoplasm. The first reaction of glutathione synthesis takes place in chloroplasts and is catalyzed by the enzyme  $\gamma$ -glutamylcysteine synthetase with the formation of the dipeptide  $\gamma$ -glutamylcysteine, and the second reaction takes place in the cytosol with the participation of the enzyme glutathione synthetase. Glutathione ( $\gamma$ -L-Glutamyl-L-cysteinylglycine) is a small molecule containing a tripeptide with a  $\gamma$ -peptide bond between the glutamate carboxyl group of the side chain and an amino group attached by a normal peptide bond to glycine with a molecular weight of 307 Yes [Kocsy et al., 2001, Noctor et al., 2011, 2012]. The study of the process of biological synthesis of glutathione in plant cells confirmed the prediction of the predominantly chloroplast localization of both glutathione and enzymes of its synthesis. This hypothesis is supported by the cessation of the production of this compound when the ultrastructure of chloroplasts changes and the fairly rapid incorporation of L-cysteine into glutathione in *Chlorella*

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*sorokiniana* (Shichira ex Krauss) and *Lemna paucicostata* Hegelm. [Jamai et al., 1996, Grant et al., 1997].

Thanks to the work of specific transporters, glutathione or its conjugates can be pumped out of the cell. An enzyme that hydrolyzes the  $\gamma$ -glutamyl bond in oxidized and reduced glutathione and glutathione conjugates is located on the outside of the cytoplasmic membrane -  $\gamma$ -glutamyltransferase ( $\gamma$ -GT) (another name for this glycoprotein is  $\gamma$ -glutamyltranspeptidase). This enzyme ensures the transfer of the  $\gamma$ -glutamyl residue to an amino acid, usually neutral, which will be transported inside the cell, which is carried out with the help of special carriers. On the outside of the membrane, the dipeptides cysteinylglycine and cysteinylglycine formed after the action of  $\gamma$ -GT are cleaved by dipeptidases with the formation of cysteine (cystine) and glycine, which are transported into the cell by amino acid transporters. For example, cysteine can be pumped into the cell with the help of ACS transporters (from English alanine-cysteine-serine), and cystine - due to the work of the cystine/glutamate antiporter Xc<sup>-</sup>, which consists of two subunits: xCT is specific for the cystine/glutamate exchanger, and 4F2hc is common to several classes of transport systems [Lewerenz et al., 2013]. At the same time, cystine inside the cell is reduced to two cysteine molecules, for example, with the participation of ascorbic acid [Lu, 2013]. The inclusion of selenium instead of sulfur in cysteine enhances the antioxidant functions of enzymes, the constituent part of which is cysteine, and can disrupt the protein structure, because the selenide bond is less strong than the disulfide bond [Leustek, Saito., 1996]. The inclusion of selenium in the metabolism of plants occurs along the same assimilation pathways as the inclusion of sulfur, and therefore a rather important determining factor in the influence of selenium on the protein metabolism of the plant organism will be the nonspecific inclusion of selenium instead of sulfur in selenium-containing amino acids [Zayed et al., 1999]. Thus, the influence of selenium on amino acid synthesis in plants deserves

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special attention. At the same time, it is important to understand the participation of selenium not only in the antioxidant protection of plants through the influence on individual amino acids and enzymes, but also a holistic idea of the role of selenium in enhancing the synthesis and increasing the content of all amino acids in the plant [Kyryushyna, 2017]. In the presence of glutathione, 5-phosphoselenate turns into selenite with the help of adenosine-5-phosphosulfate reductase. And then, in the presence of glutathione, selenite turns into selenide, which then, thanks to synthesis with O-acetylserine in the presence of cysteine synthetase, glutathione reductase, glutathione and NADP, can turn into selenocysteine. All these processes take place in the chloroplasts of cells [Ng, Anderson, 1978; Terry et al., 2000]. Selenocysteine has a lower dissociation constant than cysteine (5.47) and a higher reducing potential. Thanks to this, selenocysteine is a part of proteins that perform antioxidant functions. It should also be noted that selenocysteine can harm the cell in its free state due to its reactivity [Byun, Kang, 2001]. It is known that cysteine is part of many enzymes, including those that perform antioxidant functions, in particular glutathione peroxidase, catalase, superoxide dismutase. The inclusion of selenium instead of sulfur in cysteine strengthens the antioxidant functions of these enzymes, but at the same time, it can disrupt the protein structure, since the diselenide bond is less strong than the disulfide bond. Synthesis of selenocysteine is carried out on specialized tRNAs, which include it in the growing peptide chain [Levine et al., 1996].

Subsequently, the synthesis of selenomethionine takes place in the cytosol of cells [Van Hoewyk, 2013], since the enzymes methionine synthase and methionine methyltransferase involved in this process are cytosolic [James et al., 1995; Terry et al., 2000]. It can only be assumed that, by analogy with sulfur, the existence of a number of regulatory mechanisms for the reduction of selenite

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assimilation is possible, including enzyme activity, substrate concentration, and gene expression [Leustek, Saito, 1999; Saito, 2000].

Remaining in the cell, cysteine and glycine become substrates for  $\gamma$ -glutamylcysteine synthetase and glutathione synthetase, respectively. The bond created by glutamyltransferase in the dipeptide of the  $\gamma$ -glutamyl residue with an amino acid already inside the cell is broken by  $\gamma$ -glutamylcyclotransferase, forming 5-oxoproline and a free amino acid, and the subsequent destruction of the cycle in 5-oxoproline by oxoprolinase leads to the formation of glutamic acid, which also becomes a substrate for  $\gamma$ -glutamylcysteine synthetases. As a result, glutathione from the extracellular space can be destroyed, and its component amino acids can appear again inside the cell and be involved in a new glutathione molecule. Thanks to such reactions, this cycle is repeated many times, and with each such cycle, an amino acid bound to a  $\gamma$ -glutamyl residue is transported inside the cell. The availability of cysteine, the level of which in the cell is two orders of magnitude lower than the level of glutamate, is a regulatory factor in the work of  $\gamma$ -glutamylcysteine synthetase, which determines the rate of synthesis of reduced glutathione [Gladyshev et al., 2001].

After synthesis, reduced glutathione from the cytoplasm enters the nucleus, endoplasmic reticulum, mitochondria and peroxisomes, while the share of cytosolic glutathione remains quite high - about 85-90% [Markovic et al., 2007]. The mitochondrion is a cellular organelle that is the site of constant generation of ROS, and therefore the balanced work of the redox system "reduced glutathione to oxidized glutathione" is extremely important for maintaining the redox homeostasis of the mitochondrial matrix for the protection of DNA, RNA, proteins and lipids against the action of ROS [Ghosh et al., 2005]. It has also been shown that, in addition to the function of oxidative phosphorylation, mitochondria play a rather important role in the cell's response to stress [Borovsky et al., 2011, Brykov and Shugaev, 2011]. Stress in plants is an integral, i.e. a single, response

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of the plant organism to a damaging effect, aimed at its survival due to the mobilization and formation of protective systems [Usmanov et al., 2001]. It is known that any stressor provokes oxidative stress in the cell, in which the enzyme peroxidase plays not the least role [Zhyvetiev et al., 2010].

In millimolar concentrations, glutathione is present in the cells of all gram-negative and some gram-positive bacteria, where it plays a key role in protection against oxidative and other stresses [Fahey et al., 1978, Smirnova et al., 2001]. It is a donor of reducing equivalents in the glutathione-ascorbate cycle (Halliwell–Asada cycle). During this process, the reduced form of glutathione is oxidized to regenerate dehydroascorbate, which is converted to ascorbate. The transformation of the oxidized form of glutathione into its reduced form is catalyzed by glutathione reductase. Glutathione also takes an active part in the direct detoxification of peroxide. In the course of this reaction, reduced glutathione interacts with hydrogen peroxide or another organic peroxide to form water or water and alcohol and glutathione dimer. This process is catalyzed by glutathione peroxidase or glutathione-S-transferase [Noctor et al., 2002]. The concentration of reduced glutathione increases not only thanks to the process of de novo synthesis, but also due to the functioning of glutathione reductase, which reduces oxidized glutathione to two molecules of reduced glutathione in the presence of NADPH(H<sup>+</sup>) [Lu, 2013]. Recovery of oxidized glutathione is provided by glutathione reductase. This FAD-dependent enzyme catalyzes the oxidation of NADPH by glutathione in the cytoplasm and in mitochondria [Carlberg, Mannervik, 1975, Savvides, Karplus, 1996, Can et al., 2010]. Glutathione reductase has a rather high specificity for glutathione and possesses glutathione-reducing, transhydrogenase, diaphorase activity, and therefore is part of the enzymatic antioxidant system of plants. The highest activity of glutathione reductase was recorded in the leaves of the onion. Its activity does not change

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statistically significantly depending on the place of germination and the type of population [Balaeva-Tikhomirova et al., 2015].

This tripeptide glutathione, synthesized without the participation of ribosomes, is the precursor of phytochelatin. Phytochelatin is a short peptide synthesized from glutathione by the enzyme phytochelatin synthase and takes an active part in the detoxification of heavy metals by chelating them [Cobbett, Goldsbrough, 2002, Permyakov, 2012]. Phytochelatin forms chelates with cadmium ions ranging in size from 2500 to 3600 Da. Complexes of phytochelatin with cadmium are transported in the vacuole [Clemens, 2006]. The general empirical formula of phytochelatin is  $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ , where  $n = 2-11$  [Sharma et al., 2015]. The formation of phytochelatin is one of the constituent parts of the cell's response program to the influx of heavy metals into the cytoplasm. Having a fairly high affinity for SH-groups, cadmium ions are one of the strongest activators of their synthesis [Seregin, 2001]. Phytochelatin is not a gene product and is synthesized from glutathione with the participation of the phytochelatin synthase enzyme located in the cell cytoplasm [Grill et al., 2007; Joshi et al., 2015]. The synthesis of phytochelatin is induced by ions of various heavy metals (Ag, Bi, Cd, Cu, Hg, Ni, Sn, Sb, Te, W and Zn), which indicates the non-specificity of this detoxification mechanism, but the following elements do not induce the formation of phytochelatin: Al, Mn, Cr, Co, Fe, Cs, V [Prasad, 2004; Sharma et al., 2015]. But among all heavy metals, cadmium and copper are the most effective in this process [Souza, Rauser, 2003]. Currently, it is believed that the binding of heavy metal ions by phytochelatin is one of the most important mechanisms of their detoxification [Reese, Wagner, 1987, Howden et al., 1995, Cobbett, 2000, Nakasawa et al., 2002, Clemens, Simm, 2003, etc.]. It was established that the formed cadmium-chelatin complexes are 10-1000 times less toxic for metal-sensitive plant enzymes than free cadmium ions [Kneer, Zenk, 1992]. For understanding the role of phytochelatin in plants, the results of



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research conducted on cadmium-sensitive objects are of particular importance. For example, the role of phytochelatins in resistance to cadmium was demonstrated in *cad1* and *cad2* mutants of *Arabidopsis* [Howden et al., 1995]. Phytochelatins are not formed in the cadmium-sensitive mutant, although glutathione synthesis is not impaired. In another non-cadmium-resistant mutant *cad2* with a mutation in the  $\gamma$ -glutamylcysteine synthetase gene, the concentration of glutathione is significantly reduced, and under the action of cadmium, very few phytochelatins are formed, and as a result, the plants die. In addition, *Vigna angularis* suspension culture cells sensitive to cadmium also did not synthesize phytochelatins [Inouhe et al., 2000]. The participation of phytochelatins in the detoxification of heavy metals is also confirmed by the fact that treatment of plants or cell cultures with buthionine sulfoximine, an inhibitor of the synthesis of  $\gamma$ -glutamylcysteine synthetase, which takes part in the formation of glutathione, increases their sensitivity to heavy metals [Rauser, 1990; Jemal et al., 1998].

Synthesis of glutathione is carried out in two stages. The first of them involves the formation of  $\gamma$ -glutamylcysteine from glutamate and cysteine and is catalyzed by the enzyme  $\gamma$ -glutamylcysteine synthetase (GCS). The second stage involves the connection of  $\gamma$ -glutamylcysteine with glycine and is catalyzed by the enzyme glutathione synthetase (GS) [Meyer, 2008; Estrella-Gomez et al, 2012]. Synthesis of glutathione in a plant cell occurs in chloroplasts and cytoplasm [Mendoza-Cozatl et al., 2002; Grishko, Syshchikov, 2012]. Synthesized mainly in the leaves, it is transported to the root and fruit cells through the vessels of the phloem and xylem [Gomez, Pallas, 2004]. An increase in the activity of glutathione synthesis enzymes (GSH) correlates with increased expression of the corresponding  $\gamma$ -GCS and GS genes, while the amount of glutathione in cells also increases [Xiang, Oliver, 1998; Li et al., 2006]. There is also information that a noticeable increase in the biological synthesis of glutathione under stress is observed mainly with an increase in the expression

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level of both  $\gamma$ -GCS and GS genes. The breakdown of glutathione is catalyzed by  $\gamma$ -glutamyltransferase and is quite closely related to the transmembrane transfer of amino acids, as it results in the formation of  $\gamma$ -glutamyl-derived amino acids and the dipeptide cysteinylglycine [Mendoza-Cozatl, Moreno-Sanchez, 2006].

Glutathione is a precursor of phytochelatin - low-molecular peptides containing a large number of SH-groups [Grill et al, 1985], which ensures plant resistance due to the activation of the metal detoxification mechanism and an increase in the content of glutathione and the synthesis of phytochelatin. One of the most important mechanisms of plant resistance to metal exposure is the detoxification of heavy metal ions in the cell by binding them to thiol SH-groups of some low molecular weight peptides and proteins [Hall, 2002]. Thiol compounds are biologically active substances, the wide range of which is due to the presence of sulfhydryl ( $-\text{SH}-$ ) functional groups, which are highly reactive [Torchinsky, 1971].

From a chemical point of view, the presence of a  $\gamma$ -glutamyl group and a free SH-group in glutathione is of particular interest, which ensure that glutathione performs quite important functions in plants, in particular: participation in the metabolic processes of reduced sulfur, regulation of the expression of genes encoding the formation of some cellular glycoproteins walls and enzymes of lignin synthesis, as well as the synthesis of phytochelatin and other antioxidant systems [Noctor et al., 1998, Loscos et al., 2008].

Oxidized glutathione can enter into a thiol-disulfide exchange with active SH-groups of proteins with the formation of a mixed disulfide complex or can oxidize endogenous SH-groups with the formation of disulfides. Under oxidative stress, reduced glutathione is oxidized to an oxidized state and accumulates in the cytosol. To avoid a shift in the redox balance of the cell, oxidized glutathione can be quite actively removed from the cell or interact with the SH-group of the protein to form a mixed disulfide [Lu, 1999].

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Analysis of the effect of cadmium chloride on changes in the expression of four key enzymes for the synthesis of glutathione, homoglutathione and phytochelatins ( $\gamma$ -glutamylcysteine synthetase), glutathione synthetase, homoglutathione synthetase and phytochelatin synthetase in pea nodules was carried out. Research was conducted using the SGE pea line and the SGECdt mutant, which is characterized by increased accumulation of cadmium in plant biomass and resistance to toxic concentrations of this heavy metal. Also, the SGECdt mutant is able to form symbiotic systems more resistant to cadmium compared to the SGE line. After 4 weeks of growing peas in a medium containing 0.5  $\mu$ M cadmium chloride, no change in the expression level of the GSH1 gene was observed in the original SGE line, while a slight decrease in the expression of this gene was detected in the SGECdt mutant. At the same time, in control conditions, the expression level of the GSH1 gene did not differ in both lines. The expression level of the GSHS gene under control conditions did not differ in the original SGE line and the SGECdt mutant. Treatment of plants with cadmium chloride led to a slight weakening of the expression of this gene in the mutant, while in the SGE line the expression of GSHS increased. The expression of the hGSHS gene increased under the action of cadmium chloride both in the SGECdt mutant and in the SGE line. A comparison of the expression levels of the studied genes showed that the gene hGSHS, which encodes homoglutathione synthetase, was expressed most strongly under control conditions in pea nodules. Treatment of plants with cadmium chloride led to increased expression of the PsPCS gene both in the SGE line and in the SGECdt mutant. At the same time, in control levels of PsPCS gene expression was higher in the original SGE line. Thus, the increased resistance of nodules of the SGECdt mutant line against cadmium chloride is quite likely not related to the nature of the expression of the analyzed genes [Kulaeva, Tsyganov, 2014].

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Glutathione is a rather unique peptide found in cells not only of all eukaryotic organisms, but also of many prokaryotes. Unlike other peptides produced by matrix synthesis or post-translational modification, it has its own metabolic pathway. This compound plays a rather important role in cellular metabolism, taking an active part in maintaining the redox potential, in the detoxification processes of xenobiotics of endo- and exogenous origin, both directly and as a substrate for a number of enzymes of biological transformation. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ( $H^+ + e^-$ ) to other molecules, such as reactive oxygen species, to neutralize them, or to protein cysteines to maintain their reduced forms. When an electron is supplied, glutathione itself becomes reactive and quite easily reacts with another reactive glutathione to form glutathione disulfide [Newton et al., 1996, Smirnov, Sukhovskaya, 2014].

Oxidized and reduced glutathione form a redox pair, which, together with the pairs NADPH/NADP<sup>+</sup>, Trx(SH)<sub>2</sub>/TrxSS (reduced thioredoxin/oxidized thioredoxin) and cysteine/cystine, make up the intracellular redox buffer [Jones et al., 2004]. These ratios are regulated by such enzymes as thioredoxin reductase and glutathione reductase [Kalinina et al., 2008]. The redox status of the cell plays a key role in the regulation of its life. And specifically, in the conditions of a more restored environment, the proliferation process is launched, and a slight shift towards an oxidized environment – differentiation. With a strong shift towards the oxidized state, cell death is induced by the mechanism of apoptosis or necrosis [Schafer, Buettner, 2001; Moriarty-Craige, Jones, 2004].

It has been shown that oxidative stress and a decrease in the ratio of reduced glutathione to its oxidized form inside the cell cause a change in the activity of redox-sensitive enzymes, primarily tyrosine kinases and tyrosine phosphatases, which leads to an increase in the process of tyrosine phosphorylation [Staal et al., 1994; Rao et al., 2000; Forman, Torres, 2002].

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It is known that a violation of the balance of reduced to oxidized glutathione in the cell can lead to the induction of pathological processes, and specifically, cause the oxidation of membrane lipids and a number of target proteins, which in the end can lead to a violation of their functions, as well as the destruction of DNA under the action of radicals, which can eventually lead to cell death. The ratio of reduced to oxidized glutathione can change both due to a decrease in the level of reduced glutathione and due to an increase in the amount of oxidized glutathione. In case of pathological changes, this ratio can be changed both to rather low and to rather high values. At the same time, cells have a number of protective mechanisms aimed at maintaining this balance, for example, ABC transporters and others capable of exporting oxidized glutathione and glutathione-S-conjugates from the cell [Homolya, et al., 2003]. In conditions of oxidative stress, there is an increase in the expression of the enzyme glutamylcysteine synthetase, which is also a compensatory mechanism [Griffith, 1999]. It is also known that reduced glutathione takes an active part in plant resistance reactions against cold stress [Ohno et al., 1991; Kocsy et al., 2001]. Therefore, it is quite likely that the variability of the level of glutathione in tissues during fluctuations in the temperature of the surrounding environment may be associated with a change in the intensity of redox processes and, accordingly, with the amount of ROS [Sukhovskaya et al., 2014].

The process of splitting glutathione is more complex than its biological synthesis and differs significantly from that in mammals by the presence of several pathways for its passage in plants. It was proved that this process takes place along the first path. In this case, the main decomposition reaction is the cleavage of glycine by carboxypeptidase. The dipeptide  $\gamma$ -glutamylcysteine is further degraded to cysteine and glutamine through 5-oxo-L-proline by the sequential action of  $\gamma$ -glutamylcyclotransferase and 5-oxoprolinase [Steinkampf, Rennenberg, 1985]. In plants, the degradation of extracellular glutathione can

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occur without the participation of  $\gamma$ -glutamyltranspeptidase, by direct absorption of glutathione from the environment [Rennenberg, 1982]. In addition to the transport systems in the cytoplasmic membrane, a transport system of reduced glutathione was found in the inner membrane of mitochondria. A proton gradient is necessary to maintain reduced glutathione in the matrix and to transport it into mitochondria - this process is stimulated by ATP. This process plays a rather important role in protecting cells against oxidative damage and cytokinin-induced cell death [Matsumaru et al., 2003, Lash, 2006].

It is known that the peroxisome is the main source of hydrogen peroxide in the cell. There is a significant amount of crystalline catalase in the peroxisome, the main function of which is the neutralization of hydrogen peroxide formed during photorespiration. Later, a glyoxylate amination reaction takes place in the peroxisome. At the same time, glutamate serves as the amino group donor - the glutamate:glyoxylate reaction is catalyzed by aminotransferase. The formed glycine is transferred to the mitochondria, where serine is formed from two of its molecules through the intermediate stage of methylene-tetrahydrofolate. At the same time, carbon dioxide is released, ammonia is formed, which enters the chloroplast, and NADP, which, as a rule, is oxidized in the respiratory ETC with the participation of alternative dehydrogenases of type 2 and alternative oxidase [Peterhansel et al., 2010, Rakhmankulova, 2019]. Ammonia formed in mitochondria is partially released from the cell, and most of it is fixed in chloroplasts, where glutamine is converted into glutamate with the participation of the glutamine synthase/glutamine:oxoglutarate aminotransferase (Fd-dependent) system. Glutamate enters the peroxisome, where it interacts with photorespiratory glyoxylate resulting in the formation of glycine, which then enters mitochondria [Pérez-Delgado et al., 2016]. Since reduced glutathione is not synthesized in mitochondria, it must be transported into them from the cytosol. Previously, transporters for glutathione were identified based on homology with

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highly specific glutathione transporters hgtlp from *Saccharomyces cerevisiae* [Meyer, Rausch, 2008].

The chromatographic method established that the use of ultra-small amounts of coordination compounds of iron, iridium, rhodium, and palladium as catalysts leads to the selective oxidation of glutathione by hydrogen peroxide only to the disulfide form [Lavrov, 2007].

The dynamics of glutathione oxidation by hydrogen peroxide and the composition of the products were investigated by mass spectrometry on an LTQ Orbitrap device (Thermo Scientific, USA) using MS-MS systems. It is shown that as a result of oxidation, a single product with a disulfide bond is formed, which is another advantage over using unitiol as a model substance. At the same time, glutathione has a lower rate of autoxidation by air oxygen, which increases the accuracy of measurements. The effect of low-frequency treatment of the reaction mixture with an alternating magnetic field with a frequency of 7.8 Hertz and an intensity of about 10-2 Hz on the speed of the reaction was also investigated. The volume of the processed reaction mixture was 15 ml, the degree of homogeneity of the magnetic field in the sample volume was no worse than 2%. The concentration of glutathione in the studied solution was 10<sup>-3</sup> M, and the temperature was 18 ± 10 C. Oxidation rate measurements were carried out on a SF-26 spectrophotometer according to the standard method using a color reaction with Ellman's reagent, which fixes the amount of free SH-groups [Berezkina et al., 2015].

The place of de novo synthesis of reduced glutathione is the cytoplasm, and its steps are included in the so-called  $\gamma$ -glutamyl cycle, which consists of six reactions that take place both in the cytoplasm and on the zoanic side of the cell membrane. The direct synthesis of this tripeptide is carried out with the participation of two ATP-dependent reactions. First,  $\gamma$ -glutamylcysteine is synthesized, in which there is a unique  $\gamma$ -peptide bond between the  $\gamma$ -carboxyl

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group of glutamic acid and the  $\alpha$ -amino group of cysteine. This reaction is catalyzed by cytosolic  $\gamma$ -glutamylcysteine synthetase and is rate-limiting in the synthesis of reduced glutathione. Cytosolic glutathione synthetase then catalyzes the binding of  $\gamma$ -glutamylcysteine to glycine to form reduced glutathione. The concentration of reduced glutathione is replenished not only thanks to the process of de novo synthesis, but also due to the functioning of glutathione reductase, which reduces oxidized glutathione to two molecules of its reduced form in the presence of NADPH(H<sup>+</sup>) [Lu, 2013].

In addition to a rather important contribution to antioxidant protection, glutathione also works as a mediator of numerous processes in which the reverse oxidation of cysteine residues in various protein molecules is carried out. It is likely that both non-enzymatic and enzymatic S-glutathioneation, that is, the addition of a molecule of reduced glutathione to these residues with the formation of a disulfide bond, that is, the formation of mixed disulfides, which protects cysteine residues from possible irreversible oxidation to sulfonic acid (Cys–SO<sub>3</sub>H) through stages of sulfenic (Cys–SOH) and sulfinic acids (Cys–SO<sub>2</sub>H), and accordingly protects the proteins themselves from losing the ability to perform their functions. The formation of sulfenic acid is the first stage of thiol group oxidation with the formation of an oxygen-containing intermediate. Sulfenic acid is not stable and usually serves as an intermediate in the oxidation of thiols to disulfides or to sulfinic acid. The processes of reverse and non-reversible oxidation of thiol residues are facilitated by the presence of ROS, mainly hydrogen peroxide and active forms of nitrogen. During the interaction of thiols with hydrogen peroxide, sulfenic acid is formed in the first stage, and when the oxidant is in excess, sulfinic acid is formed, and then sulfonic acid, which cannot be restored under physiological conditions, that is, such protein modification is irreversible [Rehman et al., 2014]. Organic peroxides such as hydrogen peroxide, as well as various natural compounds, for example, lehydroascorbic acid, flavins



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and quinones, or chemical reagents: 5,5'-dithio-bis(2-nitrobenzoic acid), nitrogen compounds, redox dyes, halogens and selenites – can contribute to the oxidation of thiols to sulfenic acid [Jocelyn, 1972, Bindoli, Rigobello, 2013].

Another most significant way of disulfide formation in cells is thiol-sulfide exchange reactions, when two electrons are transferred from a pair of reduced cysteine residues to cysteines already united by a disulfide bond. At the same time, such a bond disintegrates with the formation of two free thiols, but the first pair of thiols is oxidized with the appearance of a new disulfide bond. Enzymes contributing to this exchange carry it out by catalyzing the exchange of disulfide bonds between bonds localized in their own structure and in target proteins. A classic example of such enzymes is disulfide isomerase. Disulfide isomerase is present in significant quantities in the endoplasmic reticulum of eukaryotes and contains a pair of redox-active cysteine residues in the active site. These residues change their status, becoming either free thiols or forming a disulfide bond. In the endoplasmic reticulum, disulfide isomerase catalyzes the oxidation of newly formed proteins and takes an active part in the isomerization of proteins with improperly formed disulfide bonds, achieving the construction of their native structure [Wilkinson, Gilbert, 2004].

The reverse transition of thiol  $\leftrightarrow$  disulfide is a fairly common biochemical redox process that occurs under mild conditions with the formation of a relatively unstable disulfide bond. Such a connection can be formed between the thiol radicals of two independent free thiols located near each other, or in the composition of one protein or two different proteins. The formation of disulfide bonds in polypeptide chains accompanies the formation of the tertiary structure of proteins, but redox changes of sulfhydryl residues are quite important not only for protein folding, but also for the modulation of protein activity and transmission of redox signaling [Novichkova, 2018].

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And another most significant way of formation of disulfides in cells is the reaction of thiol-disulfide exchange, when two electrons are transferred from a pair of reduced cysteine residues to cysteines already united by a disulfide bond. At the same time, this bond disintegrates with the formation of two free thiols and the appearance of a new disulfide bond. Enzymes promoting this exchange carry it out by catalyzing the exchange of disulfide bonds between bonds localized in their own structure and in target proteins. A classic example of such enzymes is disulfide isomerase. It is present in significant quantities in the endoplasmic reticulum of eukaryotes; and at the same time contains a pair of redox-active cysteine residues in the active site. These residues change their status, becoming either free thiols or forming a disulfide bond. In the endoplasmic reticulum, disulfide isomerase catalyzes the oxidation of newly formed proteins or participates in the isomerization of proteins with incorrectly formed disulfide bonds, stimulating the construction of their native structure [Wilkinson, Gilbert, 2004].

The process of S-glutathioneation is accompanied by the formation of mixed disulfides, and if a protein with a modified cysteine residue in the form of a sulfenic acid reacts, reduced glutathione takes an active part in the reaction. The importance of the S-glutathione process is based not only on its protective role, but also on the fact that similar reverse modification of proteins due to the sensitivity of cysteine residues, which is carried out under the conditions of a change in the redox status of the cell, entails a change in the activity of the modified protein, which is a response to a change in its microenvironment. Depending on what functions the protein has, as a result of S-glutathionylation, bound with it, metabolic processes are either activated or inhibited, and the effect on protein-protein interactions is also revealed. Such changes are directly reflected in numerous signal transmission processes. And especially those responsible for cell survival or death. Among such proteins, we can point to

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proteins that form the cytoskeleton and ion channels, signaling proteins, and more specifically, kinases and phosphatases, transcription factors, ras proteins, heat shock proteins, and others [Lindahl et al., 2011]. It was shown that glutathioneation of the  $\alpha$ 1-subunit of Na,K-ATPase with the help of oxidized glutathione inhibits the activity of the enzyme. Preincubation of the drug with adenosine triphosphate protects the enzyme from inhibition in a concentration-dependent manner with the maximum effect at adenosine triphosphate concentration above 1 mM. At the same time, complete restoration of SS bonds between glutathione and cysteine residues of the  $\alpha$ 1-subunit of the enzyme (deglutathione) cannot be achieved even under the action of strong reducing agents (25 mM tris-2-carboxyethylphosphine and 3% NaBH<sub>4</sub>) under denaturing conditions (6 M urea, 2% sodium dodecyl sulfate) [Dergousova, 2018].

$\gamma$ -glutamyltranspeptidase plays a rather important role in the degradation of glutathione.  $\gamma$ -glutamyl transpeptidase is present in the cells of all living organisms - from bacteria to mammals [Tate, Meister, 1981]. In *Escherichia coli* bacteria,  $\gamma$ -glutamyl transpeptidase is a soluble periplasmic protein [Suzuki et al., 1986], and in addition, these bacteria are capable of assimilating exogenous  $\gamma$ -glutamyl peptides [Suzuki et al., 1993]. The only known exception where  $\gamma$ -glutamyl transpeptidase is associated with the inner surface of the cytoplasmic membrane is *Neisseria meningitides* [Takahashi, Watanabe, 2004].  $\gamma$ -glutamyl transpeptidase interacts with glutathione and other  $\gamma$ -glutamyl compounds to form an enzyme-bound  $\gamma$ -glutamyl residue and L-cysteinylglycine. With the participation of dipeptidase, cysteinylglycine is then decomposed into cysteine and glycine, which are absorbed by the cell. The  $\gamma$ -glutamyl form of tryptidase can interact with amino acids, water, or with another glutathione molecule. If the acceptor of the  $\gamma$ -glutamyl group is water, then hydrolysis occurs [Meister, Anderson, 1983; Suzuki et al., 2001; Tate, Meister, 1981].

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During the interaction of selenite with two molecules of reduced glutathione, selenodiglutathione (GS—Se—SG) is formed, which is a key product in further ways of selenium conversion in the cell. It can either be reduced with the participation of glutathione peroxidase to labile selenium persulfide in the presence of an excess of glutathione, or catalytic oxidation of selenodiglutathione with the release of intracellular selenite is possible, which depends on the ratio of the concentration of selenite and glutathione in the cell. At low concentrations of selenite, a reaction of catalytic oxidation of glutathione occurs. At high concentrations of selenite, hydrogen selenide is formed. It can form on proteins. Selenodiglutathione can also interact with other thiols, especially with sulfhydryl groups of some proteins. These reactions explain the toxicity of large doses of selenium for biological organisms. Hydrogen selenide, as one of the toxic forms of selenium, can be oxidized to elemental selenium by air oxygen or undergo methylation with the formation of volatile selenium compounds. Hydrogen selenide can also be released from the cell under anaerobic conditions. At the same time, it can be attached to an acid-labile protein such as albumin and be included in the process of synthesis of selenium-containing amino acids - methionine and cysteine. Both of these ways are the mechanism of selenium detoxification by the cell [Gracheva, Ivanova, 2006].

Sequences of genes that control the biological synthesis of glutathione and their role in various biological processes have been found for many plant species, including field peas [Matamoros et al., 1999; Moran et al., 2000]. A comparison of the expression levels of the studied genes showed that the hGSHS gene encoding glutathione synthetase was expressed most strongly under control conditions in pea nodules. At the same time, increased expression of the PsPCS gene encoding phytochelatin synthase was detected in pea nodules [Kulaeva, Tsyganov, 2014].

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In the conditions of a low rate of glutathione recycling due to the limitation of glutathione reductase during oxidative stress, maintenance of its reduced pool can be carried out due to the adaptive growth of the total pool of this compound. The possibility of increasing the content in chloroplasts has also been shown for carotenoids [Balalaeva, Chernysheva, 2003, Balalaeva, 2004].

**CHAPTER 2. ECOLOGICAL MANAGEMENT, METHODS OF  
DETERMINING GLUTATHIONE CONTENT**

The literature offers several ways to determine glutathione in plants.

The total content of glutathione (GSht) and the content of oxidized glutathione (GSSG) is determined by the method of enzymatic recycling according to a modified method [Griffith, 1980]. For this, plant material ( $m = 0.2$  g) is homogenized in a 5% solution of sulfosalicylic acid ( $V = 1.5$  ml) cooled to  $2-4^{\circ}\text{C}$ . The homogenate is centrifuged for 10 minutes at  $20,000$  g ( $4^{\circ}\text{C}$ ), and the resulting supernatant is immediately used for analysis. Three working solutions –  $0.3$  mM NADP·H,  $6$  mM 5,5'-di-thiobis(2-nitrobenzoic acid) (DTNB) and  $50$  units/ml of the preparation GR – prepared in  $125$  mM Na-phosphate buffer (pH  $7.5$ ) containing  $6.3$  mM Na<sub>2</sub>EDTA. The standard incubation mixture for determining the content of GSht and GSSG consists of  $700$   $\mu\text{l}$  of a solution of  $0.3$  mM NADP·H,  $100$   $\mu\text{l}$  of  $6$  mM DTNB solution and  $200$   $\mu\text{l}$  of supernatant. Determination of GSSG content is carried out after the procedure of binding GSSG with 2-vinylpyridine. To do this,  $2$   $\mu\text{l}$  of 2-vinylpyridine is added to  $200$   $\mu\text{l}$  of the supernatant, then the samples are mixed to form an emulsion and the mixture is incubated for 1 hour at room temperature. The reaction is initiated by adding  $10$   $\mu\text{l}$  of GR solution ( $50$  units/ml) to the incubation medium at  $30^{\circ}\text{C}$ . The absorption intensity of the 5-thio-2-nitrobenzoic acid (TNB) solution is recorded after 30 minutes at  $412$  nm. The preparation GSSG is used to construct the calibration curve. The GSH content is calculated by subtracting the GSSG content from the GSht content ( $\text{GSH} + 2 \text{GSSG}$ ). The content of GSht, GSSG and GSht is expressed in nmol/g of raw weight.

Glutathione in a weakly alkaline environment with mercury acetate gives a precipitate of mercury glutathione:  $2\text{C}_{10}\text{H}_{16}\text{O}_6\text{N}_3\text{SH} + \text{Hg}(\text{CH}_3\text{COO})_2 =$

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$\text{Hg}(\text{C}_{10}\text{H}_{16}\text{O}_6\text{N}_3\text{S})_2 + \text{CH}_3\text{COOH}$ . The precipitate is washed with water and dissolved in hydrochloric acid:  $\text{Hg}(\text{C}_{10}\text{H}_{16}\text{O}_{16}\text{N}_3\text{S})_2 + 2\text{HCl} = \text{HgCl}_2 + 2\text{C}_{10}\text{H}_{16}\text{O}_6\text{N}_3\text{SH}$ . The solution is separated from water-insoluble substances, and glutathione, which has passed into the solution, is titrated with a 0.001N solution of potassium iodate in the presence of potassium iodide and starch. Potassium iodide is added in excess to bind mercury ions into a soluble, colorless complex compound. The course of the analysis is as follows. 4 g of raw plant material is weighed and thoroughly ground in a mortar with 2 ml of 0.3 N mercuric acetic acid solution and 2 ml of 30% sodium acetic acid solution. The homogenate from the mortar is transferred to a centrifuge tube, washing the rest with 10 ml of distilled water, mixed with a glass rod and left for 10 minutes for complete sedimentation. After that, it is centrifuged, the solution is separated, and the sediment is washed twice with water in portions of 10 ml with stirring. In the sediment, together with other substances, there is also glutathione, which later dissolves in hydrochloric acid. For this, 10 ml of 1 N hydrochloric acid solution is added to the residue in the test tube and stirred with a glass rod for 5 minutes. Then 1 ml of 20% potassium iodide solution is added, mixed and centrifuged. The centrifuge is transferred to a 100 ml titration flask, and the precipitate is washed with 10 ml of water while stirring. After centrifugation, the solution is added to the first centrifuge. 0.5 ml of starch solution is added to the resulting solution and titrated with 0.001 N  $\text{KIO}_3$  solution until a permanent blue color appears. 1 ml of 0.001 N  $\text{KIO}_3$  solution corresponds to 0.307 mg of glutathione. Glutathione content is calculated by the formula:

$$X = \frac{30700 \cdot a \cdot K}{n}$$

where X is the content of glutathione in mg per 100 g of the substance under study; a – the volume of 0.001 n of potassium iodate solution spent during titration of glutathione in ml; K – normality of potassium iodate solution; n – weight of the

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substance under investigation in g; 30700 is the normal titer of glutathione in mt, multiplied by 100 to convert to 100 g of the substance [Pochynok, 1976].

Modified version. To detect the level of total glutathione, a reaction mixture containing K,Na-phosphate buffer, 0.15 mm EDTA, 0.3 mm 5,5-dithiobis(2-nitrobenzoic acid) and 1.5 units of active glutathione reductase is used. The reaction is initiated by the addition of 80  $\mu$ M NADPH and the result is evaluated by the change in optical density at 412 nm. To determine the amount of oxidized glutathione before the analysis, 2-vinylpyridine is added to the plant extract. The level of reduced glutathione is calculated as the difference between the total content and its oxidized amount [Griffith, 1980].

To determine the reduced form of glutathione, a 20% homogenate of plant tissues prepared in a 0.3 M potassium phosphate buffer is used with a pH of 7.5 (4.26 g of  $K_2HPO_4$  dissolves in 100 ml of  $H_2O$ ). The resulting homogenate is centrifuged for 20 minutes at 6,000 rpm. 3 ml of the precipitation reagent is added to 2 ml of the supernatant (100 ml of which contains 1.67 g of  $HPO_3$ , 0.2 g of Trilon B, 30 g of NaCl) and repeated centrifugation is carried out for 10 minutes at 6,000 rpm. Then 2 ml of 0.3 M potassium phosphate buffer, 0.05 ml of 1 mM Ellman's reagent solution, 2 ml of the obtained supernatant are added to the cuvette, and the optical density is measured at 412 nm on a KFK-3 photoelectrocolorimeter [Gryshko, Syshchikov, 2002].

Weights of 0.5 g of barley or tobacco leaves, as well as 0.5 g of rat liver are ground in a porcelain mortar in 4 ml of a mixture containing 0.1 M of potassium phosphate buffer at pH=8.0 and a 25% solution of metaphosphoric acid in the ratio 3.75:1 by volume. The homogenate is first centrifuged for 10 minutes at 8,000 g, after which the supernatant is re-centrifuged for 5 waves at 13,000 g. The resulting supernatant is used for quantitative determination of glutathione. To determine reduced glutathione, the pH of selected extracts is brought to 8.0 with the help of sequential addition of 0.1 M potassium phosphate buffer at pH=8.0,



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namely: 4.5 ml of buffer is added to 0.5 ml of supernatant. After that, 1.8 ml of 0.1 M potassium phosphate buffer at pH=8.0, containing 0.005 M EDTA, 0.1 ml of orthophthalaldehyde solution is added to 0.1 ml of the diluted addition liquid, and after 15 minutes of incubation at room temperature, the fluorescence intensity is recorded. For quantitative study of reduced glutathione, it is necessary to construct a calibration graph using commercial glutathione. The dependence of the fluorescence intensity of the conjugate of orthophthalaldehyde with reduced glutathione, registered at the maximum when recording the fluorescence kinetics, is directly proportional to the concentration of reduced glutathione and linear in the range of its concentration from 0.2 to 2 µg/ml. The sensitivity of the determination is 3.6 pM of reduced glutathione. To determine the oxidized glutathione, 0.2 ml of 0.04 M N-ethylmaleimide is added to 0.5 ml of the supernatant and incubated for 30 minutes to prevent the oxidation of reduced glutathione to oxidized glutathione. Then 4.3 ml of 0.1 N caustic soda solution is added. Then, 1.8 ml of 0.1 N caustic soda solution is added to 0.1 ml of the resulting mixture, 0.1 ml of orthophthalaldehyde is added, and the fluorescence intensity at 420 nm is recorded after 5 minutes. For the quantitative study of oxidized glutathione, it is necessary to construct a calibration graph using oxidized glutathione from the company "Fluka". The sensitivity of the determination is 17 pM of oxidized glutathione [Shalygo et al., 2007].

Determination of the total content of glutathione, as well as the reduced and oxidized form, is carried out spectrophotometrically [Zang, Kirkham, 1996]. The content of total glutathione in the samples is estimated during the color reaction during the formation of a complex with 5,5'-dithiobis-2-nitrobenzoic acid (DTNBA) and reduced glutathione. To evaluate the content of oxidized glutathione, 2-vinylpyridine is used, which binds to reduced glutathione. The following reagents are required to perform the task: 1) 5% sulfosalicylic acid (dissolve 1 g of sulfosalicylic acid in 20 ml of deionized water); 2) 0.5 M sodium

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phosphate buffer (pH 7.5) is prepared using the following solutions: dissolve 1.55 g of  $\text{NaH}_2\text{PO}_4$  in 50 ml of distilled water, dissolve 0.7 g of  $\text{Na}_2\text{HPO}_4$  in 10 ml of distilled water. To prepare 100 ml of buffer, mix the corresponding amounts of stock solutions (40.5 ml of  $\text{NaH}_2\text{PO}_4$  and 8 ml of  $\text{Na}_2\text{HPO}_4$ ) and make up to 100 ml with distilled water. Check and, if necessary, adjust the pH using 5% NaOH or concentrated  $\text{H}_3\text{PO}_4$ ; 3) 0.18% solution of Na-EDTA (dissolve 36.8 mg of Na-EDTA in 20 ml of 0.5 M sodium phosphate buffer); 4) 0.12% solution of 5,5-dithiobis(2-nitrobenzoic acid) (dissolve 12 mg of 5,5-dithiobis(2-nitrobenzoic acid) in 10 ml of ethanol); 5) 0.16% NADPH solution (dissolve 1.6 mg of NADPH in 1 ml of deionized water).

Grind a portion of callus tissue (250 mg) in 1 ml of 5% sulfosalicylic acid solution and centrifuge for 5 min at 10,000 g. Add 0.375 ml of 0.5 M sodium phosphate buffer (pH 7.5) to 25 ml of the supernatant, then add 12  $\mu\text{l}$  of distilled water (this sample must be used to determine the total glutathione content). Add 0.375 ml of 0.5 M sodium phosphate buffer (pH 7.5) to 0.25 ml of the supernatant, then add 12  $\mu\text{l}$  of 2-vinylpyridine (97% 2-vinylpyridine, stabilized 0.1% 4-tertbutylcatechol) to mask the reduced form of glutathione. Close the lid tightly and shake well to form an emulsion, then incubate at room temperature in the dark for 1 hour under draft. This sample is used to determine the content of oxidized glutathione. The reaction mixture for 10 samples contains: 6 ml of 0.18% Na-EDTA solution in phosphate buffer; 1 ml of 0.16% NADPH solution; 2 ml of 0.12% solution of 5,5-dithiobis(2-nitrobenzoic acid). To carry out the reaction, immediately before the measurement, add 0.9 ml of the reaction mixture to the test tube, and then add: 1 ml of extract, 2  $\mu\text{l}$  of glutathione reductase. The control cuvette contains all reagents except for the extract and glutathione reductase enzyme. Healed 1 time for all samples. Optical density is measured every second for 1 minute at a wavelength of 412 nm.

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Construction of calibration curve. To construct the calibration curve, stock solutions of oxidized and reduced glutathione are used: 100  $\mu\text{M}$  of reduced glutathione (dissolve 3 mg of reduced glutathione in 10 ml of mQ); 100  $\mu\text{M}$  of oxidized glutathione (dissolve 3 mg of oxidized glutathione in 5 ml of mQ). Glutathione is added to the reaction mixture instead of the extract in the specified concentration: 1  $\mu\text{M}$  - 10  $\mu\text{l}$  from the stock solution; 5  $\mu\text{M}$  - 50  $\mu\text{l}$  of stock solution; 10  $\mu\text{M}$  – 100  $\mu\text{l}$  of stock solution; 20  $\mu\text{M}$  – 200  $\mu\text{l}$  of stock solution. In the future, the process takes place according to the procedure described above. Based on the obtained values of the optical density, a calibration curve is constructed in the coordinates optical density-glutathione concentration.

Calibration curves are used to calculate the concentration of reduced and oxidized glutathione. The concentration of oxidized glutathione is determined by a calibration curve constructed from known concentrations of oxidized glutathione. The optical density for determining the content of reduced glutathione is calculated as  $\Delta D = D_{\text{total}} - D_{\text{oxidized}}$ , where:  $\Delta D$  is the optical density for determining the content of reduced glutathione;  $D_{\text{total}}$  – optical density obtained by measuring the total content of glutathione in the sample;  $D_{\text{oxidized}}$  is the optical density of the sample in which the content of oxidized glutathione is measured. In the future, with the help of the obtained optical density, the concentration of reduced glutathione is determined according to the calibration curve constructed based on known concentrations of reduced glutathione. To calculate the reduced and oxidized glutathione in the sample, the following formula is used:

$$K = (A \cdot V \cdot X) / (m \cdot \Delta m),$$

where: A – concentration of glutathione,  $\mu\text{mol/ml}$ , V – volume of extract, ml, X – dilution (if 100  $\mu\text{l}$  of extract is added to the final volume of the reaction mixture of 1002  $\mu\text{l}$ , then the dilution will be equal to 10.02); m is the weight of the weight;  $\Delta m$  is the ratio of dry mass to raw mass; K is the content of the reduced form of

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glutathione,  $\mu\text{mol/g}$  of dry weight. Glutathione activity is measured in several (at least three) biological and analytical replicates. The standard error is calculated in the Microsoft Office Excel program [Zang, Kirkham, 1996].

To determine the extracellular amount of reduced and oxidized glutathione, 2.5 ml of a culture sample is taken and passed through a MFAS-OS-2 membrane filter with a pore diameter of 0.45  $\mu\text{m}$ , and the resulting filtrate is used in the reaction. The essence of the method is based on the interaction of reduced glutathione with the sulfhydryl reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent) with the formation of a colored product absorbing in the region of 412 nm. Oxidized glutathione in the process of this reaction is released again thanks to the presence of NADPH and glutathione reductase in the reaction mixture. The reduced glutathione again interacts with DTNB to form a colored product, the amount of which depends on the reaction time and is proportional to the concentration of glutathione in the reaction mixture. The cyclic nature of the enzymatic reaction gives this method a fairly high sensitivity and specificity, allowing to determine up to  $1 \times 10^{-9}$  g of glutathione in the sample. The reaction mixture contains 0.6  $\mu\text{M}$  DTNB, 10  $\mu\text{g}$  glutathione reductase and 0.2  $\mu\text{M}$  NADPH in 1 ml of 0.1 M Na-phosphate – 0.005 M EDTA buffer with pH 7.5. Before starting the reaction, the basic absorbance at  $\lambda=412$  nm is measured on a Bio-Rad SmartSpec Plus spectrophotometer (USA). The reaction begins immediately after the addition of NADPH. After 6 minutes of incubation at 25°C, the absorbance at  $\lambda$  412 nm is measured. All reagents, except glutathione, are added to the control cuvette. Calibration solutions contain from 1 to 100 ng of reduced or oxidized glutathione. In the course of the described reaction, the total amount of reduced and oxidized glutathione is determined. For their separate determination, reduced glutathione is preliminarily bound with an excess of sulfhydryl reagent N-ethylmaleimide (NEM), which is added to the sample to a final concentration of 2 mM. After a 40-minute incubation, excess NEM is

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removed by 10-fold ether extraction. The reaction mixture is freed from traces of ether by blowing with air under pressure. The concentration of reduced glutathione is determined by the difference between the concentrations of total and oxidized glutathione [Tietze, 1969].

### **3.1 Accumulation of glutathione in plants**

The content of reduced and oxidized glutathione in plants changes under the influence of various factors [Creissen et al., 1999, Camera, Picardo, 2002]. A number of works carried out on plant cells showed that the process of expression of genes sensitive to the accumulation of glutathione in the nucleus and its reduction in the cytoplasm [Voehringer et al., 1998]. The reduction of the restored potential in the cytoplasm and its growth in the nucleus affects not only gene expression, but also the ability of proteins to bind to their targets in the nucleus. It was established that at the beginning of the G1 phase of the mammalian cell cycle, the activation of oxidation processes in the cytoplasm, caused by epidermal growth factor, leads to the accumulation of ROS, which stimulates the activation of cascade phosphorylation processes and leads to the activation of DNA replication and the initiation of cell division [Carpenter, Cohen, 1990 ].

A different concentration of glutathione is also characteristic of intracellular structures. According to scientific publications, the main pool of glutathione in plant cells is located in the cytosol, chloroplasts and mitochondria. While the presence of glutathione in the vacuolar compartment is still questionable, and according to some researchers, it may be tissue-specific or species-specific [Fricker et al., 2000, Hartmann et al., 2003, Noctor et al., 2011, Pradedova et al. , 2015]. It was also established that the compartmentalization of glutathione in cells is significantly influenced by environmental factors. In some cases, under stressful conditions, glutathione was transported in the vacuole,

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which led to a multiple increase in its concentration in this compartment [Queval et al., 2011].

One of the most important functions of reduced glutathione is the reserve and preservation of cysteine, because this amino acid is extremely unstable in extracellular conditions and is quickly oxidized to cystine in processes whose products are potentially toxic ROS. There is a  $\gamma$ -glutamic acid cycle that allows the use of reduced glutathione as a continuous source of cysteine [Meister, 1988].

In millimolar concentrations, glutathione is present in the cells of all gram-negative and some gram-positive bacteria, where it plays a key role in protection against oxidative and other stresses [Fahey et al., 1978, Smirnova et al., 2001]. Both the compartmentalization and the concentration of glutathione in different organelles change at different stages of the cell cycle [Markovic et al., 2007]. The importance of glutathione in the cell is determined by its antioxidant properties. In fact, it not only protects the cell from such toxic agents as free radicals, but also generally determines the redox-status of the intracellular environment. In addition, glutathione is a substrate for the synthesis of phytochelatins, peptides that play a key role in protecting cells from the effects of heavy metals [Rafikova, Postrygan, 2012].

In table beet vacuoles, glutathione is contained in micromolar quantities and mainly in a reduced state, which correlates with its content in the tissue extract and exceeds its content in the enriched fraction of plastids of root crops [Trukhan, 2013]. The main role of the vacuole in detoxification processes is to deposit some toxic compounds in the form of conjugates with carbohydrates, amino acids, and glutathione [Abhilash et al., 2009]. At the same time, it is assumed that glutathione reductase functions in vacuoles [Edwards et al., 1990; Anderson et al., 1990; Marty et al., 2009, Noctor et al., 2011]. In wheat, two isoforms of this enzyme were detected in leaves, while three isoforms were detected in roots [Yannarelli et al., 2007]. It was also shown that vacuoles contain glutathione in the cells of

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the reserve parenchyma of beet roots, but mitochondria have the largest pool of it among all the studied organelles [Pradedova et al., 2015]. It was found that glutathione is transported in the vacuole mainly in the oxidized form and, according to today's ideas, is degraded inside the vacuole by  $\gamma$ -glutamyltranspeptidases and dipeptidases [Noctor et al., 2011]. Glutathione was also found in mitochondria [Zechmann et al., 2008]. Mitochondria are one of the most important intracellular structures, the scope of which includes not only the provision, transformation and transfer of energy in the cell, but also a number of unique functions. Among them: synthesis of heme, iron-sulfur clusters, steroids, generation of signal molecules, participation in detoxification, heat production, cell proliferation, differentiation and programmed cell death [Zorov, 2008].

It was shown that the chloroplasts of higher plants contain approximately half of the total amount of glutathione in the cell, and its concentration in these organelles is about 5 mmol. Information about its distribution in the rest of the cellular structures and organs of plants is insufficient and does not always coincide [Aristarchov et al., 1992]. It was established that in the elements of the generative sphere of *Acer L.* species, the lowest amount of this tripeptide is found in the seeds. At the same time, it is found 30-420 times more in anthers and pericarps [Hrytsai, 1997]. At the same time, it was established that a lower content of glutathione - less than 1 mmol/g of raw material - is characteristic of the leaves of herbaceous plants. At the same time, in the leaves of most trees, this indicator ranges from 1 to 3 mmol/g of raw material [Bezsonova et al., 1989].

It was noted that the compartmentation of glutathione in plant cells is significantly influenced by environmental factors. In some cases, under stressful conditions, glutathione accumulates in vacuoles, which led to a multiple increase in its concentration [Queval et al., 2011]. Numerous studies have established the selective transport of glutathione to vacuoles by means of ABC transporters of the tonoplast, which mainly transport oxidized glutathione. This fact made it possible

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to predict that the central vacuole makes a significant contribution to maintaining a fairly high redox ratio of reduced to oxidized glutathione [Coleman et al., 1997; Dixon et al., 1998; Zechmann, Müller, 2010; Noctor et al., 2011, etc.]. Glutathione homologues are found in some plants, for example in legumes. They are structurally quite close to glutathione, but in such molecules the glycine residue is replaced by  $\beta$ -alanine, and its synthesis requires the participation of a special hGSH synthetase [Matamoros et al., 1999]. For example, the presence of hGSH in soybeans is more useful than reduced glutathione for the detoxification process of acifluorfen, a xenobiotic used against weeds in soybean crops [Sugiyama et al., 2005]. Another alternative to reduced glutathione is the compound hydroxymethylglutathione, in which the glycine residue is replaced by serine. This compound is found in rice, wheat and barley [Okumura et al., 2003].

Convincing evidence of the presence of glutathione in the apoplast was obtained as a result of the study of the  $\gamma$ -glutamyl cycle, which participates in the recycling of glutathione [Martin et al., 2007]. The concentration of glutathione in leaf homogenates is usually 0.3-0.4 mM. It is also known that it is unevenly distributed between cell compartments. Its concentration is highest in mitochondria, and minimum in vacuoles and apoplast. Thus, the subcellular level of glutathione in *Arabidopsis* leaves is as follows: mitochondria - 9-10 mM, nucleus - 6-10 mM, cytosol and peroxisomes - 3-5 mM, chloroplasts - 1-1.5 mM, vacuoles - 0.01-0.14 mM [Koffler et al. ., 2013]. In the cell walls of *Arabidopsis* leaves, the concentration of glutathione does not exceed 0.03 mM [Ohkama-Ohtsu et al., 2007, Trentin et al., 2015]. One of the criteria for plant resistance to stress is the content of ascorbic acid and glutathione. These are one of the most important protectors of lipid peroxidation processes. Their increase in the autumn period, compared to the summer, in all types of studied plants is evidence that the reaction of the plant organism to the action of stress factors is taking place. [Smirnoff, 2000, Zaiko, Lyholat, 2011].



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We also determined the content of glutathione in different varieties of tomato plants during the growing season (Table 1).

Table 1 – Glutathione content in tomato plants

Varieties	Glutathione content in tomato plant tissues, μg/g:	
	Flowering phase	Fruiting phase
Gentle	4.56 ± 0.24	4.61 ± 0.21
Bobrytskyi	4.64 ± 0.23	4.69 ± 0.20
Borivskyi	4.62 ± 0.22	4.67 ± 0.21
Choirs	4.60 ± 0.21	4.65 ± 0.22
Boyan	4.58 ± 0.24	4.64 ± 0.23
Dawn	4.43 ± 0.27	4.48 ± 0.26
Zoren	4.49 ± 0.28	4.53 ± 0.27
Flora	4.38 ± 0.30	4.41 ± 0.29

It is quite likely that some increase in glutathione content in tomato plants was caused by a 30 C increase in ambient air temperature (a stress factor).

A unique feature of glutathione is the presence of a  $\gamma$ -glutamin bond, which protects this tri-peptide from degradation by intracellular peptidases and a free sulfhydryl (SH-) group, which serves as an electron donor. The one-electron reaction of glutathione with free radicals stimulates the formation of the thiyl radical GS\*, which upon dimerization with another GS\* radical gives glutathione disulfide GSSG [Mannervik et al., 1989]. The second type of redox reactions involving glutathione are thiol-disulfide exchange reactions, which play a central role in the formation of mixed disulfides with proteins (GSSG) [Kosower, Kosower, 1978]. In reactions of the third type, a two-electron oxidation occurs with the formation of an intermediate, which then reacts with a second molecule,

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identical or different from the first. In this case, glutathione disulfide GSSG is formed in the first case, and a mixed disulfide is formed in the second [Cohen, Hochstein, 1963].

A comparison of the rate constants of osmotic compression of root and stem plasmolemma vesicles with the content of SH-groups of membrane proteins revealed a negative correlation between these parameters, i.e., with high osmotic permeability, the dithiol-disulfide balance is shifted toward the oxidized state of SH-groups [Ampylogova, 2007].

The nature of the change in the content of low molecular weight antioxidants ascorbic acid and glutathione in the tissues of the embryo according to the microphenological phases of spring barley seed germination under conditions of optimal moisture is shown. The content of these antioxidants increases in the tissues of the embryo during the germination of spring barley seeds. A significant increase in the content of ascorbic acid and glutathione occurs before seed ringing: ascorbic acid by 5.9 times, and glutathione by 2.4 times. During the entire period of germination - from dry seed to seedling - on average, the content of ascorbic acid increases by 8-10 times, and glutathione - by 4.5 times [Kovalyova, 2011].

A significant difference in the levels of reduced glutathione accumulation and its metabolism in reed plants (*Phragmites communis* Trin.) from ecotypes adapted to different edaphic and climatic conditions was established [Chen et al., 2003].

There are significant differences between the content of ascorbic acid during the growing season and during the transition of plants to a state of rest. The same dependence is observed for glutathione. This indicates the reaction of the plant organism to harmful external factors. In addition, the peculiarities of the activity of enzymes of the glutathione-ascorbine protective system have been established [Zaiko, Lyholat, 2011].

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Glutathione is found in the meristem, conducting bundles and in uninfected parenchyma cells. Glutathione accumulated most intensively around young infectious filaments. Glutathione was not detected in cells where old suberized infectious threads are located. If bacteria were released from the infectious droplet in individual cells and nodules, active accumulation of glutathione was observed in cells containing bacteroids. The localization of glutathione synthetase in the nodule meristem of both original and mutant lines was also shown. It was found that glutathione is involved in the functioning of infectious threads, infectious droplets, and symbioses [Ivanova, Tsyganov, 2015]. It has been shown that the content of glutathione in intracellular layers is about 0.5 mM, but sometimes reaches 10 mM [Al-Turk et al., 1987].

To detect the spatial localization of glutathione, enzymes of its synthesis ( $\gamma$ -glutamylcysteine synthetase, glutathione synthetase) and glutathione reductase in the tissues of the symbiotic nodule of pea, we used the original SGE line and obtained on its basis mutants blocked at various stages of the development of the symbiotic nodule. In the nodules of the original line, the most intense signal associated with glutathione was observed in the zones of infection and nitrogen fixation. In nodules of the sym40 gene mutant (the ortholog of the *Medicago truncatula* EFD gene, which regulates the work of the negative regulator of the cytokinin response in nodules), which are characterized by hypertrophied infectious droplets, the localization of glutathione was also observed in infected cells. In a mutant of the sym33 gene (ortholog of the *M. Truncatula* IPD3 and *Lotus japonicas* CYCLOPS genes, which encode a key transcription factor activating the process of nodule organogenesis), there is no bacterial exit into the cytoplasm of plant cells from "locked" suberized infection threads. In such infectious threads, the signal was associated with the bacteria present in them. If bacteria were released from an infectious droplet in individual cells, a rather active accumulation of glutathione was observed in cells containing bacteroids. In all

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studied variants, the most intense signal was observed around juvenile bacteroids, which testifies to the important role of glutathione specifically at the stage of bacterial exit from infectious droplets and differentiation into bacteroids. In addition, glutathione can participate in protective reactions, the active manifestation of which was shown for mutants of the *sym33* genes and *sym40*. In the nodules of the wild type, an active accumulation of glutathione reductase was observed, compared to enzymes of glutathione synthesis. It can be predicted that in nitrogen-fixing nodules, maintenance of a certain redox status of cells is more important than *de novo* synthesis of glutathione. The expression of genes encoding glutathione and homoglutathione synthesis enzymes in nodules was also investigated: GSH1 ( $\gamma$ -glutamylcysteine synthetase), GSHS (glutathione synthetase) and hGSHS (homoglutathione synthetase). At the same time, a significant increase in the expression of the GSH1 gene was observed, compared to the original line, in all mutant lines. It was also shown that the highest expression level of the GSHS gene is characteristic of the *sym40* gene mutant, and the hGSHS gene is characteristic of the *sym33* gene mutants. The obtained information allows predicting the presence not only of different regulatory mechanisms for the GSHS and hGSHS genes, but also, quite likely, of different functions of these thiols in nodules [Ivanova, Tsyganov, 2016].

Accumulation of glutathione under the influence of low temperatures was found in cells of cold-resistant crops - barley [Radyuk et al., 2009] and wheat [Repkina, 2014], as well as in heat-loving plants of tobacco plants [Cui et al., 2013]. Moreover, the increase in the level of glutathione was due to the synthesis of new molecules and its recovery from the oxidized form [Radyuk et al., 2009; Repkina, 2014]. It has been shown that glutathione takes a fairly active part in the disinfection of excess ammonium in plants [Kretovych, 1971].

A high intracellular content of reduced glutathione is characteristic of dividing cells and meristems, and in aging cells, the content of reduced

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glutathione decreases, and oxidized glutathione increases. Morphogenic calli of Tatar buckwheat preserve genetic stability and the ability to morphogenesis during a long time of cultivation - up to 10 years, while the non-morphogenic calli obtained from them are characterized by polyploidy and aneuploidy, rapid growth, lack of differentiation ability. Glutathione can be one of the internal factors that allows cells of morphogenic cultures to maintain homeostasis under the influence of stressors. It was established that the content of total glutathione in the cells of morphogenic and non-morphogenic calli differed slightly and varied during the passage in the range of 400-600  $\mu\text{M/g}$  of dry weight. The main differences related to the ratio of reduced to oxidized glutathione in the cells: in non-morphogenic calli it varied from 2.8 to 0.48 (until the end of the passage), and in morphogenic calli it was maintained throughout the passage at a sufficiently high level (from 1.5 to 7). It is also important to note that in different lines of morphogenic callus, the cycle of formation of proembryonic cell complexes was characterized by the same dynamics of changes in glutathione content. And therefore, in order to maintain the morphogenic ability associated with the processes of differentiation and dedifferentiation, it is quite important for cultures to maintain a high ratio of reduced glutathione to non-reduced glutathione [Khaertdinova, Rumyantseva, 2013]. It was established that glutathione disulfide and, to a lesser extent, glutathione are transported in the vacuole of plant cells with considerable probability and quite actively [Tommasini et al., 1993]. There is an opinion that vacuoles are involved in the deposition of heavy metal ions with the participation of glutathione derivatives of phytochelatins [Morelli, Scarano, 2004]. In addition, glutathione sulfide actively accumulates in it [Norcton et al., 2011]. At the same time, glutathione and glutathione-S-transferase activity were detected in the vacuoles of fodder beet roots, and therefore it can be predicted that these compounds can conjugate with glutathione inside the vacuole [Pradedova et al., 2010, 2013]. Conjugation of electrophilic xenobiotics and toxic metabolites

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with glutathione is considered as one of the main detoxification mechanisms [Dixon et al., 2010]. It is believed that phytochelatins are indicators of the entry of a number of heavy metals into the cytoplasm [Zeller, Feller, 2000]. It has also been shown that organic acids, phytochelatins and metallothioneins take an active part in the detoxification of heavy metals, but do not determine the ability of plants to hyperaccumulate [Seregin, Kozhevnikova, 2011]. The formation of phytochelatins in the roots of corn seedlings under conditions of cadmium pollution is also noted [Barsukova, 1997]. In phytochelatins, the main load for binding heavy metals is carried out by r-Glu-Cys motifs through complexation. To create transgenic plants to be used in phytoremediation of soils contaminated with heavy metals, a synthetic pseudophytochelatins gene encoding a phytochelatins analog with the formula Met(GluCys)<sub>6</sub>Gly was constructed de novo and cloned. Unlike natural phytochelatins, which are produced enzymatically, this peptide is able to be synthesized by the matrix method. A genetic engineering construct was created based on the binary vector pCAMBIA 1305.1, which carries the studied gene under the control of the viral constitutive 35S promoter. Model transgenic tobacco plants were obtained, in which such a design, upon expression of the pseudophytochelatins gene, caused additional resistance to the action of cadmium ions in concentrations up to 200 μM relative to the non-transgenic control [Postrygan et al., 2011].

The smallest amount of the reduced form of glutathione was recorded in *Salsola soda*, which indicates the possible participation of this compound in the inhibition of free radical oxidation of lipids in this species. For the glycohalophyte *Euphorbia peplus*, the highest amount of glutathione was established, which may be due to the activation of the antioxidant reutilization system against the background of fairly intense free radical processes [Kosakovskaya et al., 2012].

It was established that the change in the content of ascorbic acid and glutathione in seedling tissues during the phases of seed germination of all

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varieties of winter barley for all years of reproduction has general patterns: in general, in all varieties, it increases from dry seeds to the appearance of a full-fledged seedling, but the curve acquires two minima. as the content of ascorbic acid and glutathione in the microphenophase "roots" of interest to researchers and "sprout" does not decrease significantly. And therefore, when the main charging root breaks through the coliorhiza, and then the second and third roots appear, but all of them are up to 2 mm long, there are significant changes in the activity of the antioxidant system in the tissues of the seedlings, which can be a reproduction of the restructuring of the metabolism as a whole. From the moment of seed ringing, which is considered the point of seed germination, to the "root" microphenophase, on average, for all studied varieties, the content of ascorbic acid decreases by 2 times (from 10 to 5  $\mu\text{g/g}$ ), and the content of glutathione - by 1.2 times (from 59.3 to 48.6  $\mu\text{g/g}$ ). Then, in a short period of time from the microphenological phases of "roots-1" to the microphenological phases of "roots-2" (short roots), the content of ascorbic acid increases by 3 times (from 5 to 14.8  $\mu\text{g/g}$ ), and the content of glutathione - by 2 times (from 48.6 to 93.4  $\mu\text{g/g}$ ). In the "root" microphenological phase, when a coleoptile less than half the length of the seed appears in the presence of growing roots from the flower scales of the seed, a secondary decrease in the content of ascorbic acid and glutathione occurs. From the previous phase of "roots-3" (long roots) to the microphenophase of "sprout", on average, the content of ascorbic acid decreases by 2 times (from 18.5 to 9.4  $\mu\text{g/g}$ ), and the content of glutathione - by 1.9 times (from 138.5 to 72.8  $\mu\text{g/g}$ ). Then, in a short period of time from the "sprout" microphenophase to the "sprout" microphenophase, the content of ascorbic acid increases by 3.3 times (from 9.4 to 31.3  $\mu\text{g/g}$ ), and the content of glutathione - by 1.6 times (from 72.8 to 116.3  $\mu\text{g/g}$ ). Thus, the revealed patterns of changes in the amount of ascorbic acid and glutathione during the microphenophases of winter barley seed germination indicate that the microphenophases of "roots" and "sprouts", which are short in time, are points of

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change in the activity of the ascorbate-glutathione cycle, while the consumption of ascorbic acid and glutathione initially exceed their synthesis/regeneration, and then their content is rapidly restored. This can also show the orientation of physiological and biochemical processes during the period of seed germination and seedling growth. It is obvious that it is during these phases that the metabolism is restructured, and therefore it is necessary to find the key limiting factors of growth processes [Kazakova, 2017].

It was established that the complex of adaptive reactions of the studied tree introducers is reduced to the following groups: 1) the main component of the antioxidant system is low-molecular antioxidants - ascorbic acid and glutathione, their level is high enough and increases under stress, the activity of ascorbate peroxidase remains at the control level; 2) with technogenic stress, the level of ascorbic acid and the activity of ascorbate peroxidase increase, while the concentration of glutathione, which most likely performs the function of ascorbic acid recovery, decreases; 3) under conditions of stress caused by motor vehicle emissions, the concentration of ascorbic acid and glutathione increases, the activity of ascorbate peroxidase increases, that is, the ascorbate-glutathione cycle is the main factor in the inactivation of ROS; 4) in conditions of man-made stress, the pool of low molecular weight antioxidants ascorbic acid and glutathione is depleted and at the same time the activity of ascorbate peroxidase increases; 5) the activity of the studied components of the ascorbate-glutathione cycle remains unchanged under man-made stress caused by highway emissions [Gorelova et al., 2013]. It was found that under conditions of osmotic stress, the content of thiol compounds in beetroot corms changes, which can be used as a stress diagnosis [Nesterkina et al., 2013].

It was shown that the oxidized form of glutathione in chloroplasts can participate in the allosteric regulation of Calvin cycle enzymes - phosphoribulokinase and sedoheptulose-1,7-bisphosphate phosphatase



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[Rennenberg, 1982]. It was found that the main role in these processes is played by the level of the oxidized form of glutathione in the cell during the dark phase of photosynthesis [Foyer, Halliwell, 1976]. In addition, glutathione takes an active part in such an important physiological and biochemical process as sulfur metabolism. In most sulfur-containing organic compounds, this element is in a reduced form accessible to plants. However, it is absorbed by the roots mainly in the form of sulfates, since less oxidized ( $\text{SO}_2$ ) or more reduced ( $\text{H}_2\text{S}$ ) inorganic sulfur compounds are toxic to plants [Polevoy, 1989, Herschbach, Rennenberg, 1994, Schwenn, 1994]. All this means that the sulfate that entered the plant is reduced in the chloroplasts of the leaves through the formation of cysteine as the first stable product in which organic sulfur is in a reduced form. In this form, it can move through the phloem to the roots to meet the plant's nutritional needs. In this sense, glutathione is considered as one of the long-distance transport forms of sulfur. After all, more than 40% of the sulfate entering the cells is converted into glutathione, 99% of which is taken outside the cells [Shevyakova, 1979, De Kok, 1986]. Then this peptide can be decomposed with the release of reduced sulfur, which is involved in further cellular metabolism [Rennenberg, 1982].

In the ripening grain of wheat, lipoxygenase, which is capable of oxidizing SH-groups of proteins, and the system - NADP, NADP-dependent glutathione reductase, glutathione and protein-disulfide reductase, which catalyzes the reactions of restoration of SS bonds in protein molecules and the regeneration of oxidized glutathione, are present. The dynamics of protein-disulfide reductase activity in the course of wheat grain development is quite closely correlated with the activity of glutathione reductase ( $r = 0.76$ ), as well as with the content of reserve proteins of SS bonds and SH groups. Three molecular forms of protein-disulfide reductase and two glutathione reductases are most likely present in fully mature soft spring wheat kernels. At the same time, it was established that the specific activity of protein-disulfide reductase does not depend on the type of

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glutathione reductase [Trufanov et al., 1993, Permyakov, 2004]. It has been established that the activities of thiol:oxygen oxidoreductase and glutathione-dependent protein disulfide oxidoreductase are present in the ripening grain of wheat, which respectively catalyze the formation and restoration of disulfide bonds in reserve proteins. Glutathione-dependent protein disulfide oxidoreductase of grain was isolated, purified to electrophoretic homogeneity and characterized. This enzyme has a molecular weight of about 167 kDa and consists of two subunits, about 73 and 77 kDa. It was established that wheat grain disulfide reductase has enzymatic characteristics similar to enzymes from animal tissues belonging to the superfamily of thiol oxidoreductases. The influence of disulfide reductase on the physical properties of gluten indicates the specificity of this enzyme for the intermolecular SS-bonds of glutenin, which distinguishes it from the thioredoxin h known in grains [Osypova, 2011].

In transgenic tobacco plants under conditions of low temperature, the level of both total and reduced ascorbate increased while the level of its oxidized form remained unchanged, which is most likely caused mainly by de novo synthesis of reduced ascorbate. It was also shown that along with the increase in ascorbate content in transformants under stress conditions, there is a significant increase in glutathione content. In Fe-superoxide dismutase transformants under low-temperature stress, the amount of reduced glutathione increased by 70%, and the level of oxidized glutathione increased by 30%, compared to the control. An increase in the level of total glutathione with a simultaneous increase in the content of its reduced form indicates de novo synthesis of the latter [Pavlyuchkova, 2010]. Information was also obtained on the increase in the content of reduced glutathione during cold adaptation of Scots pine [Wingsle et al. 1999], strawberries [Luo et al., 2011], barley [(Radyuk et al., 2009] and many other types of plants. Usually, indicators of the content of reduced glutathione and ascorbate are mutually dependent [Galiba, 2013].

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It has been proven that glutathione and phytochelatin take an active part in the binding of toxic ions in the cytoplasm of cells with the formation of complexes, thereby ensuring the resistance of plants against cadmium [Sanità di Toppi, Gabbrielli, 1999]. Phytochelatin are low-molecular peptides, unlike metallothioneins, which are not gene products and are synthesized from glutathione with the participation of phytochelatin synthase [Grill et al., 1985; Rauser, 1999; Seregin, 2001, 2009; Clemens, 2001; Lee et al., 2002; Nakasawa et al., 2002; Schat et al., 2002; Heiss et al., 2003; Souza, Rauser, 2003]. It was shown that in plant cells stressed by heavy metals, in the process of transferring the ions of these metals through the plasma membrane, the SH-groups of carrier proteins are able to retain part of the ions, performing a barrier function [Garmash et al., 2008].

The participation of phytochelatin in the long-distance transport of copper ions in calendula plants was confirmed, especially with relatively low copper content in the environment. It is likely that at high concentrations of this heavy metal, the need for chelating agents in the cytosol increases, which limits the possibility of phytochelatin participating in the long-distance transport of copper ions throughout the plant. In addition, it is known that phytochelatin form high-molecular complexes with heavy metal ions in the vacuole, thereby isolating toxic ions from the metabolically active cytosol [Volkov et al., 2009]. In most higher plants, up to 90% of metals are detoxified thanks to phytochelatin, small cysteine-rich peptides [Gekeler et al., 1988, Grill et al., 1988].

At the same time, glutathione is a precursor of phytochelatin, and it can also take part in the chelation of metal ions. It was found that in the presence of cadmium, the amount of glutathione in plant cells significantly decreased, and more strongly, by 2.4 times in the leaf, which is most likely connected with the consumption of its molecules for the synthesis of phytochelatin. However, its content in the leaves remained almost 2 times higher than in the root. The noted

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effect can be explained by the fact that under normal environmental conditions, leaves synthesize glutathione in excess, then export it to other tissues. In conditions of increased concentrations of heavy metals in the external environment and in the plant, the export of glutathione decreases, maintaining its relatively high content in the leaf [Heiss et al., 2003]. At the same time as the level of glutathione in plant cells decreased in the presence of cadmium, the content of phytochelatins increased sharply. At the same time, their number was higher in the leaf than in the root. A more noticeable increase in the concentration of phytochelatins in the leaves, compared to the roots, despite their lower potassium content, was previously noted in corn [Wójcik, Tukiendorf, 1999], barley [Akhter et al., 2012] and tobacco [Wojas et al., 2008]. The authors believe that all this is a rather important mechanism of plant metal resistance, which provides protection of mesophyll cells against toxic ions and allows maintaining the photosynthetic apparatus at the required level. Proven studies have confirmed that the green sedge has the ability to successfully vegetate and develop in conditions of increased content of cadmium ions in the soil, forming a significant biological mass, and what is especially important for phytoremediation, accumulates ions of this metal in significant quantities. Judging by the information obtained, a rather important role in the resistance of this plant against cadmium is played by phytochelatins, the content of which increases significantly in the roots and leaves. A particularly sharp, almost order-of-magnitude increase in the level of phytochelatins occurs in the leaf, which probably contributes to the active binding of cadmium ions and the protection of mesophyll cells against toxic effects [Kaznina et al., 2014].

The effect of dextrorubicin on glutathione concentration, activity of DNA-repairing metabolic pathways and Yap1-dependent redox-sensitive pathways was studied using the eukaryotic cell model *Saccharomyces cerevisiae*. As a result, it was demonstrated that dextrorubicin causes an increase in the concentration of reduced glutathione in *Saccharomyces cerevisiae*, an increase in the intracellular

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concentration of oxidized glutathione and the total content of the sum of oxidized and reduced glutathione in the cell, but at the same time causes a decrease in the ratio of reduced to oxidized glutathione. These effects of dexorubicin on glutathione metabolism are at least partially mediated through DNA repair mechanisms [Kryuchkov et al., 2011].

To identify the spatial localization of glutathione in the tissues of the nitrogen-fixing pea nodule and the stages of nodule development at which its synthesis is observed, we used the original SGE pea line and mutants obtained on its basis with abnormalities in the development of symbiotic nodules at different stages. The expression of genes encoding glutathione and homoglutathione synthesis enzymes in symbiotic pea nodules was investigated: GSH1 ( $\gamma$ -glutamylcysteine synthetase), GSHS (glutathione synthase) and hGSHS (homoglutathione synthase). At the same time, it was shown that mutations in the sym33 and sym40 genes, which are characterized by "locked" infectious threads from which no rhizobia emerge, and the formation of hypertrophied infectious threads and infectious droplets, respectively, lead to a significant increase in the expression level of genes encoding these enzymes, compared to the baseline. When analyzing the level of expression of the GSH1 gene, its significant increase was observed, compared to the original line, both in the single mutant of the sym33 gene and in the double mutants of the sym33 and sym40 genes. At the same time, it was shown that the highest level of expression of the GSHS gene is characteristic of the sym40 gene mutant, and the hGSHS gene is characteristic of the sym33 gene mutants. Immunolocalization of glutathione in the tissues of the symbiotic nodules of the original pea line revealed its predominant localization in the zones of infection and nitrogen fixation, while in the sym33 and sym40 mutant lines, glutathione was detected around defective structures or cells. It was previously shown that mutants of the sym33 and sym40 genes are characterized by increased production of ROS in nodules [Ivanova et al., 2014].

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The conducted studies made it possible to establish that at the monitoring sites located on the territory of OJSC "Surikovy Zavod" and OJSC "ArcelorMittal Kryvyi Rih" in areas of heavy pollution, the intensity of oxidation processes in Bolle poplar leaves increased, which led to a statistically significant increase in the content of the reduced form of glutathione by more than 40%, compared to the control. The given information indicates a significant activation of the glutathione-dependent antioxidant system, probably by accelerating the functioning of the redox cycle of the antioxidant by the appropriate glutathione-dependent enzymes and possible intensification of its *de novo* synthesis. The concentration of this tripeptide did not differ from the control in the lightly polluted areas of the specified enterprises. Along with this, a slightly different strategy of deactivation of ROS and their metabolites, which are formed in cells under the action of industrial emissions of both enterprises, was noted in the leaves of the alder tree. Thus, in this species, no statistically significant difference in the content of the reduced form of glutathione in the assimilation apparatus was established in relation to the control at all monitoring sites. The obtained information most likely indicates a significant intensification of the enzymatic reutilization of the studied tripeptide, rather than its active synthesis. Thus, as a result of the research conducted by the researchers, several strategies for the deactivation of ROS and their metabolites, which are formed in cells under the influence of adverse environmental factors, were noted. One of them is the intensification of the accumulation of the reduced form of glutathione during industrial pollution, possibly due to the acceleration of its synthesis. The second is the stabilization of the content of this antioxidant at the level of control due to the activation of the functioning of enzymes for its recovery – glutathione- and ascorbate-dependent reductases. According to researchers, fluctuations in the content of the reduced form of glutathione can be considered as one of the

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indicators of resistance of plants against the effects of industrial pollution [Syshchikov, 2010].

It was established that a statistically significant increase in antioxidants is observed in oat leaves with an increase in the concentration of the fluorine toxicant in the nutrient medium. Thus, the amount of glutathione increases by more than 70% under the influence of the minimum concentration of fluorine (5 mg/l). With an increase in the concentration of this element up to 10 mg/l, a more than 2-fold increase in the content of glutathione is observed, and a further increase in the concentration of fluorine in the growing medium causes an even greater activation (almost 3 times) of the process of synthesis of this antioxidant. A similar dependence was established for corn, but the concentration of glutathione increases at a slightly lower rate (by 30-85%). At the same time, the amount of reduced glutathione increases by 3 times in oat roots, even with a minimal concentration of fluorine. As the concentration of fluorine in the cultivation medium increases, the content of this antioxidant also increases, reaching its maximum at the maximum concentration of the toxicant in the medium. A similar dependence is observed in the roots of corn, although the rate of growth of the amount of antioxidant is much lower than in the leaves (by 12%). However, in terms of absolute values, the content of glutathione in the roots is 2.5 times higher than the similar indicator for the leaves, and therefore the antioxidant is most likely enough to bind the intermediates of the toxic action of fluorine. At the same time, a dose-dependent decrease in glutathione concentration was established in pea roots. Thus, the minimum concentration of fluorine causes the use of 13% of its reduced form, while at 50 mg/l and 100 mg/l in the environment, a decrease in the amount of the antioxidant by 70% and 98% was noted, respectively [Gryshko, 2007].

The conducted studies showed that the total content of glutathione in the non-morphogenic callus was slightly higher than in the morphogenic callus.

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However, in non-morphogenic callus, the oxidized form of glutathione predominated, i.e., the ratio of reduced to oxidized glutathione was always below unity, and in morphogenic callus, the ratio of reduced to oxidized glutathione ranged from 1.5 to 2.5. Under the action of 3-amino-1,2,4-triazole - a specific inhibitor of catalase - the main enzyme that destroys hydrogen peroxide - the total content of glutathione in both cultures decreased already after 1 day of cultivation, and its growth was observed only on the 7th day of cultivation. At the same time, the increase of total glutathione in the non-morphogenic callus was lower than the maximum value in the control, and in the morphogenic callus it was higher than in the control. The influence of 3-amino-1,2,4-triazole was also expressed in a decrease in the ratio of reduced glutathione to oxidized glutathione in both cultures already after 1 day of cultivation. But during further cultivation, if in non-morphogenic callus this indicator decreases to almost zero by the third day, then in morphogenic callus it increased to 2.5, which correlated in morphogenic callus with early activation of glutathione reductase - by 1-3 days. A certain increase in the ratio of reduced glutathione to oxidized glutathione in non-morphogenic callus on the 7th day of cultivation to 0.5 was most likely due to both the activation of glutathione synthesis and a slight increase in the activity of glutathione reductase, probably due to the synthesis of a new isoenzyme. It should also be noted that the content of the oxidized form of glutathione in the non-morphogenic callus was always higher than the reduced form, both in the control and in the experiment. In the morphogenic callus, on the contrary, both in the control and in the experiment, the content of the reduced form of glutathione exceeded the oxidized form. Taking into account the information about viability, which decreased in non-morphogenic callus for 7 days of cultivation by 20%, while in morphogenic callus - only by 10%, it can be argued that the change in the redox status of glutathione reflects the alapsatory capabilities of the culture and is consistent with previously obtained



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results about greater sensitivity of non-morphogenic cultures against various stress factors [Khaertdinova and etc., 2011].

A wide range of fluctuations in the content of reduced glutathione (from 19.2 to 46.6 mmol/g of mass) in the leaves of shrubby plants of the genus *Ribes* L. from regions of different geographical origins was shown [Syshchikov et al., 2009].

The obtained scientific results indicate that the export of reduced glutathione from *Escherichia coli* cells is quite closely related to the transport of electrons along the respiratory chain: the output of glutathione is inhibited when glucose is depleted and stimulated when it is added to the growing culture. The modifying effect of *qor* and *relA* mutations on the export of reduced glutathione indicates the role of the *rkdox* state of quinones and the possibility of the participation of the alarmon (p)ppGpp in the regulation of the transmembrane circulation of reduced glutathione. The revealed parallelism in changes in the export of reduced glutathione and the expression of antioxidant genes may be a consequence of the participation of these different antioxidant systems in the detoxification of ROS produced during respiration. This information confirms that one of the functions of the export of reduced glutathione can be the fight against superoxide in the periplasm of bacterial cells [Tyulenev et al., 2013].

Experimental studies have shown that: 1) inhibition of protein synthesis in *Escherichia coli* cells leads to an increase in intracellular glutathione and glutathione obtained during the division of the *relA* gene, *Escherichia coli*; 2) chloramphenicol stimulates the release of reduced glutathione to a greater extent than valine; 3) mutations in the components of the respiratory chain modify the change in the levels of reduced glutathione when protein synthesis is inhibited; 4) (p)ppGpp and the ArcAB regulatory system can be involved in the control of processes associated with the transmembrane circulation of reduced glutathione [Ushakov et al. 2013]. It was shown that in the growing aerobic culture of

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*Escherichia coli* there is a constant circulation of glutathione between the cells and the environment. As a result of the dynamic balance between the entry and exit of this thiol, a constant concentration of extracellular glutathione per unit of biological mass is maintained. The value of this concentration strictly depends on the pH values of the environment. Treatment of cells with protonophores, ionophores and weak acids, as well as a change in extracellular pH leads to stimulation of glutathione release. A rather close relationship between glutathione export and inorganic phosphate availability was also noted. The authors predict that glutathione circulation may be involved in the regulation of the redox status of the periplasm, the protection of this compartment against oxidative stress, and the regulation of transmembrane ion fluxes [Smirnova et al., 2012].

The ability of salicylic acid to maintain an increased content of reduced glutathione under stress was revealed, probably through the positive regulation of glutathione reductase activity [Daneshmandi et al., 2010].

Quantitative content of reduced and oxidized forms of glutathione in tissues of rye and barley infected with causative agents of brown rust and reticular helminthosporiosis was investigated, the analysis of the obtained results showed that the ratio of reduced to oxidized forms of glutathione can serve as a criterion for assessing oxidative stress arising in plant tissues caused by damage by pathogens and depends from the type of ratios that are included in pathosystems: obligatory or optional [Nedved et al., 2007].

The main reservoirs of glutathione are, firstly, the cytosol, in which almost 90% of it is located, and 10% falls on mitochondria, and a small percentage falls to the fate of the endoplasmic reticulum [Hwang et al., 1992].

The intracellular level of reduced glutathione is quite an important factor on which the cell's ability to enter the path of apoptosis depends. Several aspects related to reduced glutathione should be mentioned here: first, a change in cellular redox homeostasis, namely a decrease in the ratio of reduced to oxidized

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glutathione, either due to the oxidation of reduced glutathione or due to its transport from the cell, which is reflected in the initiation or the performance of the cascade of stages of apoptosis itself, and secondly, there is evidence of belonging S-glutathione to modulate the activity of proteins involved in the activation of cell death [Novichkova, 2018].

The influence of sodium selenite and 4,4-di[3(5-methyldipyrzoly)]selenite (DMDPS) on the microphenological phases of swelling and germination of winter wheat of the "Alay Zorya" variety was studied. The influence of sodium selenite and DMDPS on amylolytic, proteolytic activity on the content of the reduced form of glutathione was studied. The opposite effect of DMDPS and selenite on biochemical processes during grain germination was established: for DMDPS it is stimulating, and for sodium selenite it is inhibiting. Under the influence of sodium selenite, a 30% decrease in proteolytic activity was established, and under the influence of DMDPS - 5%. It was also established that the amylolytic activity of wheat under the influence of DMDPS shows a tendency to reach the same level as in the control "Wheat + H<sub>2</sub>O", however, the maximum is reached 4 hours earlier. In the sample with sodium selenite, a 15% decrease in amylolytic activity was established, compared to the control. The stimulating effect of DMDPS on the accumulation of glutathione has been established. The maximum content of the reduced form of glutathione was noted for a sample of wheat germinated with DMDPS - 8.53 mg%. This is 22% more than the control sample and 36.1% more than the sodium selenite sample. The extreme value of the indicator is reached after 28 hours of germination for the control sample, and after 16-20 hours – for samples with DMDPS and Na<sub>2</sub>SeO<sub>3</sub>. The duration of the microphenological phases of seed germination when using DMDPS as part of soaking water is reduced by 2-4 hours, compared to tap water. The obtained results are used to control the germination process of wheat seeds in the

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production of selenium-enriched grain-based supplements [Glotova, Galochkina, 2017].

Under the conditions of anthropogenic pollution of the reservoir, high levels of reduced glutathione accumulation and significant activation of glutathione-dependent enzymes in the roots and stems of *Phragmites australis* (Cav.) Trin. ex Steud) and *Typha angustifolia* L. indicated the adaptive orientation of metabolic changes. The species specificity of the adaptive strategy consisted in the fact that cattail plants are characterized by relatively more significant activation of glutathione recovery processes and the accumulation of its pool, while reed plants are characterized by an increase in toxicant neutralization processes. The studied features of cattails and reeds are fully consistent with the data on the effectiveness of the use of these species in self-purification processes of water bodies and determine the expediency of further research into the protective metabolic mechanisms of aquatic plants resistant to the effects of toxicants [Krot, 2006].

Exposure of cultivated plants to heavy metals has been shown to decrease glutathione levels [Kukkola et al., 2000; Madhava Rao, Sresty, 2000] and ascorbate [Mishra, Agrawal, 2006] in tissues.

It was found that the *Escherichia coli* mutant, defective in the synthesis of  $\gamma$ -glutamyltranspeptidase, accumulated more glutathione in the medium compared to the parent strain, but retained, albeit to a lesser extent, the ability to reduce the concentration of extracellular glutathione in the stationary phase of growth. All this indicates that  $\gamma$ -glutamyltranspeptidase is not the only mechanism that returns glutathione to cells [Suzuki et al., 1987].

As is known, the resistance of plants against heavy metals is related to the effective operation of cellular mechanisms of their detoxification, among which the binding of metal ions in the cytoplasm by non-protein thiols, such as reduced glutathione and phytochelatins, plays a rather important role. In the conducted

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experiments, cadmium and lead significantly activated the synthesis of glutathione and phytochelatins in *phleum pratense* (L.) plants. Thus, in the presence of cadmium, the amount of phytochelatins in the roots increased by 6 times, compared to the control, and by 12 times in the leaves; in the presence of lead - by 3 and 9 times, respectively of glutathione, testifying to the launch of its synthesis, which also contributes to the growth of metal resistance of the studied plants [Kaznina et al., 2016].

It was shown that a significant part of the copper pool in the cell can be formed by Cu(I) complexes with glutathione [Miras et al., 2008; Poger et al., 2008; Banci et al., 2010]. Reduced glutathione has a fairly high affinity for binding to monovalent copper; the dissociation constant of this Cu(I)–GSH complex is of the order of 10<sup>-11</sup> [Banci et al., 2010]. Glutathione is contained in cells in rather high concentrations - up to several millimoles [Valko et al., 2006], which is almost two orders of magnitude higher than the total copper content in them under normal conditions [Rae et al., 1999]. It is quite likely that the copper ions entering the cell first bind specifically to the molecules of reduced glutathione, since its concentration in the cell exceeds the content of transporter and metallochaperone molecules in it by several orders of magnitude [Maryon et al., 2013]. From the composition of complexes with reduced glutathione, copper can quite easily pass to the comparatively few molecules of metallochaperons; the affinity of metallochaperones to Cu(I) is significantly higher than that of reduced glutathione, and therefore *in vivo* metallochaperones in micromolar concentrations and glutathione in millimolar concentrations have a comparable ability to bind copper [Banci et al., 2010]. In some cases, glutathione can directly participate in the delivery of copper to copper-containing proteins, such as Cu/Zn-Superoxide dismutase [Carroll et al., 2004; Jensen, Culotta, 2005]. One of the most important properties of the copper complex with reduced glutathione is that the copper in it is stabilized in the form of Cu(I), and oxidation-reduction

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transitions  $\text{Cu(I)} \leftrightarrow \text{Cu(II)}$  are impossible until the  $\text{Cu(I)}$  ion associated with a molecule of reduced glutathione [Hanna, Mason, 1992; Milne et al., 1993; Spear, Aust, 1995; Kachur et al., 1998; Pecci et al., 1997; Rigo, 2004]. However, oxidation of thiol complexes with copper can lead to the formation of thiol radicals  $\text{RS}\cdot$ , which then serve as a source of superoxide generation [Spear, Aust, 1995; Pecci et al., 1997]. However, the superoxide radical is much less reactive than the hydroxyl radical, which is formed during the oxidation-reduction of copper ions; in addition, the cell has a sufficient number of superoxide radical detoxification mechanisms, which primarily include superoxide dismutase [Leitch et al., 2009].

Reduced glutathione can potentially serve as one of the important zinc ligands in the cell, as its concentration in the cytoplasm is significantly higher than that of individual free acids, such as cysteine or histidine. It is quite likely that zinc does not form binary  $\text{Zn-GSH}$  complexes, but ternary complexes, the composition of which, in addition to zinc and reduced glutathione, also includes other ligands, for example, histidine or nucleotides [Krezel et al., 2003; Krezel, Bal, 2004; Krezel, Maret, 2006].

When conducting research in barley seedlings under the influence of cadmium ions, an increase in the total number of SH-containing compounds was noted. Thus, in the variant using the maximum concentration of this metal (100  $\text{mg/dm}^3$ ), the number of thiols increased by 5 times compared to the control. In addition, compared to the control, an increase in the total pool of glutathione was found, and the amount of its reduced form increased to a greater extent than the oxidized one [Tytov et al., 2008].

It was found that in barley leaves under the influence of excessive soil moisture and (low temperature + excessive soil moisture) there is an increase in the activity of glucose-6-phosphate dehydrogenase. Thus, after 3 days of the combined action of low temperature and excess soil moisture, the activity of

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glucose-6-phosphate dehydrogenase exceeded the control by 32%, and under the action of only excess soil moisture - by 76%, which is due to hypoxia, which develops in plants under the action of excess soil moisture . In the post-stress period, the activity of this enzyme in the leaves of the experimental plants gradually decreased to the control values. The conducted correlation analysis showed that there is a close positive relationship between the activity of glucose-6-phosphate dehydrogenase and excess soil moisture ( $r = 0.69$ ,  $p < 0.05$ ), as well as between the content of reduced glutathione and the activity of glucose-6-phosphate dehydrogenase ( $r = 0.73$ ,  $p < 0.05$ ). Thus, under the combined effect of low temperature and excess soil moisture, the pool of reduced glutathione in barley leaves was replenished due to the restoration of its oxidized form with the help of excess soil moisture, and in this process, the enzyme glucose-6-phosphate dehydrogenase plays a rather important role [Dremuk, Shalygo, 2016 ] .

An increase in the level of phytochelatins in the tissues of wheat stalks when grown in soil containing cadmium was shown. At a cadmium concentration of 10 mg/kg of soil, the synthesis of phytochelatins in the stems of untreated plants occurred more intensively than at a concentration of 200 mg/kg. Simultaneously with an increase in the level of phytochelatins in the stems of plants not treated with *Bacillus subtilis* 26D bacteria, a decrease in the concentration of reduced glutathione was observed [Smirnova et al., 2015]. It is likely that the main part of the reduced glutathione was used for the synthesis of phytochelatins [Yadav, 2010]. In addition, some part of the reduced glutathione could be oxidized to its oxidized form, the increase in the level of which may indirectly indicate the development of oxidative stress. An increase in the level of phytochelatins and a decrease in the concentration of reduced glutathione were also observed in the stems of plants treated with bacillus cells when grown in soil containing cadmium ions. However, the level of phytochelatins in the stems of plants inoculated with bacteria was higher compared to untreated plants. In addition, it is worth noting

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that at a concentration of 200 mg/kg, the level of reduced glutathione in the stems of plants treated with bacteria *Bacillus subtilis* 26D was higher than in untreated plants. Thus, the increase in resistance of plants treated with *Bacillus subtilis* 26D bacteria to the action of cadmium ions can be explained by a higher level of phytochelatins in plant tissues compared to untreated plants [Smirnova et al., 2015].

Treatment of plants with retardant doses of chlorocholine chloride and choline chloride leads to an increase in the resistance of the photosynthetic apparatus against ultraviolet radiation and high temperatures. The protective effect of these retardants was also found to be associated with an increase in the activity of antioxidant enzymes and the content of low-molecular-weight antioxidants, in particular glutathione, in leaves and chloroplasts, as well as with an increase in abscisic acid in leaves [Kreslavsky, 2010].

The coordinated response of two groups of genes encoding high molecular weight chelators (metallothioneids, MT1 and MT2 genes) and small polypeptides (phytochelatins, phytochelatin synthase gene PCS) to the effect of copper in high concentrations on plants was demonstrated. This coordination is manifested in the dominant expression of the PCS gene compared to the A/77 and MT2 genes in the first hours of copper excess action, which at a later stage of adaptation is replaced by the predominant transcription of the MT1 and MT2 genes when the intensity of the PCS gene expression drops [Ivanova, 2011].

Abscisic acid selectively activates NADP·H-oxidase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase and increases the concentration of glutathione, tocopherol, carotenoids, and ascorbate [Prats et al., 2005, Adie et al., 2007]. It has also been shown that exposure of plants to heavy metals reduced glutathione levels [Kukkola et al., 2000; Madhava Rao, Sresty, 2000] and ascorbate [Mishra, Agrawal, 2006] in tissues. In addition, heavy metals



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can cause a decrease in the activity of antioxidant enzymes [Sasanova et al., 2012].

It is also noted that the content of glutathione depends on the metal concentration in the nutrient medium, varying in different types of plants. It was shown that glutathione content increases with increasing cadmium concentration in sorghum (*Pisum sativum* L.) [Gupta et al., 2002], sedge (*Sedum alfredii* Hance.) [Sun et al., 2007], corn (*Zea mays* L.) [Rüegsegger, Brunold, 1992], as well as in tomato cell culture (*Solanum lycopersici* L.) and tobacco (*Nicotiana glauca* L.) [Estrella-Gomez et al., 2012].

Reduced glutathione can potentially serve as one of the zinc ligands in the cell, as its concentration in the cytoplasm is significantly higher than that of individual free acids, such as cysteine or histidine [Krezel et al., 2003]. It is quite likely that zinc does not form binary Zn-GSH complexes (zinc – reduced glutathione), but ternary complexes, the composition of which, in addition to zinc and reduced glutathione, also includes other ligands, for example, histidine or nucleotides [Krezel et al., 2003; Krezel, Bal, 2004; Krezel, Maret, 2006].

As a component of the antioxidant system, glutathione takes an active part in protecting cellular structures from the damaging effects of ROS. As the body ages, the antioxidant fund of cells decreases, however, according to literature, the content of glutathione in yeast increases with aging. In the work carried out, the content of protein and low-molecular thiol reduced (SH) and oxidized (SS) substances in baker's yeast was determined by amperometric titration. The aging model was yeast aging for 35 days at a temperature of  $0 \pm 50$  C. Each week, selected yeast samples were frozen and thawed; 1.5 and 10% extracts were obtained from them, which were centrifuged, and then the liquid above the sediment was collected and studied. At the same time, the influence of dilution on the openness of reduced and oxidized substances in the extracts of the indicated concentrations was not detected. In the proteins of yeast extracts, the content of

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SH-groups decreased in 35 days by an average of 31% (from 353 to 243  $\mu\text{mol/g}$ ), and the number of SS-bonds did not change reliably (147 and 129  $\mu\text{mol/g}$ , respectively). Under the same conditions, the content of low-molecular-weight thiol reduced substances increased by 2 times (from 60 to 120  $\mu\text{mol/g}$ ), while the content of oxidized substances did not change (36 and 39  $\mu\text{mol/g}$ , respectively). Since anabolic processes, including the energy-dependent synthesis of glutathione, which accounts for the majority of non-protein SH in biological media, cannot take place efficiently in cells without a nutrient medium, and the restoration of its oxidized form would lead to a decrease in the amount of SS, the probable reasons for the growth of low-molecular-weight SH in aging yeast can be either a decrease in the use of reduced glutathione, or the activation of partial proteolysis of intracellular proteins with the formation of SH-peptides [Dudenko, Shleykin, 2008].

Based on the obtained experimental data, a number of species can be distinguished according to the decrease in the content of reduced glutathione: *Amelanchei ovalis*, *Amelanchei canadensis*, *Amelanchei florida* and *Amelanchei spicata*. Representatives of the genus *Amelancheir* Med. (*Amelancheir spicata* and *Amelancheir florida*) are characterized by greater resistance to adverse conditions of the steppe environment compared to *Amelancheir ovalis* and *Amelancheir canadensis*. Based on this, more resistant species can be recommended for use in green construction and decorative gardening. Less resistant species can be used to create green plantations, taking into account their specific reaction to adverse environmental conditions [Dolgova, SamoiloVA, 2009].

The total content of glutathione in the leaves of control wheat plants of the Ballada variety was on average 2.5 times higher than that of the Beltskaya variety. Under the influence of drought, the total content of glutathione in the leaf increased by 80% in the Ballada variety and by 130% in the Beltskaya variety.

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One of the main functions of reduced glutathione is to maintain ascorbic acid in a reduced form, which functions in the ascorbate-glutathione cycle. An increase in the level of glutathione, which takes an active part in the detoxification of hydrogen peroxide in conditions of water deficit, is a positive adaptive response aimed at reducing the effects of oxidative stress. In the leaves of experimental wheat plants, the activity of enzymes of the ascorbate-glutathione cycle increased, compared to the control. Thus, the activity of ascorbate peroxidase increased by 25% in the Ballada variety, and by 29% in the Belskaya variety plants. The activity of glutathione reductase, which plays a key role in protecting plants from oxidative stress, in Ballada and Beltskaya varieties increased by 45% and 56%, respectively. The absence of significant changes in the activity of ascorbate peroxidase and glutathione reductase is indirect evidence that the ROS content in leaves did not significantly increase under conditions of progressive soil drought [Nikolaeva, Maevskaya, 2010].

The biological significance of the glutathione system is diverse and affects almost all aspects of cellular activity: 1) conjugation of xenobiotics and their metabolites; 2) protection against the damaging effect of ROS and reactive metabolites of xenobiotics; 3) maintenance of thiol-disulfide balance; 4) maintaining a restored cell environment; 5) regulatory influence on carbohydrate, lipid and protein metabolism through the interaction of the reduced and oxidized form of glutathione at physiological concentrations with a number of proteins and enzymes; 6) transport of amino acids through the cell membrane; 7) maintaining the optimal condition and functions of biological membranes; 8) participation in the synthesis of nucleic acids and proteins; 9) regulation of cell proliferation processes; 10) participation in cell aging mechanisms; 11) cysteine reserve in the cell; 12) regulation of the synthesis of heat shock proteins, which provide protection of the cell against protein denaturation; 13) participation in adaptation mechanisms against hypoxia; 14) hindering the implementation of cell

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death mechanisms [Schroder, 2001, Cummings, 2005, Circu, Aw, 2008]. At the same time, it is only necessary to note that the latest modern scientific research only expands our imagination about the biological functions of the glutathione system in the plant world.

A comparison of the content of low-molecular antioxidants (ascorbate, glutathione, proline, flavonoids) in plants revealed no unequivocal relationship between their amount and the level of water pollution. At the same time, it turned out that the maximum values for all these indicators were distinguished by those types of macrophytes, which are characterized by the maximum accumulative capacity in relation to heavy metals. The maximum amount of glutathione was also recorded in the leaves of *Ceratophyllum demersum* [Chukina, 2010].

The content of reduced glutathione increases in the tissues of the embryo during the germination of spring barley seeds. A significant increase in the content of glutathione occurs before seeding - by 2-4 times, and during the entire period of germination - from dry seed to seedling - by an average of 4-5 times [Kovalyova, 2011].

Foliar treatment with sulfur and phosphorus of 14-day-old seedlings of Smuglyanka winter wheat plants leads to an increase in the content of SH-groups in the roots. The most significant effect was produced by treatment with  $\text{KH}_2\text{PO}_4$  (100  $\mu\text{M}$ ), in which the content of sulfhydryl groups in the roots increased by 34%. The dissolution of the nutrient mixture leads to a corresponding decrease in the content of SH-groups in the roots. When treated with the fungicide Amistar Extra 280 SC with 0.12 mM orthophosphate in the nutrient solution, an increase in the content of SH-groups in the roots by 29% was observed [Sandetskaya et al. 2012].

With the help of fluorescence microscopy, reduced glutathione was detected in the vacuoles of beet roots in a relatively low concentration, which was on average 104  $\mu\text{M}$  and was lower than the concentration of this tripeptide in

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mitochondria (448  $\mu\text{M}$ ) and plastids (379  $\mu\text{M}$ ). At the same time, it was also found that the pool of oxidized glutathione in vacuoles was higher compared to the pool of glutathione in other organelles [Pradedova et al., 2018].

## **2.2 The role of glutathione in redox-dependent processes**

The reaction of plants to the adverse effects of the external environment is aimed at adaptation against them. As you know, the adaptation syndrome is a set of non-specific protective reactions of plants to the action of adverse factors. Protective reactions of plants in response to stressors is a complex process that develops in space and time in a certain sequence. One of the signs indicating the involvement of compounds in the formation of a non-specific adaptation syndrome is the increase in their activity and the phase of these changes in response to the stressor. The process of plant adaptation is conditionally divided into two main stages: stress reaction and specialized, long-term adaptation. Which perform various biological tasks. The stage of stress reaction – a non-specific response of cells – is formed quite quickly in response to the action of the stressor and ensures the short-term survival of the organism, as well as induces the formation of more reliable specialized mechanisms of adaptation [Timofeeva, 2009].

Stress factors acting on a living organism have a double effect: damaging and irritating [Urmantsev et al., 1986]. The damaging effect is the destruction of the integrity of cell membranes, disruption of the processes of photosynthesis, cell respiration, phosphorylation and other processes related to the vital activity of the cell. All this leads to a partial or complete loss of cell performance or its death. The irritating effect, on the contrary, causes a whole complex of precautionary measures in the cell, which prevent the negative effect when the stress itself appears [Henkel, 1982, Ishikawa et al., 1995, Franco et al., 1999].

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Currently, sufficient scientific information has already been accumulated, which indicates that the formation of ROS is a fairly universal response of plants to stresses caused by the action of abiotic and biotic factors [Yarullina et al., 2011, Kolupaev Karpets, 2014]. It should also be noted that redox reactions occur in all living organisms, the by-product of which is ROS [Apel, Hirt, 2004]. The generation of ROS can be induced by photooxidative stress, damage by ultraviolet rays, changes in temperature, moisture conditions, salinity of the environment, the action of heavy metals, herbicides and agrochemicals, and air pollution by hypotoxic stress [Molodchenkova et al., 2007, Kolupaev, 2007]. At the same time, it was also found that ROS are formed during incompatible interactions of plants with pathogens, as well as during foliar treatment with their elicitors [Naton et al., 1996, Jabs, Slusarenko, 2000, Minibayeva, Gordon, 2003, Yarullina et al., 2003, Maksimov, Cherepanova, 2006]. It is also believed that the main causes of ROS generation are the imbalance of the electron transport chain in chloroplasts and mitochondria [Neill et al., 2002; Mori, Schroeder, 2004; Ślesak, 2007; Sharma et al., 2012].

Taking into account the fundamental role of ROS in the response reactions of living organisms and the variety of protective reactions of organisms, the issue of possible management of plant resistance has recently been quite actively considered [Lukatkin, 2003]. At the same time, it is believed that the development of appropriate approaches will primarily contribute to the growth of the level of low-molecular antioxidants and the activity of antioxidant enzymes with the aim of increasing the resistance of plants against the effects of anthropogenic and biotic stressors. As numerous studies show, the balance of both components of antioxidant protection is extremely important. However, the assessment of the necessary proportional changes in the various components of the antioxidant system is still open and requires further research in this direction [Veselova et al., 1993, Cakmak et al., 1993, Kenya et al., 1993, Baraboy, Sutkovoy, 1997,

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Kurganova et al. ., 1997, Bolwell et al., 1998, Kirlin et al., 1999, Loewus, 1999, Merzlyak, 1999, Dat et al., 2000, Lukatkin, 2002, Rivero et al., 2003, Antonova et al., 2005, Devy, Prasad, 2005, Gazaryan et al., 2006, Kwak et al., 2006 , Kolupaev, 2007, Chiang et al., 2008, Polovnikova, Voskresenskaya, 2008, Garifzyanov, 2011].

It is assumed that the occurrence of oxidative stress in cells when plants are exposed to adverse conditions is a universal link of the body's response to various types of stress. At the same time, oxidative stress, on the one hand, triggers processes that cause plant damage, and on the other hand, promotes the development of adaptive reactions. The consequences of oxidative stress are an increase in the activity of antioxidant enzymes, which stimulates a decrease in the level of ROS in cells; metabolic changes aimed at repairing damage caused by ROS; a sharp increase in antioxidant protection and others. As a result, the adaptive activity of the plant increases and its stress resistance increases, and not only to the active factor. All this happens in the plant organism only if the action of the stressful external factor does not exceed lethal limits [Lukatkin, 2007]. Among all known ROS, hydrogen peroxide plays the greatest role, regulating a set of molecular, biochemical, and physiological reactions in the plant cell [Udyntsev et al., 2011]. A significant increase in the concentration of hydrogen peroxide, in turn, is one of the reasons for the inactivation of a number of Calvin cycle enzymes [Charles, Halliwell, 1981; Takeda et al., 1995; Kreslavsky et al., 2007, Sharova, 2016].

Thiols play a rather important role in determining the redox status of proteins, cells, and plant organisms. Oxidation of these groups leads to the formation of disulfides or other highly oxidized products, and sometimes with loss of biological activity. Redox control in the cell due to interconversions of dithiols  $\rightleftharpoons$  disulfide is considered as one of the rather effective ways of adaptation of plants against stresses. A shift in the intracellular or membrane

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balance towards the predominance of SH-groups with high lability and reactivity can change the activity of a wide range of enzymes and proteins with a different function, and as a result, the intensity and direction of numerous metabolic pathways. However, the nature of endogenous factors capable of regulating the balance of oxidized and reduced dithiols in plants, as well as the physiological consequences of changes in this balance, have not yet been fully established. In the study of this problem, as a rule, two techniques are used: artificially changing the ratio of dithiols  $\square$  disulfide by blocking SH-groups with SH-reagents on model systems of various complexity and analysis of the activity of reactions or processes carried out by them. And on the other hand, the quantitative determination of SH-groups in cells, tissues or organs of plants that have been exposed to stressors, which allows us to judge the dynamics of changes in the status of thiol groups under physiological conditions [Ampylogova et al., 2007]. Under optimal conditions, ROS is produced at a low level, mainly in chloroplasts, mitochondria, and peroxisomes. Studies using isolated mitochondria have revealed several sites of superoxide radical formation. Theoretically, one-electron reduction of oxygen with the formation of a superoxide radical can occur on all components of the respiratory chain. However, research using isolated mitochondria allowed us to show that superoxide radical formation occurs mainly in the first and third complexes [Adam-Vizi, Chinopoulos, 2006]. NADPH oxidases, cell wall peroxidases, amine oxidases, flavin-containing oxidases and some other enzyme systems can also serve as potential sources of ROS [Mynybaeva, Gordon, 2003; Glyanko et al., 2009, Kolupaev, Karpets, 2010; Swanson and Gilroy, 2010]. And the source of ROS in the apoplast are cell wall-bound oxidases, peroxidases, and polyamine oxidases [Mittler, 2002; Minibayeva et al., 2009]. Quite a few enzymes can also contribute to the formation of ROS, including those localized in the plasma membrane and cell wall [Tarchevsky, 2002].



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Maintaining a normal internal redox status plays a rather significant role in the vital activity of plant cells. A change in this status is often a consequence of stressful actions or the result of the activity of the cells themselves, which regulate cellular processes. Under certain conditions, exceeding the rate of ROS production over the rate of their detoxification can lead to damage and death of cells, which is caused by oxidative stress. Due to their high reactivity, ROS can damage DNA, proteins, and lipids, but under normal conditions, the presence of antioxidant protection allows cells to maintain the intracellular concentration of oxidants at a safe level [Zadvornova et al., 2009, Reczek, Chandel, 2015, Samokhina et al., 2016, Holovatskaya et al., 2017]. The protective effect of the antioxidant system is determined both by the activation of antioxidant enzymes and by the significant accumulation of various low-molecular organic antioxidants, which reduce the damaging effect of stress factors [Radyukina, 2015].

ROS are formed most often in those compartments of the plant cell in which electron transport chains are located - in chloroplasts and mitochondria. In addition, significant amounts of ROS are formed in peroxisomes, plasmalemma, and apoplast [Rio et al., 2002; Miller, Mittler, 2006; Zhigacheva et al., 2013, Sagi, Fluhr, 2006]. The main mechanisms of ROS generation are associated with malfunctions of electron transport chains of mitochondria or microsomes, especially at low ADP concentrations, as well as with changes in the properties of dehydrogenases [Dontsov et al., 2006]. Radical forms of oxygen and their transformation products can change the activity of enzymes and cause protein degradation, etc. , Chirkova, 2002, Sagi, Fluhr, 2006, Martynovych, Cherenkevych, 2008, Shetty et al., 2008, Van Dongen et al., 2009, Jin et al., 2011, Pucciariello et al., 2012, Demidchik, 2015, Dietz et al., 2016].

Plant proteins are the most frequently oxidized components, accounting for 68% of all macromolecules oxidized in the cell [Rinalducci et al., 2008]. And

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therefore, quite likely, protein oxidation is quite often used as a marker of oxidative stress. ROS can oxidize proteins directly or indirectly through redox-sensitive molecules, such as reduced glutathione and thioredoxins, which control the cellular redox status [Thannickal, Fanburg, 2000]. It has been shown that most types of protein oxidation are irreversible, but reversible modifications have also been found [Moller et al., 2007].

Most often, ROS can change the structure and activity of proteins by acting on the thiol groups of amino acids [Spadaro et al., 2010]. As you know, sulfur-containing amino acids in the cell are cysteine and methionine residues, which are very sensitive to oxidation by all forms of ROS. During the oxidation of the cysteine residue, intra- and intermolecular disulfides (R1-SS-R2), derivatives of sulfenic (R-SOH), sulfinic (R-SO<sub>2</sub>H) and sulfonic (R-SO<sub>3</sub>H) acids can be formed. The first three of these modifications are reversible, and at the same time, the formation of sulfonic acid is already irreversible. The reversibility of oxidation and reduction plays a key role in changing enzyme activity [Spadaro et al., 2010]. In addition, disulfides with glutathione can be formed. At the same time, it is believed that the formation of such "mixed" disulfides can protect the protein against further, stronger oxidation [Ghezzi, Bonetto, 2003]. Methionine residues can also be oxidized with the sequential formation of methionine sulfoxide (MetSox) and methionine sulfone. Methionine sulfoxide can be reduced to methionine under the action of the enzyme methionine sulfoxide reductase. Small heat shock proteins in chloroplasts work precisely according to this mechanism [(Gustavsson et al., 2002]. It is also known that some peripheral methionine residues can work as endogenous antioxidants, protecting active centers and other ROS-sensitive protein domains [Levine et al., 1996].

Some heavy metals (Cr, Cu, Fe), participating in Haber-Weiss and Fenton redox reactions, can directly generate ROS in plant cells, while other metals (Cd, Hg, Ni, Pb, and Zn) cause ROS accumulation indirectly - due to the disruption

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caused by them in the structure of chloroplasts and mitochondria or inhibition of the activity of enzymes of the antioxidant system [Pinto et al., 2003; Krämer, Clemens, 2005; Chen et al., 2009; Sharma, Dietz, 2009; Sazanova et al., 2012]. There are 4 mechanisms that explain the formation of ROS in a plant cell. One of them functions at the level of the outer surface of the plasma membrane and is mediated by the PR protein, an NADPH-dependent oxidase enzyme. The other 3 mechanisms are implemented at the level of the cell wall matrix and include the participation of poly(di)amine oxidase peroxidase and oxalate oxidase. Unlike the last two enzymes, which take part in the processes of the formation of hydrogen peroxide directly, NADPH-dependent oxidase and peroxidase catalyze the initial formation of superoxide anion, which subsequently dismutates into hydrogen peroxide [Szajdak, Maryganova, 2007, Almagro et al., 2009] .

It is well known that the formation of plant resistance against stress factors is a complex, multi-component process that includes both specific and general, i.e. non-specific reactions [Drozdov et al., 1984; Alexandrov, 1985, Hartung et al., 1998].

The redox homeostasis of the plant cell is formed as a result of the balance between the accumulation of ROS, the functioning of antioxidant enzymes and the system of low-molecular antioxidants [Seredneva et al., 2017]. At the same time, other redox intermediates with extraordinary negative redox potentials are present in chloroplasts. They include plastoquinones, thioredoxins, ferredoxin [Sigler et al., 1999, Pinto et al., 2003; Apel, Hirt, 2004, Krämer, Clemens, 2005; Jones, 2006, Kyselevsky et al., 2008, Chen et al., 2009; Sharma, Dietz, 2009; Sazanova et al., 2012, Przybytko, 2013, Kumar et al., 2014, Upadhyay, 2014]. The peculiarity of chloroplasts is that they produce all ROS when illuminated [Ivanov et al., 2014]. ROS are formed in the cell as well as in the process of normal vital activity of the plant organism. Under normal conditions, the main sources of ROS in plants are the processes of photosynthesis and photorespiration [Sluse et

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al., 1998, Feussner, Wasternack, 2002, Apel, Hirt, 2004, Foyer, Noctor, 2005; Gechev et al., 2006; Groß et al., 2013, Demidchik, 2015]. It was established that the half-life of singlet oxygen is  $2 \times 10^{-7}$  seconds [Gorman, Rodgers, 1992], superoxide anion –  $2 \times 10^{-6}$  –  $4 \times 10^{-6}$  seconds [Gechev et al., 2006], and hydrogen peroxide –  $2 \times 10^{-3}$  seconds [Henzler, Steudle, 2000, Gechev, Hille, 2005]. The cell itself is not capable of detoxifying the hydroxyl radical with the help of enzymatic antioxidants [Vetoshkina, 2016]. During the reactions of the light stage of photosynthesis, accumulation of singlet oxygen is possible with the participation of pigments [Lukatkin, 2002]. In chloroplasts, in the process of electron transport, part of the electrons can also be intercepted by the oxygen of their electron transport chain with the formation of a superoxide radical. Its occurrence is connected with the work of the I and II photosystems [Polesskaia, 2007].

Mitochondria, which perform the function of ATP synthesis, are one of the important organelles in plant cells. In the case of incomplete reduction of oxygen in ETC respiration, the formation of ROS is possible, which include the superoxide anion radical, hydroxyl, hydroperoxide radicals, and hydrogen peroxide [Shugaev et al., 2011]. Nonspecific toxins induce the generation of ROS, and in many cases the development of disease symptoms is explained by this mechanism [Knoche, Duvick, 1987, Desmond et al., 2008, Sapko et al., 2011]. It was shown that ROS activate the germination of spores of the rust fungus *Puccinia striiformis* f. *tritici* [Yin et al., 2016] and affect the viability of the phytopathogen *Cladosporium fulvum* Cook. [Lu, Higginsf, 1999]. It was also shown that the processes of oxidation of low molecular weight substrates in the mitochondria and oxidation of proteins in the cytoplasm and other organelles also make a significant contribution to the disruption of the redox balance of the cell under normal conditions. There is also an enzymatic way of ROS formation, connected with the work of enzymes - lipoxygenase, peroxidase, NADPH-oxidase, xanthine oxidase,

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superoxide dismutase [Ishikawa, 1995, Yamasaki, 1997; Sherwin, Farrant, 1998, Blokhina et al., 2003; Makarova, 2003, Mittova et al., 2003, Hassan, Alla, 2005, Gechev et al., 2006, Baranenko, 2006, Tkach, Storozhenko, 2007, Flora, 2007, Romanova, 2008]. Superoxide dismutase is a key enzyme limiting the transformation of superoxide radical into other ROS (prooxidants), as it catalyzes the reaction of hydrogen peroxide formation from the superoxide anion radical [Fridovich, 1986, Kirillova, 2004, Romanova, 2005]. It was found that the activity of superoxide dismutase of transgenic corn plants under oxidative stress was 7 times higher than that of non-transformed plants [Danilova et al., 2007].

Traditionally, ROS are considered primarily as radicals that damage cellular structures. Radicals are atoms or groups of atoms containing unpaired electrons. Radicals, as highly reactive compounds, can participate in a number of cellular processes even in the absence of enzymes. This property of theirs is quite suitable for the activation of signaling cascades [Foyer, Noctor, 2005; Jimenez-Del-Rio, Velez-Pardo, 2012]. Under normal conditions, the increased formation of ROS, which is observed during the transition from one stage of ontogenesis to another or when the time of day changes, is quickly inactivated by the constitutive level of antioxidant enzymes or low molecular weight antioxidants [Pobedimskij, Burlakova, 1996; Hernandez et al., 1999, Kumutha et al., 2009, Gill et al., 2010]. The balance of formation and utilization of ROS is tightly regulated by a large network of genes [Pucciariello et al., 2012]. As is known, the most stable and long-lived form of ROS in plant tissues is hydrogen peroxide, which is formed as a result of the two-electron reduction of an oxygen molecule during the dismutation of the superoxide anion radical [Droge, 2002, Kolupaev, Karpets, 2009]. It was shown that heat action of different intensity (37 and 43°C) on wheat seedlings has different effects on the content of hydrogen peroxide, generation of superoxide anion radical and activation of superoxide dismutase. At the same time, on the one hand, hydrogen peroxide and the superoxide anion radical can

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participate in the launch of protective mechanisms in plant cells, and on the other hand, activation of superoxide dismutase and neutralization of ROS are also quite important elements of resistance [Nylova et al., 2017]. The earliest response of a plant organism to a pathogen attack is the local generation of ROS - an oxidative burst that triggers a whole chain of subsequent protective reactions [Hippeli et al., 1999; Dröge, 2002, Yarullina, 2006].

It has also been established that hydrogen peroxide is a unique cheap, environmentally friendly anti-stress drug that promotes the survival of cultivated plants in extreme conditions of low temperatures, soil salinity and moisture deficit [Apasheva, Komissarov, 2011], hyperthermia and osmotic shock [Obozny, Kolupaev, 2014] . At the same time, the ability of exogenous hydrogen peroxide to induce phytofluorescence resistance of potato tubers was shown [Panina, 2005]. It was established that after treating plants with hydrogen peroxide, its exogenous level increases, which triggers a cascade of transduction reactions and activates protective mechanisms for a long time, including the redistribution of electron carriers in the electron transport chain of chloroplasts. When a pathogen attacks, the level of exogenous hydrogen peroxide increases, a signal is transmitted, and at the same time the development of biotic stress is inhibited [Pshybytko, 2018].

At the same time, it was found that hydrogen peroxide causes the cell walls of corn seedlings to stiffen only with a sufficiently high peroxidase activity. If the production of hydrogen peroxide by oxidases of the cell walls is not accompanied by an increase in peroxidase activity, then hydrogen peroxide accumulates in the apoplast and takes part in spontaneous reactions that cause oxidative damage to cells and, as a result, slow their growth. These results confirm the participation of apoplast hydrogen peroxide, as well as enzymes that maintain its balance, in the inhibition of cell growth during differentiation and in the elimination of stress [Bylova, Sharova, 2011]. It was established that the introduction and expression

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in the plant genome of the heterologous *gox* gene, which encodes the glucose oxidase enzyme, leads to a constant increased content of hydrogen peroxide in plant tissues. The researchers predict that this could increase plant defenses without changing other valuable traits of agricultural plants. The created transgenic forms of plants with the glucose oxidase gene show an increased level of resistance against a wide range of fungal and bacterial phytopathogens. In addition, on the basis of the phenomenon of cross-tolerance, one can also expect an increase in resistance against abiotic factors of the environment, but this issue has not yet been studied deeply enough. It is also unclear how the increased constant level of signaling molecules will affect the properties of the plant in a comfortable situation and its ability to respond to adverse changes in the surrounding environment. The conducted scientific studies showed that the increased level of the signal stress molecule of hydrogen peroxide does not lead to the development of general non-specific resistance of plants - the resistance against hyperthermia even decreases. The high level of ROS in the modified potato causes an increase in resistance against oxidative stress, which accompanies the adverse effect of low positive temperature. It also contributes to the resistance of such plants against microorganisms, probably due to the direct action of hydrogen peroxide as an antimicrobial agent. Life "under constant stress" did not lead to permanent stability of cells [Borovsky et al., 2017].

In fact, glutathione not only protects the cell from such toxic agents as free radicals, but also generally determines the redox status of the intracellular environment [Stružnka et al., 2005]. The ratio of reduced to oxidized glutathione in the middle of the cell is one of the most important parameters that shows the level of intracellular toxicity, it can change depending on the preference of certain reactions, which in turn is determined by the state of the cell under oxidative stress. At the same time, glutathione interacts quite effectively with metal ions of

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variable valence, protecting cells against their toxic effects [Kosower, Kosower, 1978; Shelley, Jasvinder, 2002, Molodchenkova, 2007, Circu, Aw, 2008].

An extremely important part of the antioxidant defense system are enzymes that prevent the formation of ROS or destroy them, the main role of which is performed by various peroxidases, including catalase, as well as the ascorbate-glutathione cycle (Halliwell-Asada cycle), the enzymes of which ensure coordinated work and effective recovery of low-molecular antioxidants - ascorbate and glutathione. In natural and agricultural biogeocenoses, plants quite often face the problem of oxygen deficiency and absence, which are paradoxically related to oxidative stress, which occurs immediately after the restoration of oxygen access [Emelyanov et al., 2015]. It is reported that the resistance of plants against hypoxia is explained by the level of activity of systems of enzymatic and chemical antioxidant protection of their lipids from the processes of peroxidic destruction by ROS [Crawford et al. 1996].

We determined the degree of antioxidant effect of glutathione when stimulating its formation by various signaling structures when spraying plants with them during the growing season (Table 2).

It was shown that the expression of all families of the ascorbate-glutathione cycle is organ-specific. Thus, among the 8 genes encoding ascorbate peroxidases, OsApx4, 5, 6 were mainly expressed in the stem, and OsApx1 in the root. In addition, the total expression level of these genes in the root was an order of magnitude lower than in the stem. The study shows that the regulation of the Halliwell-Asada cycle at the level of expression occurred mainly at the level of genes encoding the first and last enzymes of the cycle - ascorbate peroxidase and glutathione reductase, respectively [Prykazyuk et al., 2015]. It has been shown that the oxygen concentration in the mitochondria of mammals reaches 0.1  $\mu\text{M}$ , while in the mitochondria of plant cells it is more than 250  $\mu\text{M}$ . At the same time, according to researchers' estimates, approximately 1% of oxygen absorbed by



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plants is transformed into its active forms, which is necessarily connected with incomplete step-by-step recovery of molecular oxygen [Asada, 1999].

Table 2 – The role of glutathione in stimulating the antioxidant activity of tomato plants

Options research	Content of total glutathione in tomato tissues, $\mu\text{g/g}$ :	
	in 24 hours	after 72 hours
CONTROL, without treatment	$4.62 \pm 0.23$	$4.71 \pm 0.22$
Chitosan complex, 2.0 kg/ha	$7.46 \pm 0.19$	$7.75 \pm 0.20$
Salicylic complex, 0.04 kg/ha	$7.24 \pm 0.20$	$7.68 \pm 0.19$
Brassinosteroid complex, 20 mg/ha	$7.35 \pm 0.18$	$7.72 \pm 0.16$
Jasmine complex, 0.004 kg/ha	$7.49 \pm 0.18$	$7.77 \pm 0.15$
Mikosan complex, 10.0 l/ha	$7.32 \pm 0.18$	$7.70 \pm 0.19$

In the course of scientific research, numerous attempts were made to understand the role of redox processes in the regulation of plant growth through their treatment with oxidizing agents and reducing agents:  $\text{NAD(F)}\cdot\text{H}$ , hydrogen peroxide, ascorbate, glutathione, dithiothreitol, oxidase inhibitors, and peroxidase inhibitors. But such actions, as a rule, caused inhibition of growth, which was quite likely a stress reaction to a sharp change in the redox balance. Acceleration

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of plant growth was sometimes achieved by stimulating weak radical reactions in them [Sharova, Medvedev, 2017].

In stressful conditions for the cell, the content of ROS begins to increase quite quickly, and at the same time, oxidative stress develops. As a result of contact with ROS, membranes are destroyed, proteins are damaged, and chlorophyll is degraded. Oxidative stress occurs when the balance between the mechanisms that trigger oxidative phenomena and cellular antioxidant protection is disturbed [Bolwell, Wojtaszek, 1997, Ayushynova, 2015]. Universal and quite important for plants, enzymatic and non-enzymatic ROS deactivation systems play a key role in preventing oxidative damage [Blochina et al., 2003; Bukhov, 2004; Foyer, Noctor, 2005, 2015; Logan et al., 2008]. The antioxidant status of plants is determined by the balance between pro-oxidant and antioxidant reactions occurring in the cell [Golubenko et al., 2017]. The interaction of ROS and hormones plays a rather important role in the life of plants. On the one hand, this interaction is manifested in the ability of ROS to influence the content of hormones and the transmission of hormonal signals. And on the other hand, it is implemented through the influence of hormones, both on production and activation of ROS [Kudoyarova, 2017]. ROS causes oxidation of lipids, proteins, Fe-S-centers of enzymes, fragmentation of peptide chains, has a direct and indirect effect on DNA [Imlay, 2008, Bose et al., 2014]. An integral part of the stimulation of metabolism when leaving the state of rest is the increase in the concentration of ROS and the activation of the antioxidant system [Molchan et al., 2017]. It has been shown that maintaining a balance between the processes of synthesis and degradation of ROS is a necessary condition for the normal germination of pollen grains and the growth of the pollen tube of blue spruce [Evmenyeva, Breygina, 2017].

ROS formed during stress spontaneously interact with various substrates, which, being oxidized, form polymers with their help, strengthening the cell wall.

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That is, in the zone of ROS generation, there is an acceleration of oxidation processes, called "oxidative flash". A significant increase in the content of ROS outside the cell reveals a direct antifungal effect on the development of pathogens, which have been shown to be quite sensitive to them. Evidence of this hypothesis was obtained during the study of the effect of hydrogen peroxide on the germination of fungal spores and inhibitors of O<sub>2</sub>- formation on lipid peroxidation in bacteria [Maksimov, 2017]. In the first case, inhibition of the growth of the pathogen was observed, and in the second - its active development. Extracellular generation of ROS by the closing cells of the stomata upon contact with part of the appressorium of the fungus *Puccinia triticina* Erikss. was also detected, which leads to the stopping of the penetration of this pathogen into the tissues of wheat plants [Shtubey, 2009]. It is shown that elicitors can not only stimulate the formation of ROS, but can also be released under their action from the cells of pathogenic fungi. It was found that diffusates of germinated spores of the fungus *Magnaporthe grisea* - the causative agent of piricular disease - induced such protective reactions as necrotization and the release of fungitoxic exometabolites in the calli of resistant rice. Therefore, the elicitor effect of exometabolites of spores is manifested in the tissues of the whole plant, and not only in isolated cells [Lapykova et al., 2000]. It was also found that carriers of catalase activity are released by the spores of the fungus *Magnaporthe grisea*, the causative agent of rice piricular disease, both immediately after the spores are washed off the mycelium, and later, during their germination [Abramova et al., 2008]. It was shown that the *Septoria nodorum* mushroom strains secrete catalase into the culture medium. At the same time, in the culture medium of the highly virulent strain 9MN, the activity of the enzyme was significantly higher than that of the low virulent strain 4VD. It is likely that the ability to secrete catalase contributed to the rather active growth and development of strains in the presence of hydrogen peroxide. Since this was particularly evident in the 9MN strain, it can be predicted

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that through the production of this metabolite, the fungus *Septoria nodorum* regulates the content of hydrogen peroxide in infected plant tissues [Troshina et al., 2008]. It was established that hydrogen peroxide determines the cytotoxic effect of humic substances, and specifically, in relation to pathogens *Fusarium* sp. Trans [Saito et al., 1980]. It was established that infection of *Pinus sylvestris* seedlings with *Heterobasidion annosum* HA-6-96 caused an increase in peroxidase activity, which is a protective reaction of the plant against the pathogen [Chemerys, Chepyzhko, 2018]. The pathogen's ability to damage and develop in the plant organism largely depends on the activity of its extracellular hydrolases, specifically proteinases. In response to the action of proteinases, the synthesis of protein inhibitors is induced in the plant, the action of which is aimed at suppressing the activity of these enzymes [Mosolov, Valueva, 2005]. Established, that some pathogenic fungi are able to regulate the level of ROS in the infection zone by synthesis and extracellular release of catalase, which destroys hydrogen peroxide [Ibragimov et al., 2010]. It was shown that the activity of catalase in the mycelial zone of the highly virulent strain of powdery mildew is significantly higher than that of the avirulent one [Talieva, Myshina, 1996]. Catalase secretion by the causative agent of cereal corns was also detected [Garre et al., 1998].

But when the oxidative burst was suppressed with the help of ROS scavengers or when plants were pretreated with Ca<sup>2+</sup> channel inhibitors, an increase in the development of infectious structures of rust fungi was noted [Heath, 1998; Xu and Heath, 1994].

Under optimal conditions, ROS is produced at a low level, mainly in chloroplasts, mitochondria, and peroxisomes. NADPH oxidases, cell wall peroxidases, aminooxidases and some other enzyme systems can also serve as potential sources of ROS [Fenyk et al., 1995, Nair et al., 1996, Kulinsky. 1999, Gilad et al., 2000, Akimova et al., 2002, Minibayeva, Gordon, 2003; Millenaar,

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Lambers, 2003, Rogozhyn, 2004, Passardi et al., 2005, Navrot et al., 2007, Polesskaya, 2007, Kolupaev, Karpets, 2010; Swanson, Gilroy, 2010, Borovsky et al., 2011, Kravchuk et al., 2011, Ershova, Berdnykova, 2013, Pradedova et al., 2016]. Specifically, the source of ROS in the apoplast are cell wall-bound oxidases, peroxidases, and polyamine oxidases [Mittler, 2002; Minibayeva et al., 2009]. The plant cell wall is considered as a composite material in which various polymers with deterministic structural details interact with each other, forming an extremely complex supramolecular structure and modifying it in the process of plant development [Ibragimova et al., 2009]. Polysaccharide elicitors are currently the most studied of the cell walls of fungal pathogens. The most studied elicitor isolated from the cell wall and culture fluid of *Phytophthora megasperma* f. sp. *glycinea*, which induces soybean phytoalexin glyciolin [Usov, 1999].

Under conditions of stress, the formation of ROS can increase dramatically and cause oxidative stress. At the same time, plants produce a fairly significant amount of ROS in response to the action of biotic and abiotic stressors [Foyer, Noctor, 2005]. The study of the action of highly active oxygen radicals as regulators of carotenoid biosynthesis in chromoplasts showed that they induce the expression of multiple carotenogenic genes, acting as secondary messengers that initiate carotenoid synthesis (Bouvier et al., 1998).

It was shown that the enzymes bound to the cell wall by hydrogen and ion bonds have the highest redox activity compared to other cell wall enzymes. Thus, by changing the level of ROS under various physiological loads on cells, mobile redox enzymes of the apoplast can act as a regulator of the formation of adaptation processes [Kolesnikov et al., 2007, Elato et al., 2013]. It has been shown that ion exchange reactions in cell walls are a rather important specific link in the development of plant resistance reactions against adverse environmental factors [Meychyk et al., 2007]. ROS is a central component of stress responses in plant cells, with the level of ROS determining the type of response. If at low

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concentrations, ROS induce adaptive responses and the expression of protective genes, promote cell division, and at high concentrations, they trigger processes that lead to cell death. In this way, the dual function of ROS is carried out [Mynybaeva, 2007]. At the same time, it has been proven that ROS are a rather important link in the development of a hypersensitivity reaction, under their action oxidation of lipid membranes occurs, which leads to disorganization of cell structures and its collapse [Baraboy, 1991, Rusterucci et al., 1996, Kombrink, Schmelzer, 2001 ].

It is likely that the main reason for the occurrence of oxidative stress in plant cells in the presence of heavy metals, regardless of the redox ability of the metal, is a decrease in the functional activity of antioxidant enzymes. As is known, as a result of violations of the structure of enzymes, the cause of which can be the formation of strong bonds of metal ions with functional groups, mainly with –SH groups that are part of enzymes, as well as the replacement of native metal ions with toxic ions in the active centers of enzymes , their activity is inhibited. Moreover, as a result of the metabolism of reduced glutathione during the synthesis of phytochelatins, necessary for chelation and sequestration of metals, the content of this non-enzymatic antioxidant in cells can decrease quite sharply [DalCorso, 2012; Sytar et al., 2013; Sharma et al., 2015].

However, despite the rather strong toxic effect of heavy metals on the components of the antioxidant system, cultivated plants have a fairly high resistance against oxidative stress, which is due to the mutual substitutability of the functional components of the multi-level system of antioxidant protection. Thus, antioxidants include a number of enzymes and non-enzymatic low-molecular-weight chemical compounds that are localized in different compartments of the cell and form a single antioxidant system that takes a rather active role in maintaining cellular redox homeostasis [Scandalios, 2005; Gill, Tuteja, 2010; Caverzan et al., 2016]. The vacuolar compartment itself plays a

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rather important role in maintaining stable cell homeostasis. All this is achieved by directing complex compounds and various ions that are harmful to the functioning of intracellular systems, especially in excess entering the cytoplasm from the external environment, into the middle of the vacuole through tonoplast transporters [Gulevych, Baranova, 2007].

The interaction of plants with pathogens is accompanied by the induction of protective reactions, which include the synthesis of compounds that strengthen the cell wall, as well as various antimicrobial compounds, such as phytoalexins, PR-proteins, antimicrobial peptides, and others [Odyntsova et al., 2007].

PR proteins (pathogenesis-related proteins) are extracellular proteins synthesized in a plant cell when it is attacked by a pathogen [Gianinazzi et al., 1969, 1970, White, 1979, Bol et al., 1990, Malamy et al., 1990, Malynovsky, 2010]. They were first detected in the leaves of a resistant variety of tobacco infected with the necrosis virus [Dyakov et al., 2001]. Currently, it has already been proven that PR proteins take a rather active part in the implementation of various plant protection mechanisms [Valueva, Molosov, 2002, Filipenko et al., 2013; Sharypova et al., 2013]. PR-9 proteins are classified as peroxidases [Whipps, 2001]. PR-proteins implement the cell protection mechanism associated with the growth of ROS formation in the plant cell. It is known that one of the mechanisms for the formation of hydrogen peroxide functions at the level of the outer surface of the plasma membrane and is mediated by the PR protein - the enzyme NADPH-dependent oxidase [Udyntsev et al., 2011]. Hydrogen peroxide is a signaling molecule that regulates many physiological processes, including phytoalexin production, stomatal opening, expression of protective PR proteins, resistance formation, and lignification of cell walls. But at the same time, long-term accumulation of hydrogen peroxide is toxic to plants [Novo-Uzal et al., 2013]. As is known, the expression of genes of the PR family is activated under biotic stress, but their activation was observed both under the influence of heavy

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metals and under other abiotic stresses, and therefore their participation in the protective reactions of plants to the action of a fairly wide range of stress factors is assumed [Edreva, 2005]. It has been shown that ROS, through the signaling molecules of salicylic and jasmonic acids, regulates the expression of protective PR-protein genes, and thus determines the interaction of signaling pathways in the formation of the protective response of plants against a fairly wide range of pathogens. Moreover, the salicylate-dependent pathway is mainly activated during the attack of biotrophic pathogens, leads to a hypersensitive response and local resistance, and the jasmonate-dependent pathway takes part in the defense against necrotrophic pathogens and insects [Gimenez-Ibanez, Solano, 2013]. It has also been shown that the functions of PR proteins are significantly broader than just participation in protective mechanisms: they are always present in certain amounts in cells, playing a rather important role in various processes of plant growth and development regulation [Scherer et al., 2005, Garcia-Brugger et al., 2006, Loon et al., 2006].

Induced resistance of plants is based on genes of species and varietal immunity against incompatible, but congruently preserved forms of phytopathogens. Defense reactions controlled by these genes can be activated not only by living microorganisms, but also by chemical biologically active compounds. In the latter case, it will be acquired artificially induced immunity. Induced protection against pathogens in this case is based on the stimulation of defense mechanisms through metabolic changes that help the plant to defend itself more effectively. Induction of plant resistance against pathogens is carried out by pre-treatment of seeds or vegetative plants with chemicals that have the properties of plant immune system stimulators [Tyuterev, 2002].

When pathogens and their elicitors act in plants, the protection system (NADFN-oxidase, peroxidase, phytoalexin-forming, phenolic, etc.) is activated, a set of proteins toxic to pathogens is synthesized, as well as enzymes that destroy



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the cell walls of pathogens, or strengthen the cell walls of plants, etc. . The protection of plants, which is based on inducing their resistance with the help of their own components of the immune system, allows to avoid pollution of the surrounding environment and is ecologically safe [Pavlovskaya, Gagarina, 2018].

The study of the effect of the regurgitant (oral secretion) of larvae of the Colorado potato beetle in the 4th age on mechanically damaged leaves of common bean showed that the oxidative burst associated with the formation of hydrogen peroxide by means of NADPH oxidase and superoxide dismutase is a necessary link, along with biological synthesis and reception of ethylene, which leads to an increase in the activity of peroxidase and polyphenol oxidase in leaves [Steinite et al., 2004].

The discovery of new and research of already found elicitors for various types of cultivated plants, the detailed study of all the diversity of their mechanisms of action are of great practical importance, paving the way for the development of new methods of combating significant crop losses [Sokolov, 2015]. It has been established that the structural and functional interaction of a necrotrophic pathogen with a plant during the period of penetration is almost similar to that of a biotrophic pathogen and is characterized by the manifestation of exo- and endocytosis and lysosomal participation depending on the resistance of the plant against the pathogen. In plants, exocytosis and endocytosis occur in the areas of attachment and penetration of pathogens through the cell wall, as a result of which recognition occurs between the cells of the fungus and the plant. In phytopathological systems created by biotrophic pathogens, there is exocytosis of substances directed at the protoplasm of the host into the extrahaustorial matrix, in the absence of the reverse process due to the absorption of evaginations by the extrahaustorial membrane by haustoria; at the same time, restoration of the relationship between the various vector processes of cytosynthesis becomes possible only at the final stages of the parasitic interaction [Karpuk, 1996].

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For many years, biochemistry was dominated by the idea of the destructive role of ROS during stress. In numerous scientific works carried out in recent years, it is indicated that the formation of ROS in the plant organism is not only an integral property of the normal vital activity of cells, but also a non-specific reaction of the body's response to the action of stresses of various nature. At the same time, it was shown that when the intracellular level of ROS changes, the activity of almost all classes of effector proteins, which take an active part in signal transmission from the plasmolemma to the nucleus, changes. The activity of a number of protein kinases, phosphatases, phospholipases, transcription factors, ion channels and pumps depends on the level of oxidants and reductants in the cell. Therefore, redox regulation of cellular processes is one of the fundamental mechanisms for regulating the functional activity of cells, and the preservation of redox homeostasis parameters is vital both for individual cells and for the entire organism as a whole [Scandalios, 2005; Pang, Wang, 2008, Bechtold et al., 2009, Kolupaev, Karpets, 2009; Foyer, Noctor, 2011; Kreslavsky et al., 2012; Bilova et al., 2016, Paudel et al., 2016, Sies, 2016].

Under the influence of weak factors of the external environment, the value of the ratio between the intracellular concentrations of electron donors and acceptors or reducing agents and oxidizing agents changes within certain physiological limits, while under conditions of stress in cells, an imbalance between the levels of oxidants and antioxidants is revealed, which can cause oxidative stress and redox disturbances signaling [Sies, Jones, 2007, Cherenkevych et al., 2009]. The increased activity of antioxidant enzymes of winter wheat plants in the period of milk-milk-wax maturity, when the intensity of photosynthesis decreases, indicates the intensification of the protective reactions of the photosynthetic apparatus against oxidative stress. During the period of grain pouring, the leaves gradually age and the rate of assimilation of carbon dioxide decreases due to the reutilization of nitrogen-containing

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compounds in the grain that is poured. The consequence of this is a decrease in the consumption of NADPH in the Calvin cycle, which leads to overreduction of the electron transport chain and the formation of ROS in chloroplasts, which damage photosynthetic membranes [Sokolovska-Sergienko, 2017].

It is known that the exogenous use of brassinosteroids changes the expression of a number of genes, as well as the activity of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase, and non-enzymatic antioxidant systems involving ascorbic acid, tocopherols, carotenoids, and glutathione [Hardike et al., 2007].

In maintaining the viability of the cell, a rather important role is played by the control of metabolism, carried out mainly due to thiol-disulfide exchange. Sulfhydryl groups of cysteine residues are quite important for the functioning of enzymes and cellular signaling processes, which are the basis of the body's responses to environmental factors. The basic mechanism of thiol-mediated redox control in cellular metabolism is the ability of SH-groups to reversibly change their redox state with the subsequent change in conformational, catalytic or regulatory functions of the protein [Kolupaev, Karpets, 2009; Kalinina et al., 2014a]. It has also been shown that during the transfer of heavy metal ions through the plasma membrane, the SH-groups of carrier proteins are able to retain some of the ions, thereby performing a barrier function [Prasad, 1999].

A rather important role in the formation of plant resistance against cadmium ions is played by the surface cell membrane (plasmolemma), which can completely block the entry of toxic ions into the cell or significantly reduce the level of their passive transmembrane transport. This function of the plasmolemma is provided by a change in the structure, specifically, a decrease in the content of unsaturated fatty acids, a decrease in membrane fluidity, or a decrease in the number of membrane targets for metal ions, for example, SH groups on its outer surface. There is also information that, under the influence of cadmium, the

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orderliness of the lipid bilayer of alasmolema can increase, which, in turn, also contributes to its lower sensitivity against the direct action of metals. The increase in the concentration of reduced glutathione is also a rather important mechanism of protection of plants, including cereals, against the toxic effect of cadmium ions. Despite the fact that reduced glutathione can independently bind ions in the cytoplasm and transport them to the vacuole, it is about 1000 times less effective in protecting against this metal than phytochelatin. Their synthesis is activated by a number of heavy metals, but specifically cadmium is their most active inducer. The binding of cadmium by non-protein thiols is considered one of the most important mechanisms of its detoxification in the cell in plants [Tytov et al., 2012].

Lead ions interact with free SH-groups, which are necessary to stabilize the three-dimensional structure of the protein. In addition to the reaction with SH-groups, the blocking of  $-COOH$  by lead ions also plays a rather important role in the inhibition of enzyme activity. Ions of this metal form a mercaptide with  $-SH$  of cysteine, and can also form complexes with phosphate groups [Levina, 1972]. Divalent lead ions do not have redox properties like trivalent iron ions, and therefore the oxidative stress caused by them occurs indirectly through the stimulation of ROS production, an increase in the pro-oxidative status of the cell due to a decrease in the amount of reduced glutathione, activation of calcium-dependent systems and effects on processes, which involve iron ions [Pinto et al., 2003]. The concerted activity of the systems of membrane transporters, metal chaperones and chelators implements at the cellular level the main strategy of resistance of plants against heavy metals and their hyperaccumulation [Kuznetsov et al., 2011].

Low-molecular antioxidants, which include glutathione, are of greatest importance at the earliest stages of activation of increased ROS production and, together with the enzyme superoxide dismutase, constitute the first line of defense

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against ROS [Yu, 1994, Karimullina, Pozolotyna, 2009, Polovynkina, Sinitsyna, 2010, Gagarina, 2012, Lushchak, 2012, Sharova, 2016]. Superoxide dismutase, peroxidase, and catalase are the main components of enzymatic antioxidant protection that directly disinfect ROS, or take an active part in the regeneration of low molecular weight antioxidants [Akimova et al., 2007]. In addition to antioxidants with a small molecular weight, antioxidant enzymes are an important link in the first line of the ROS neutralization system, namely those that prevent the formation of the strongest of all oxidants known to chemists, the hydroxyl radical. Since the hydroxyl radical is characterized by extreme aggressiveness and non-specificity of interactions, it can be argued that a specific enzyme that would neutralize HO• does not exist. If we even assume the possibility of such an interaction, it would very quickly lead to the inactivation of the enzyme. There is also no specific low-molecular-weight antioxidant that would neutralize HO•, because such a compound would have to compete strongly with other molecules in the cell to interact with HO•. Therefore, the intracellular concentration of such a substance should be as high as possible. As a result, the osmotic pressure in the cell would also be very high. Thus, the main enzymes of the first line of defense that prevent the formation of HO• are superoxide dismutase, catalase, and peroxidase [Semchyshyn, 2015]. Catalase is a fairly common enzyme that decomposes hydrogen peroxide into water and molecular oxygen [Borisova, Chukina, 2007].

Antioxidant systems are represented by enzymatic antioxidants, such as superoxide dismutase, catalase, dehydroascorbate reductase, glutathione transferase, ascorbate peroxidase, phospholipid hydroperoxidase, and others. The set of non-enzymatic antioxidants is quite large and includes ascorbic acid, glutathione, carotenoids, anthocyanins, tocopherols, ubiquinones, flavonoids, cysteine, methionine and many other compounds [Todorov, Todorov, 2003]. Superoxide dismutase carries out the recombination of the O<sub>2</sub><sup>-</sup> radical with the

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formation of hydrogen peroxide and triplet oxygen, being the only one among the known antioxidant enzymes that directly ensures the breaking of chains of oxygen-dependent free radical reactions in the cells of living organisms [Ruth, Heath, 2002]. It is claimed that superoxide dismutase is the only enzymatic antioxidant that neutralizes radical ROS, therefore this enzyme is considered as the first link of antioxidant protection [Alscher, 2002]. It is believed that catalase is in close interaction with superoxide dismutase, which actively participates in the decomposition of significant amounts of hydrogen peroxide [Guan, Scandalios, 2000]. An increase in the activity of this enzyme during freezing of naked oats was also shown [Liu et al., 2013]. Cold hardening can also cause increased gene expression and increased catalase activity. Such an effect, specifically, was found in chrysanthemums. During long-term cold adaptation of plants of this species, an increase in the expression of catalase genes was established in 21 days [Chen et al., 2014].

Glutathione transferases are a superfamily of enzymes that catalyze the conjugation of glutathione with a variety of endogenous and exogenous compounds containing electrophilic sulfur, phosphorus, nitrogen, and carbon atoms, as follows. Glutathione transferases take an active part in cellular protection against the possible toxic effects of these compounds. Due to their catalytic properties, these enzymes are among the enzymes of the second phase of the biological transformation of xenobiotics [Hayes et al., 2005, Tew, Townsend, 2012, Wu, Dong, 2012]. A detailed study of the structures and amino acid sequences of cytosolic glutathione transferases made it possible to divide them into two groups. At the same time, the analysis of the amino acid composition of the G-site served as a rather important criterion. Thus, the first group (Y-GSTs) combines enzymes whose active center includes tyrosine, necessary for interaction with reduced glutathione ( $\alpha$ ,  $\mu$ ,  $\pi$  and  $\sigma$  classes), with enzymes from the second group (S/C-GSTs) in either series ( $\varphi$ ,  $\tau$ ,  $\theta$  and  $\zeta$  classes)

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or cysteine ( $\beta$  and  $\omega$  classes) participate in glutathione activation. In isoforms of both groups, as well as in mitochondrial glutathione transferases, these amino acid residues necessary for glutathione activation are located in a region called the "catalytic loop" following the first  $\beta$ -sheet in the thioredoxin-like domain. A comparison of the structures of the H-site in isoforms of different classes indicates a rather wide variability of this site [Oakley, 2011]. Glutathione transferases are active members of the antioxidant system, they catalyze the reduction reaction of organic hydroperoxides to alcohols with the participation of reduced glutathione [Brylkina, 2002, Hayes et al., 2005].

Ascorbate peroxidase is the main enzyme that neutralizes hydrogen peroxide in the cell [Andreeva, 1988]. Glutathione peroxidase utilizes both organic and inorganic peroxides, restores oxidized glutathione, slows down the progression of lipid peroxidation [Polovnikova, 2010]. In corn seedlings, the majority of glutathione peroxidase activity is concentrated in the root system, and activation of its functioning under the action of cadmium ions is observed only in the metal-tolerant hybrid Dniprovskiyi [Syshchikov, 2011]. Under the influence of elevated temperatures, the expression of certain oxidant enzymes increases, including the thermostable cytosolic isoform of ascorbate peroxidase ARKH2, which is not expressed under normal conditions [Panchuk et al., 2002]. It was also found that the absence of significant changes in the activity of ascorbate peroxidase and glutathione reductase is indirect evidence that the content of ROS in the leaves of winter wheat varieties did not significantly increase under conditions of progressive soil drought [Nikolaeva, Maevskaya, 2010]. A correlation has been established between the resistance of wheat, pea, corn, and soybean plants to hypoxia and the activity of enzymes of the antioxidant system of plants - ascorbate peroxidase, catalase, glutathione peroxidase, NADP-peroxidase, and general peroxidase [Ershova et al., 2007].

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Reprogramming the level of expressed protein-enzymes of the antioxidant system, in particular ascorbate reductase, catalase, glutathione reductase, superoxide dismutase, and many others, as well as proteins of the regulatory system, is an integral part of the plant cell response to adverse temperature [Bilgin, 2010].

When ascorbate peroxidase activity is low, guaiacol peroxidase is activated under the action of ozone and ultraviolet radiation [Takahama, Oniki, 2000]. It reduces peroxide by reducing phenolic compounds, in particular guaiacol or pyrogallol. This type of peroxidase takes part in the biological synthesis of lignin [Roshchyna, 2009; Polovnikova, 2010].

Together with catalase, peroxidase is part of a single system of antioxidant protection of living organisms, preventing the destructive effect of ROS. The intensity of aerobic processes can be estimated by the activity of peroxidase. Therefore, the study of peroxidase activity is a very important factor in assessing the stress response of a living organism [Mika et al., 2004, Jackson et al., 2010, Mostafa et al., 2011, Naumenko et al., 2013]. Activation of peroxidase is one of the non-specific responses of plants to damage by phytopathogens. It is part of the antimicrobial system of plant protection and takes part in the processes of lignification, creating a mechanical barrier in the way of infection. Some isoforms of peroxidases are able to be among the first to come into contact with infectious structures of pathogens [Istiyeva et al., 2013]. On the active role of peroxidases in the protective response against the pathogenic fungus *Verticillium dahliae* Kleb. the results of studies on the correlation of peroxidase activity with hemigossypol oxidation during the formation of gossypol in cotton extracts [Benedict et al., 2006] show. The early accumulation of peroxidases in the intercellular fluid is considered as part of the hypersensitivity reaction after treatment of plants with bacteria of the genus *Xanthomonas*. Moreover, in hypersensitive cells, high activation of guaiacol peroxidase persists for 12 hours



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after pathogen treatment [Delannoy et al., 2003]. It was found that the active development of the phytophthora pathogen *Phytophthora infestans* (Mont.) de Bary on potato plants with suppressed anionic peroxidase activity is associated with insufficient lignification of cell walls at the rooting sites of the pathogen, which indicates the key role of this enzyme in protecting potatoes from this pathogen [Sorokan, 2013].

Peroxidase is a functionally quite labile enzyme that reacts to any changes in the environment and stressful situations and belongs to the 9th class of PR proteins [Savych, 1989, Gazaryan, 1992, Hiraga et al., 2001; Duroux, Welinder, 2003; Maksimov et al., 2004, Rogozhyn et al., 2004, Gechev et al., 2006; Cosio, Dunand, 2008, Akimova et al., 2009, Udyntsev et al., 2011, Agati et al., 2012]. The composition of peroxidase includes hemin represented by protoporphyrin 9 in a complex with trivalent iron and a polypeptide chain. The polypeptide chain includes from 203 to 308 amino acids and forms a compact tertiary structure, which is represented by a large and a small domain. The hemin part of the molecule, in turn, plays the role of an active center that takes an active part in the decomposition or activation of hydrogen peroxide, resulting in the formation of radicals of the corresponding substrates. Peroxidase is an enzyme that responds to the most diverse actions by changing the composition of its isoforms or increasing the activity of already present molecular forms. After synthesis, peroxidase isozymes can move from the cytoplasm to the interplasmic space, that is, this enzyme is secreted. The fairly high polymorphism of peroxidases is due, on the one hand, to the fact that they are encoded by a large number of loci and alleles, and on the other hand, to post-transcriptional modifications of molecules associated with the work of specific proteases and glycosylation of individual isoenzymes. In the cell, peroxidases exist in both cytoplasmically dissolved and membrane-bound forms [Rogozhyn, 2004]. The polymorphism of this enzyme increases the adaptive capacity of plants [Graskova et al., 2009]. A feature of the

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mechanism of action of peroxidase is its ability to catalyze the oxidation of organic substrates with the participation of oxygen, that is, the enzyme can perform the role of an oxidase. At the same time, the oxidase substrates of this enzyme are indolyl-3-acetic acid and dioxyfumaric acid [Rogozhyn, 2000]. Two different functions were found in peroxidase - oxidase and peroxidase. All this allows us to predict that two independent active centers, spatially separated, although located close to each other, can participate in the catalytic action of the enzyme [Kolman, Remm, 2000]. There is an opinion that the decomposition of indolyl-3-acetic acid is initiated by oxidative decarboxylation reactions carried out by plant peroxidases. It has been shown that changes in the metabolism of phytohormones, and specifically in the metabolism of indolyl-3-acetic acid, occur already in the first minutes of stress [Gazaryan et al., 1996, Ghosh, Basu, 2006]. Plant peroxidases not only accumulate locally at the site of infection or under the action of abiotic factors, but are also expressed systemically, and like other PR proteins, are always present in plants in a certain amount. They take an active part in the regulation of a wide range of physiological processes throughout the life cycle of plants, thanks to the large number of isoforms and the number of reactions catalyzed by them. The main function of peroxidases is to catalyze the hydrogen peroxide oxidation of various electron-donating substrates, and primarily of polyphenolic structures. At the same time, with an excess of substrates with a high reducing potential, peroxidases begin to show oxygenase activity, catalyzing the generation of hydrogen peroxide [Passardi et al., 2007]. The oxidizing function of peroxidases is realized in the presence of a suitable oxidant and alkalizing of the pH of the apoplast [Almagro et al., 2009]. It was established that the change in pH determines the ratio of peroxidase and oxidase activities of peroxidases. Thus, it was shown that the peroxidase of the cell wall, which was capable of forming hydrogen peroxide at neutral pH, restored its peroxide function when the pH of the surrounding solution shifted to the acidic side [Blee et al., 2001]. And the

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direct introduction of salicylic acid into the intercellular space led to an increase in the content of hydrogen peroxide and a change in peroxidase activity in the apoplast of wheat leaves, which plays a rather important role in signal transmission and ensuring the passage of biochemical processes closely related to the protection of plants under the influence of stressors of various nature [Maksyutova et al., 2009]. At the same time, it was found that the expression of a significant part of peroxidase isoforms changes dynamically depending on the stage of ontogenesis of soybean plants [Dyachenko et al., 2009].

Since a pool of soluble peroxidases and slowly oxidizing substrates is always present in the apoplast, it can be assumed that the release of a small amount of a rapidly oxidizing substrate/reductant, cofactor, or inducer is sufficient for an immediate response. The subsequent growth of the response can be carried out both due to the release of an additional amount of enzyme and the appearance of substrates. Thus, the key role of extracellular peroxidase activators is based on the regulation of the balance of ROS in the apoplast of plant cells [Chasov et al., 2014]. Also, under stress, there is an increase in the concentration of abscisic acid in plant cells, which triggers the synthesis of dehydrins. It should also be noted that the increase in peroxidase activity in some plant species is accompanied by an increase in the content of dehydrins [Zhyvetyev et al., 2010]. It is believed that the increase in the expression of dehydrin genes and the accumulation of their protein products is quite important for increasing the resistance of plants against low-temperature stress [Campbell, Close, 1997; Close, 1997; Thomashow, 2001; Allagulova et al., 2004; Shakirova et al., 2005, 2009].

All peroxidases are conventionally divided into three superfamilies: the first includes animal peroxidases; the second - animals, plants, bacteria and fungi, and the third - plants, microorganisms and fungi. The third family is divided into three classes: Class I includes plant ascorbate peroxidases, catalase-peroxidases of microorganisms and cytochrome-c-peroxidases; Class II combines Mn-

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peroxidases and lignin-peroxidases of fungi, as well as mobile catalase-peroxidases; Class III includes classic plant peroxidases, localized mainly in the apoplast, vacuole, and cell wall [Passardi et al., 2005, Gazaryan et al., 2006; Maksimov et al., 2011].

Class III peroxidases are rather strong redox enzymes of the apoplast - multifunctional enzymes that catalyze the oxidation of various electron-donating substrates due to the decomposition of hydrogen peroxide to water. Peroxidases play a rather important role in the regulation of cell growth by stretching, the generation of ROS, the formation of resistance against various biotic and abiotic factors, and the protection of cells against oxidative stress [Cosio, Dunand, 2008]. Their functioning in pro- and antioxidant modes, the number of peroxidase genes and post-translational modifications determined the key role of these enzymes in controlling the redox status in plant cells. It is shown that wheat peroxidase genes are characterized by a complex structure, which manifests itself in the presence of specific cis-elements of the promoter, which influence the expression of genes in various interactions. This feature determines the fairly high activity of wheat peroxidase genes and contributes to the manifestation of the functions of this enzyme in the protective reactions of plants [Sharifullina, Minibayeva, 2013]. Some researchers suggest using peroxidases as a marker of plant winter hardiness [Kapustyan et al., 1999, Kapustyan, 2000]. It is also claimed that the activity of this enzyme in a resistant variety of plants is always higher than in a susceptible one and increases sharply under the action of the pathogen. It was reported that the peroxidase weakly bound to the cell wall of resistant and susceptible potato varieties is competitively inhibited in the absence of the pathogen. In the first moments of infection, a sharp increase in the activity of enzymes of the resistant variety occurs due to an increase in their affinity for the substrate as a result of a decrease in competitive inhibition. And in a susceptible potato variety, there is no sharp increase in enzyme activity due to simultaneous passage of two

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multidirectional processes: a decrease in  $K_m$  and a decrease in the rate of formation of  $V_{max}$  and decomposition of the enzyme-substrate complex [Graskova et al., 2008].

Analysis of the nucleotide sequence of the peroxide gene AtPrx53in20 natural races of *Arabidopsis thaliana*, revealed the existence of two haploids [Kupriyanova, 2008]. It was also established that the isoform of apoplast peroxidase with a molecular weight of 37 kDa exhibits the highest activity in the roots of wheat seedlings under wounding stress. The 37 kDa peroxidase amino acid sequence is used to search for genes encoding this protein [Minibayeva et al., 2009]. It was also found that the resistance of cultivars against drought can be estimated by the indicators of the total activity of peroxidase in dry seeds of cultivated plants, and this assessment can be carried out based on the results of the seeds of one year of reproduction and in the absence of information about the yield of these cultivars on humid and arid backgrounds [Haydash, Kazakova, 2007]. Such phenolic compounds as ferulic acid and coniferyl alcohol, as well as NADPH and ascorbic acid, detected in the post-incubation solution after extraction of spring wheat roots from it, are slow substrates for peroxidase of wheat roots. It is believed that these substances, along with peroxidase, take an active part in the protective mechanism of plants under stress [Konovalova et al., 2007].

The direction of research on the isolation of peroxidase from wood-moving fungi *Pleurotus ostreatus* is quite interesting, since it was found that the mushroom peroxidase is not a lignin- or Mn-dependent peroxidase, but belongs to the class of secretory fungal peroxidases of the classical plant peroxide type. The ligninolytic system of white rot fungi consists of various oxidizing enzymes: lignin peroxidases, Mn-dependent peroxidases, and laccases. White mold fungi *Pleurotus ostreatus* produce both Mn-dependent peroxidases and hybrid peroxidases on peptone medium [Cohen et al., 2001]. Moreover, hybrid

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peroxidases have the catalytic activity of lignin-peroxidases and Mn-peroxidases [Gomez-Toribio et al., 2001].

Peroxidases are enzymes that are synthesized not only constitutively, but also inducibly, under the influence of various physical, chemical, and biotic factors. Their molecular and functional heterogeneity makes it possible to change the ratio between isoforms in favor of those more "adapted" to specific stressful conditions of the environment, and the increase in peroxidase activity can be considered as a protective function of the plant organism [Yevinsh, 1987, Bestwick et al., 1997, Sasaki et al. , 2002, Tognolli et al., 2002]. When pathogens and chitooligosaccharides act on plant cells, wheat anionic peroxidases manifest themselves as relatively mobile isoenzymes that are activated both in the freely soluble and in the apoplast protein fractions [Maksimov et al., 2010]. In addition, it is proposed to use various enzymes, including peroxidases, as a diagnostic feature to assess the degree of resistance of plants to the action of stress factors [Smith et al., 1976].

Unlike many other enzymes, peroxidase is characterized by polyfunctionality and high heterogeneity of its isozyme system. It is known that an increase in peroxidase activity correlates with an increase in man-made load on plants [Lukatkin et al., 2003]. Peroxidases catalyze the formation of oxidative bridges between the polymers of cell walls using hydrogen peroxide, thereby reducing the elastic stretch of the walls. Rigidification of the walls in the basal direction is accompanied by a two-fold increase in the activity of ion-bound peroxidases with the cell wall; when the growth of mesocotyls is inhibited under the influence of red light, the activity of peroxidases also increases. But at the same time, the growth of NADH-oxidase activity of cell walls was insignificant. Thus, in the process of slowing down the growth of corn mesocotyls caused by cell differentiation, there is a significant stiffening of cell walls and an increase in the activity of peroxidases, which ensures a decrease in the content of hydrogen

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peroxide in the apoplast solution, against the background of practically unchanged activity of NADH-oxidases [Avrutyna et al., 2007]. Peroxidase also oxidizes organic substances in the soil, in particular phenols, amines and some heterocyclic compounds due to the oxygen of hydrogen peroxide and other organic peroxides. It performs a protective function, disinfecting peroxides and decomposing aromatic xenobiotics in the soil, and also plays a rather important role in the process of humus formation [Zvyagintsev et al., 2005]. As the research results showed, after 2.5 months, the peroxidase activity of soil contaminated with cadmium was restored to the initial values of clean soil [Pleshakov et al., 2010]. The sensitivity of the peroxidase and proteinase inhibitor genes to the signaling molecules of salicylic and jasmonic acids was revealed [Akhatova and others., 2013].

The property of peroxidases to be activated under the influence of chitin and sorbed on it implies the possibility of their participation in the processes underlying two types of protective reactions of plants against phytopathogens. The first type includes the rapid activation of this enzyme when it comes into contact with cellular structures of pathogens, as observed, for example, when it interacts with chitin peroxidase of seed rice, potatoes, horseradish, similar to its activation during a hypersensitivity reaction. The second type of reaction can be compared with the gradual accumulation of enzyme molecules in the zone of localization of the fungus, associated with the appearance of a peculiar "attracting" center in the shape of chitin-containing structures of the phytopathogen [Cherepanova, 2005]. It was established that the property of peroxidase to be sorbed on chitin implies its participation in the processes that form the basis of protective reactions of plants against chitin-containing phytopathogens [Kuzmina et al., 2008]. Chitin-binding lectins, thanks to their specificity for N-acetyl-D-glucosamine (ClcNAc) and chitin oligomers, are the most likely candidates for a protective role in plants against fungal pathogens

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[Oka et al., 1997, Jouanin et al., 1998] . One of the ways to protect the mycelium of the fungus from sorption on its surface can be the property of the gene products, for example, *avr4* of the mushroom *Cladosporium fulvum* Cook. bind to chitin and protect it from degradation by chitinases. It is possible that this pathway may contribute to the prevention of binding to chitin and other chitin-specific proteins, including oxidoreductases [Van den Burg et al., 2003]. Of particular interest are works showing a decrease in the number and species composition of pathogenic microorganisms and nematodes in chitin-containing soil [Sarathchandra et al., 1996, Begunov et al., 2001, Tyuterev, 2002, Zinovyeva et al., 2004]. Phytofluoride resistance of immunized potato plants was prolonged and spread both to tubers during storage and to vegetative plants, in addition, the residual resistance induced by chitin was preserved in the new crop as well [Perehod et al., 1997]. A reduction in the amount of sterols, as well as a parallel accumulation of reshitin, to which the pathogen left with sterols becomes more sensitive, is one of the mechanisms of resistance induced by chitosan in the pathosystems "tomato - root nematode" and "potato - late blight pathogen" [Maximov et al., 1997].

It is known that the fungus *Phellinus robustus* (P. Karst.) Bourdot & Galzin synthesizes intracellular [Melnychuk et al., 1976, Annesi et al., 2003] and extracellular [Lyr, 1956, 1958, Reshetnikova et al., 1992] peroxidases. However, this property is not characteristic of all strains of this fungus [Gandbarov, 1989].

The participation of peroxidases weakly bound to the cell wall in the formation of protective mechanisms of potato plants under the action of a bacterial pathogen is shown. Patterns of activation of the studied peroxidase molecules or their *de novo* synthesis depending on the ability of potato varieties to show resistance against the pathogen were revealed. At the same time, two strategies for the protection of potato cells against the action of the pathogen were substantiated for the first time. One of them is characteristic of resistant genotypes and is associated with the rapid and rapid activation of peroxidases already present



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before infection, and the other is characteristic of susceptible genotypes, due to the de novo synthesis of enzyme molecules at later stages of infection [Graskova et al., 2008].

The change in the sensitivity of proton pumps of the tonoplast against redox regulators was studied depending on the change in the functional load of the tonoplast, which occurs at different phases of ontogenesis. At the same time, it was established that the sensitivity of tonoplast proton pumps against redox regulators changed depending on the phase of ontogenesis. During the period of active plant growth in the first year of vegetation, both oxidized and reduced glutathione significantly (by 40-60%) reduced the activity of H<sup>+</sup>-pyrophosphatase. And during the resting period of the root crop, the activity of this enzyme practically did not change with the addition of oxidized and reduced glutathione. In the second year of vegetation, during the generative phase of ontogenesis, the activity of H<sup>+</sup>-pyrophosphatase under the influence of oxidized and reduced glutathione was inhibited again by 40-60%. All this information allows us to talk about the rather important role of redox homeostasis, since its change in any direction negatively affects the activity of the enzyme. The activity of H<sup>+</sup>-ATPase in the first year of vegetation did not change under the influence of oxidized glutathione and increased by 20-25% when reduced glutathione was added. During the resting period, this enzyme also had little dependence on oxidized glutathione, but reduced glutathione showed an inhibitory effect, not a stimulating one, by 20-25%. In the second year of vegetation, H<sup>+</sup>-ATPase showed maximum sensitivity to reduced glutathione – its activity increased almost 2 times, although oxidized glutathione did not have a significant effect on this period [Ozolya et al., 2007].

A rather important factor in plant resistance to stress is the maintenance of a high level of low-molecular antioxidants – reduced glutathione and ascorbic acid, which not only "put themselves under attack" by radicals and peroxides, but

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also take part in the restoration of other antioxidants, and are also coenzymes that regulate the work enzymes [Chupakhina, 1997, 2009; Kenya and others, 1993; Menshikova, Zenkov, 1993; Chirkova, 2002]. It is quite likely that the increase in the ratio of reduced to oxidized glutathione and the level of total glutathione leads to a decrease in redox potential (redox potential) - a more reduced state of the intracellular environment, which contributes to a decrease in the level of ROS in cells and an increase in the resistance of plants to oxidative stress [Szalai et al., 2009]. Redox potential is one of the integral physicochemical parameters characterizing the state of the flora.

Ascorbic acid is involved in maintaining the redox potential of the cell, reducing tocopherol and glutathione [Khan et al., 2011]. It also takes an active part in the ascorbate-glutathione cycle, and also directly utilizes ROS [(Blokhina et al., 2003; Mahmood et al., 2010; Khan et al., 2011). One of the criteria for plant resistance is the content of ascorbic acid and glutathione. These are one of the most important protectors of lipid peroxidation processes [Smirnoff, 2000, Zaiko, Lyholat, 2011]. This acid plays a major role in the elimination of hydrogen peroxide, and is probably the strongest antioxidant in plants [Gill, Tuteja, 2002]. Its functional role in plants is quite large the most important function is antioxidant protection - it disinfects hypochlorous acid, as a precursor to the formation of free radicals; it restores tocopherol radical, aerating vitamin. It has antioxidant properties and protects lipids from peroxidation [Guskov et al., 2009]. The antioxidant functions of ascorbic acid are due to the fact that it is a potential donor of hydrogen atoms or electrons used for the reduction of hydrogen peroxide or some free radical products. In the process of these reactions, ascorbic acid can lose one or two hydrogen atoms with the formation of a monodehydroascorbate radical or dehydroascorbate. Oxidation of ascorbic acid with the formation of monodehydroascorbate radical can be the result of direct contact with ROS, as well as enzymatic reactions. Being an unstable radical, the

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monodehydroascorbate radical quickly transforms into dehydroascorbate. Thus, two molecules of the monodehydroascorbate radical, interacting with each other in a dismutation reaction, give ascorbate and dehydroascorbate. As a reducing agent, ascorbic acid takes an active part in the functioning of many antioxidant systems operating in chloroplasts, mitochondria, peroxisomes, cytosol, and apoplast [Polesskaya, 2007]. At the same time, it was established that the anion channels of the plasma membrane of the cells of higher plants have significant permeability to the ascorbate ion. The output of ascorbate through anion channels can be of great importance for plant physiology, providing both the intercellular transport of this rather important antioxidant and metabolite, and the repolarizing flow of ions during stress-induced leakage of electrolytes [Griusevych et al., 2018]. It is also believed that the protective function is performed not by reduced ascorbic acid, but by its oxidized form, since in the downy mildew-resistant pea variety, part of ascorbic acid was represented in the oxidized form in the cells located in the affected area. According to researchers, it serves as a barrier on the path of the causative agent of the disease. In susceptible varieties of peas, only the reduced form of ascorbic acid was detected in the places of penetration of the pathogen [Peresykin et al., 1977]. The effect of exogenous ascorbic acid on wheat plants that were exposed to an increased concentration of heavy metals was studied using the example of cadmium, which caused a decrease in growth parameters, contributed to a sharp deterioration of the energy balance and the development of oxidative stress. It was found that ascorbic acid had a protective effect, which was expressed in an increase in the values of morphometric indicators, stabilization of the energy balance and reduction of lipid peroxidation. At the same time, a positive correlation ( $r = 0.97$  at  $p < 0.05$ ) was found between the change in energy and redox balances at toxic concentrations of cadmium and the protective effect of ascorbic acid [Enikeev et al., 2013]. It was shown that, starting from a concentration of 0.3 mM, ascorbic acid inhibited the growth of the

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main root of *Arabidopsis*, and such parameters of its architecture as the diameter of the root and the length of cells in the stretching zone were modified [Voitekhovich et al., 2018]. It was established that metal nanoparticles probably activate mechanosensitive channels of the plasma membrane of root cells, and also oxidize L-ascorbic acid [Demydchyk, 2014].

The most important aspect of the action of ROS, from an overview doon its biological effect, is their interaction with DNA molecules. To date, about 100 variants of DNA damage by free radicals have been identified. In general, the consequence of ROS attack is breaks in the polynucleotide chain of the molecule, modification of the carbohydrate part and nitrogenous bases, which can lead to the appearance of various mutations [Semchyshyn, 2002]. It has been shown that under oxidative stress, mitochondria serve as the main source of ROS [Chernyak, 2010]. The main source of ROS in plants in the light is chloroplasts, in which ROS occur mainly due to the fact that oxygen can act as an alternative electron acceptor from carriers of the photosynthetic electron transport chain. The superoxide anion radical,  $O_2^-$ , is formed during the reduction of  $O_2$  molecules in the plastoquinone pool and on the acceptor side of photosystem-1 [Borisova-Mubarakshina et al., 2015].

At the same time, the activity of superoxide dismutase changes in different directions; in some cases, its growth was noted, and in others - a decrease, which depends on the intensity of the action of the stress factor, as well as on the susceptibility of the organism, the stage of plant growth, etc. [Iturbe-Ormaetxe et al., 1998; Stroinski, 1999, Kaminska-Rozek, Pukacki, 2004, Pradedova et al., 2009]. Two more variants of superoxide dismutase of barley - Sod B and Sod D - were also described, and it was established that the isoenzyme composition of superoxide dismutase isolated from the roots causes a not strictly specific reaction of plants against adverse factors in the root zone [Netsvetaev, 2000]. In *Arabidopsis*, the activity of this enzyme increased on the third day of exposure to

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a temperature of 20 C, and then decreased [Synkevich et al., 2016], and in winter wheat, it significantly decreased during short-term exposure to negative temperatures ( $-9^{\circ}$  C) for 2-6 hours [ Zagoskina et al., 2011]. Three forms of superoxide dismutase were found in beet root cells, two in vacuoles and nucleus, and only one in mitochondria [Isheeva et al., 2008].

As a result of spraying Scots pine (*Pinus sylvestris* L.) with a solution of sulfuric acid, part of the ions from the solution enters the conifers through the stomata. A change in osmotic potential affects the activity of a number of enzymes, including enzymes of redox systems [BUkharina and others., 2007]. Immediately after spraying, a decrease in peroxidase activity and an increase in ascorbate peroxidase activity were observed. And at the end of the third year of the experiment, the activity of ascorbate peroxidase equaled the control. Glutathione reductase activity was generally higher in the experiment than in the control. And for peroxidase, the opposite pattern is observed, although usually its activity increases under stress [Polovnikova, Voskresenskaya, 2008, Neverova, 2009]. The low activity of peroxidase corresponded to a high activity of either ascorbate peroxidase or glutathione reductase, compared to the control. The change in enzyme activity contributed to the detoxification of excess sulfate ions and their involvement in metabolism. One of the main ways of detoxifying the sulfate ion in a plant is its use in the synthesis of amino acids. Sulfate ion is included in the formed cysteine molecule, and cysteine, in turn, turns into cysteine acid or cystine, goes to the synthesis of methionine and other reactions [Metzler, 1980]. The enzymes of the glutathione cycle include glutathione reductase, which takes an active part in the transformation of oxidized glutathione into reduced glutathione and helps restore the disturbed balance between reduced and oxidized glutathione [Stevens et al., 1997]. The increase in the activity of this enzyme creates the possibility of the formation of glutathione complexes with metals even at a fairly high level of oxidative stress [Noctor et al., 2002] and is one of the

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important elements of plant protection against the effects of heavy metals [Verma, Dubey, 2003, Mishra et al., 2006; Yannarelli et al., 2007; Gratão et al., 2008, Anjum et al., 2012].

The redox system of glutathione is of considerable interest and is comprehensively studied not only in mammals, but also in plants. A change in the activity of this system serves as a criterion for deviation from physiological norms in case of pathological changes [Arrigo, 1999; Forman et al., 2008]. The amount of glutathione and its redox state are indicators of the resistance of a living organism against the effects of biotic and abiotic stress factors [Agrawal et al., 1992; Gaullier et al., 1994, Dixon et al., 1998, Krytskyi, Telegina, 2004, Go, Jones, 2008, Kalinina et al., 2008, Mhamdi et al., 2010, Anjum et al., 2012, Gill et al. ., 2013].

Reactions with the participation of reduced glutathione occur both non-enzymatically and catalyzed by enzymes for which glutathione is a cosubstrate. Protection of cells against the toxic effect of oxidative stress is one of the most important functions of glutathione. First of all, as a low-molecular antioxidant, as an effective scavenger ("trap") of various free radicals, it contributes to non-enzymatic antioxidant protection. For example, reduced glutathione can react with compounds such as hydroxyl radical ( $\bullet\text{OH}$ ), hypochlorite ( $\text{ClO}^-$ ), singlet oxygen and peroxynitrite ( $\text{ONOO}^-$ ) [Ookhtens, KaplowitzGalano, 1998, Galano, Alvarez-Idaboy, 2011].

The reverse oxidation-reduction of -SH groups regulates the activity of catalytic proteins, and through them, many other processes. Regulation of activity is carried out, as it is believed, in three ways. One of which is related to the cross-linking of protein structures through disulfide bonds ( $\text{RS-SR}$ ), which leads to their aggregation and high-molecular interactions. The second method is based on reverse modification of Cys residues of the active center. The third method is allosteric regulation [Kemp et al., 2008, Jones, 2008]. Redox regulation of the

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activity of catalytic proteins is mediated not only by the reverse oxidation of SH groups, but also by S-glutathione, i.e., the formation of mixed disulfides with glutathione thiol (RS-SG). For example, this type of reaction, along with the oxidation of SH-groups, is the basis of the "on-off" mechanism (an "on-off" mechanism) controlling the reaction activity of the active centers of catalytic proteins (allosteric regulation). S-glutathioneation also prevents the formation of RS-SR, corrects folding and protects Cys residues from oxidation to sulfenic (RSOH), sulfinic (RSO<sub>2</sub>H) and sulfonic acids (RSO<sub>3</sub>H), thus changing the functional state of proteins [Conte, Carroll, 2013, Kalinin and etc., 2014]. Proteins quite often contain several Cys residues, each of which can undergo irreversible changes, undergoing oxidation with the formation of RS-SR, RSOH, RSO<sub>2</sub>H, RSO<sub>3</sub>H, as well as S-thiolation, S-sulfhydration, and others. In this connection, a fairly wide range of thiol-dependent regulation is possible [Jones, 2008, Conte, Carroll, 2013].

The peculiarities of changes in the activity of ascorbate peroxidase and the amount of ascorbate and glutathione in tobacco plants transformed with a sense gene of ascorbate peroxidase under the action of contrasting abiotic factors - photooxidative and low-temperature stresses - were clarified. It was established that both under photo-oxidative stress and under the action of low temperature, the transformant plants were also characterized by a higher activity of ascorbate peroxidase compared to wild-type plants - by 30% and 20% under photo-oxidative and low-temperature stress, respectively. The amount of ascorbate, both total and reduced under such conditions, in transgenic plants was less compared to wild-type plants, while the content of glutathione, mainly its oxidized form, in transformants both under photooxidative and low-temperature stress exceeded this indicator for plants wild type Thus, the transformation of tobacco plants with a sense gene of ascorbate peroxidase leads to the intensification of the functioning of the ascorbate-glutathione cycle in them, which is manifested in a more

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intensive use of ascorbate in transformants against the background of increased activity of ascorbate peroxidase. The higher content of glutathione in the transformants is probably related to its efficient *de novo* synthesis to participate in the maintenance of the pool of reduced ascorbate. It was noted that the low level of ascorbate in transformant plants under stressful conditions is not an indicator of low antioxidant status of the cell. As it is generally believed, on the contrary, it indicates the active and effective functioning of the protective system, which is confirmed by the lower content of ROS in the leaves of transgenic plants both under photooxidative and low-temperature stress [Kozel, Shalygo, 2010].

It was shown that under conditions of photooxidative stress and under low-temperature exposure, tobacco transformant plants are characterized by a lower level of ROS accumulation in them, and as a result, a lower degree of damage to the components of cell membranes and a less pronounced violation of the functioning of the photosynthetic apparatus, compared to wild-type plants. The analysis of the components of the antioxidant system allowed us to establish that the amount of ascorbate, both total and reduced, and under photooxidative and low-temperature stress in transgenic plants was lower, compared to wild-type plants, while the content of glutathione, mainly in its oxidized form, in transformants both under photooxidative and low-temperature stress exceeded this indicator for the wild type. At the same time, under such conditions, the transformant plants were characterized by a higher activity of ascorbate peroxidase compared to the wild type - by 30% and 20% under photooxidative and low-temperature stress, respectively. The activity of glutathione reductase in the leaves of transgenic plants was higher than in wild-type plants under photooxidative stress by 40%, while at low temperature the activity of this enzyme was almost the same for both transformants and wild-type plants. Thus, the transformation of tobacco plants with a sense gene of ascorbate peroxidase leads to the intensification of the functioning of the ascorbate-glutathione cycle in them,



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which is manifested in a more intensive consumption of ascorbate in transformants against the background of increased activity of ascorbate peroxidase. The higher content of glutathione in the transformants is probably related to its *de novo* synthesis to participate in the maintenance of the pool of reduced ascorbate. The results obtained by the researchers indicate that transgenic plants, in which the ascorbate-glutathione cycle functions quite actively, are able to more effectively resist various types of stress. The results of these studies can be used in breeding to create new varieties of cultivated plants with increased resistance to abiotic stress [Kozel, 2011].

The complex effect of air and soil toxicants leads to the development of oxidative stress in plants. In the neutralization of ROS, a significant role is assigned to the system of coordinated reactions of the antioxidant system of plants. Maintaining a high level of low-molecular antioxidants - ascorbic acid and glutathione, which not only expose themselves to the attack of radicals and peroxide, but also take an active part in the recovery of other antioxidants, and also act as coenzymes that regulate the work of enzymes [Keniya et al. ., 1993; Menshikova, Zenkov, 1993, Chupakhina, 2009].

Redox-dependent processes significantly affect the functional activity of many proteins, take an active part in the regulation of the most important processes for cell life, such as proliferation, differentiation, apoptosis. Recently, special attention of researchers is focused on the study of thiol-disulfide regulation carried out by redox proteins, the activity of which is determined by a redox-active site in the form of an amino acid sequence with two or one active thiols. Among these proteins, two thiol disulfide reductases stand out – thioredoxin (Trx) and glutaredoxin (Grx), which are part of the superfamily of thioredoxins. These enzymes are multifunctional and form thioredoxin- and glutathione redoxin-dependent systems, which play a rather important role in maintaining intracellular redox homeostasis. The first system contains, in addition to thioredoxin, NADPH-

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dependent thioredoxin reductase, which restores the oxidized form of thioredoxin. The second system includes glutathione as an agent that reduces oxidized glutaredoxin, and glutathione reductase, which reduces glutathione from its oxidized form [Kalinina et al., 2008]. The main location of glutathione reductase in plants is considered to be chloroplasts (70-80%), but glutathione reductase activity is also found in the cytosol, mitochondria, and peroxisomes [Edwards et al., 1990; Rao and Reddy, 2008]. In *Escherichia coli* cells, glutathione reductase encoded by the *gor* gene consists of two subunits of 55 kDa each [Greer, Perham, 1986]. It has been shown that glutathione reductase activity is manifested as a response to stress and that mutations affecting this activity also have negative consequences for the cell, and all this proves the importance of the glutathione reductase enzyme in the metabolism of reduced glutathione [Rogers et al., 2004]. The isoenzyme composition and kinetic characteristics of the listed compartments were determined for glutathione reductase. It should also be noted that there is currently very little information about glutathione reductase of vacuoles. Only isolated facts indicate the possibility of the presence of this enzyme in this cellular structure [Rautenkranz et al., 1994, Pradedova et al., 2016]. This information is quite a strong reason to believe that vacuoles contribute to the regulation of intracellular redox processes.

Glutathione reductase is a very important enzyme of the antioxidant defense system of plants. It catalyzes the reduction of oxidized glutathione with the participation of  $\text{NADPH} + \text{H}^+$  in the glutathione-ascorbate cycle. Glutathione reductase in plants has 4 isoforms that are associated with different cellular compartments. The largest amount of this enzyme is associated with chloroplasts, but isozymes are also found in the cytosol and mitochondria [Kaigorodov, 2010]. Thanks to their catalytic properties, glutathione reductases are among the enzymes of the second phase of the biological transformation of xenobiotics. Substrates of glutathione reductase include compounds of various chemical nature, more

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specifically: arene oxidases, halonitrobenzenes,  $\alpha,\beta$ -unsaturated hydrocarbons, quinones, etc. Glutathione reductases are also involved in a number of other biological processes: biological synthesis of steroids and eicosanoids, tyrosine catabolism, and regulation of apoptosis [Jakobsson et al., 1999, Ladner et al., 2004].

Both systems, which include glutathione as an agent that reduces oxidized glutaredoxin, and glutathione reductase, which reduces glutathione from its oxidized form, and NADPH-dependent thioredoxin reductase, which reduces the oxidized form of thioredoxin, make a significant contribution to the antioxidant protection of cells against the destructive effects of oxidative stress, which causes the formation of intra- and intermolecular disulfide bonds in proteins, oxidation of functional SH-groups with the formation of sulfonic acid and subsequent proteasomal degradation of proteins [Fernandes, Holmgren, 2004].

In plants, the activity of glutathione peroxidase is low, and the antioxidant role of glutathione is expressed mainly in its participation in the ascorbate-glutathione cycle as a substrate of dehydroascorbate reductase [Noctor et al., 2012]. Therefore, glutathione is necessary for detoxification of xenobiotics and heavy metals. It is a reserve and transport form of reduced sulfur. Its content in plant cells is many times higher than the content of all other thiol (SH-containing) compounds and is 3-10 mM in the cytosol, cell organelles and phloem sap [Pivato et al., 2014]. Selenium plays a rather important role in the construction of glutathione peroxidase [Arthur 2000, Flohe et al., 2000, Lyabusheva, 2004, Vykhreva et al., 2012]. A significant effect of selenium in Chinese cabbage on the activity of glutathione peroxidase was established. The increase in the activity of this enzyme, compared to the control variant, was 30% [Skrypnyk, Chupakhina, 2008].

Many researchers believe that the binding of cadmium ions by non-protein thiols is one of the most important mechanisms in the detoxification of this metal,

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which differs in different plant species [Steffens, 1990, Chardonnens et al., 1999; Cobbett, 2000; Seregin, Ivanov, 2001; Souza, Rauser, 2003; Sharma, Angrawal, 2005; Pomponi et al., 2006; Ogawa et al., 2009; Pal, Rai, 2010, Shah, 2011, Gallego et al., 2012]. Simultaneously with the synthesis of phytochelatins, there is a decrease in the pool of glutathione, which is associated with its participation in the formation of these compounds [Mattioni et al., 1997; Cobbett, 2000; Shah, 2011]. Another example of intracellular binding (sequestration) of metal ions by bacteria is the cadmium ion-induced synthesis of low molecular weight proteins similar to phytochelatins by some marine gamma-proteobacteria [Ivanova et al., 2002]. Along with phytochelatins and metallothioneins, histidine, nicoanamine and muteic acid are also quite important chelators [Seregin, 2017]. Sequestration of cadmium ions by glutathione was also shown in *Rhizobium leguminosarum* strain cells [Lima et al., 2006]. The role of glutathione in reducing the toxicity of cadmium ions for chlorella cells (*Chlorella vulgaris* Beijer) was revealed at concentrations higher than 0.9  $\mu\text{mol/l}$  [Evseeva, 2006].

Glutathione itself is also able to form complexes with heavy metals. As is known, its composition includes thiol groups, through which it can bind to ions of metals and metalloids [Anjum et al., 2012]. It has been shown that in mammalian cells more than 50% of all copper can be in the form of complexes with reduced glutathione [Freedman et al., 1989]. But glutathione itself binds heavy metals less effectively than phytochelatins.

Glutathione takes an active part in regulating the activity of redox-sensitive proteins, the SH-groups of which are easily subject to reverse oxidation. Among them are enzymes (for example, protein phosphatases and protein kinases), cysteine-rich receptor-like protein kinases, transcription factors, ion channels, proton pumps [Schmitt et al., 2014]. Glutathione peroxidase is localized in the cytoplasm, mitochondrial matrix or plasmalemma. This enzyme takes an active part in the detoxification of various organic or inorganic peroxides, nucleic acids,

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protein molecules, for the process of reducing hydrogen peroxide, the glutathione peroxidase enzyme can use glutathione [Eshdat et al., 1997].

The antioxidant effect of ascorbic acid and glutathione is realized through their participation in the work of enzymatic organic antioxidants, especially enzymes of the ascorbate-glutathione cycle [Shang et al., 2013]. Thus, in cyanobacteria, the content of ascorbic acid is measured in meager indicators, in mosses - 0.1-0.5, in algae - about 0.5, and in higher plants (*Arabidopsis thaliana*) - 5  $\mu\text{mol/g}$  of raw material [Kaur, Nayyar, 2014]. Ascorbic acid has the ability to interact with radical ROS, singlet oxygen, and hydrogen peroxide [Putylna et al., 2008]. Glutathione peroxidases use glutathione in the enzymatic regeneration of lipid peroxides, as well as for the synthesis of metal-binding proteins, in particular phytochelatins under conditions of stress caused by adverse interactions [Miteva et al., 2010]. Phytochelatin synthesis is also induced by various heavy metals, including cadmium, copper, mercury, zinc, and iron, but not all of these metals form complexes with phytochelatins in vivo [Grill et al., 1989; Maitani et al., 1996]. Thus, in cell cultures of *Rubia tinctorum*, the synthesis of phytochelatins was induced by numerous metals, but "metal - phytochelatin" complexes were detected only for silver, cadmium, and copper [Grill et al., 1989]. The involvement of the regulation of phytochelatin synthesis in the cell was revealed - at the level of transcription for *Arabidopsis* [Clemens et al., 1999], and at the level of protein content in *Brassica juncea* [Heiss et al., 2003]. Due to binding of glutathione with cadmium ions and involvement in the synthesis of phytochelatins as a substrate, the level of this antioxidant decreases; it turns out to be quite important for the cell to maintain its level in order to resist this stress [Schützendübel, Polle, 2002]. Also, one of the manifestations of cadmium toxicity in some plant species, such as peas, is a decrease in ascorbate concentration [Rodríguez-Serrano et al. 2006].

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Despite the fact that the role of phytochelatins in the mechanisms of detoxification of heavy metals is obvious, the participation of phytochelatin synthase and phytochelatins themselves in the mechanisms of resistance against heavy metals has not yet been fully studied. For example, it is known that the overexpression of the AtPCS1 gene and the increased level of phytochelatins in transgenic *Arabidopsis* plants can increase the accumulation of cadmium without increasing plant resistance, and even more – can lead to hypersensitivity against cadmium. It was also shown that the expression of the TaPCS1 gene led to a decrease in the sensitivity of cad1-3 mutants of *Arabidopsis* plants against cadmium and, in addition, contributed to the further transport of cadmium, which in turn stimulated a decrease in its accumulation in the roots [Gong et al., 2003]. Similarity of the expression dynamics of the PCS1 phytochelatin synthase gene under the influence of cadmium and low temperature is shown. Since the expression level of the phytochelatin synthase gene increases in the case of exposure to both cadmium and low temperature, its participation in non-specific protective reactions of plants to the action of these stress factors can be predicted [Repkina et al., 2013]. It was found that the overexpression of genes that increase the level of glutathione and phytochelatins contributes to the increase of resistance of *Arabidopsis thaliana* against cadmium [Guo et al., 2008], and the deficiency of phytochelatins, on the contrary, contributes to the increase of hypersensitivity [Ha et al., 1999].

It was established that the transformation of tobacco plants with the MSD1 and FSD2 genes of *Arabidopsis* activates the antioxidant system, which is expressed in the increased content of low-molecular-weight antioxidants (ascorbate and phenolic compounds), increased activity of antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, glutathione reductase) and lower levels of ROS, compared to plants wild type [Savina, Shalygo, 2015, Savina, 2018].

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The ability of plants to homeostasis the internal environment in a wide range of external conditions plays a rather important role in adaptation against stressful actions. Regulation of the expression of genes encoding proteins of the main life support systems is crucial for maintaining viability. In the process of research, the leading role of the differential regulation of gene expression in the functioning of the plant organism in the conditions of an unstable external environment was established [Kholodova et al., 2008].

Gene expression is a rather complex multistage process. It includes transcription and processing of mRNA in the nucleus, export of mRNA from the nucleus to the cytoplasm through nuclear pores, translation and degradation of mRNA and proteins. Although initially these processes were considered and studied as completely independent of each other, recently it has become clear that the transcription of genes *in vivo* is quite closely interconnected with other stages of expression [Gergieva et al., 2008]. One of the negative consequences of inhibition of mRNA export can be the blockade of antiviral protection of the cell, which leads to an increase in the reproduction of the cytoplasmic virus [Dorokhov, Komarova, 2008]. Editing of RNA in plant mitochondria plays a rather important role in gene expression of these organelles. When using total cellular RNA, only fully edited transcripts are detected, while the process of mitochondrial isolation has a significant effect on the functioning of the editing system, which is manifested in the predominance of partially edited mRNAs [Klymenko, Konstantinov, 2008].

At the same time, it is shown that cellular glutathione takes part in maintaining the pool of reduced ascorbate; ascorbic acid is able to restore membrane-bound tocopherol. Glutathione and ascorbate play a rather important role in maintaining the redox status of the plasmalemma and proteins associated with the cell wall [Chirkova, 2002]. In the system of glutathione and ascorbic acid, the main electron donors (e<sup>-</sup>) are NADPH and NADH. On the other hand,

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these dinucleotides can be electron donors for molecular oxygen and contribute to the formation of ROS [López-Mirabal, Winther, 2008]. In vacuoles, the more oxidized state of glutathione and ascorbic acid against the background of high content and more reduced state of NADH is probably a consequence of the low efficiency of intravacuolar NADH-dependent systems that reduce oxidized glutathione and dehydroascorbic acid. At the same time, the more oxidized state of NADP in leoplasts, in which reduced glutathione was significantly less, and reduced ascorbic acid, on the contrary, was much more than in mitochondria, may indicate a rather high efficiency of NADH-dependent systems reducing these compounds. The obtained results allow us to state that the internal environment of the vacuoles in beet root cells is more oxidized compared to such cellular structures as plastids and mitochondria [Pradedova et al., 2017a].

Detoxification of ROS occurs thanks to the presence of a multicomponent antioxidant system in plants, a rather important link of this system is the ascorbate-glutathione cycle, the enzymes of which ensure coordinated work and quite effective recovery of low-molecular antioxidants - ascorbate and glutathione. The effect of anoxia and post-anoxic aeration on the content of these antioxidants and the activity of the key enzymes of the ascorbate-glutathione cycle - ascorbate peroxidase and glutathione reductase, as well as glutathione peroxidase in wheat and rice plants, contrasting in their resistance to hypoxia, was investigated. The effect of 24-hour apoxia caused a slight decrease in reduced ascorbate in the stems of wheat seedlings. The influence of postanoxic oxidation was manifested in a gradual decrease in the level of the reduced form of the vitamin and a weak accumulation of its oxidized form - dehydroascorbate. In the roots of wheat, changes in the level of ascorbic and dehydroascorbic acid were similar to those in the stem, but the changes were not too significant. In rice seedlings, oxygen deficiency led to a more than 4-fold decrease in the level of ascorbate in the stems, while in the roots, the concentration of this vitamin almost



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did not change. A further decrease in ascorbate and a progressive increase in dehydroascorbate were observed in rice seedlings as postanoxic aeration progressed. With long periods of postanoxic aeration (24 hours), the level of both forms of the vitamin in rice was restored to the control level. It is quite interesting that changes in the levels of oxidized and reduced ascorbic acid in rice seedlings under reoxygenation conditions were oppositely directed, that is, the decrease in the ascorbate level was accompanied by the accumulation of dehydroascorbate. On the basis of these studies, we can conclude that ascorbate is more actively involved as an antioxidant during oxidative postanoxic stress in rice than in wheat. This is also confirmed by the information on the change in the activity of ascorbate peroxidase. Under the influence of anoxia and subsequent aeration, the activity of this enzyme did not change in rice cells, while its more than twofold activation occurred in wheat. The oxidized form of glutathione predominated in wheat seedlings. The level of the reduced form did not change during anoxia and gradually decreased during the period of reaeration. The content of the oxidized form of glutathione increased significantly under anoxia, remained at the same level during reaeration, and then gradually decreased. In rice, the concentrations of reduced and oxidized forms of glutathione were the same. Anoxia and short-term rearing caused a decrease in the level of both forms of glutathione, and long-term post-anoxia led to their accumulation, which was more pronounced in the roots of rice seedlings. The activity of glutathione reductase remained unchanged in the stems of both plants both under conditions of anoxia and the subsequent oxidative action. Differences were found in plant roots: glutathione reductase was gradually inhibited in wheat, and activated in rice. The activity of the enzyme that provides antioxidant protection of membranes - glutathione peroxidase, remained stable in all variants of the gas regime in rice seedlings, while its irreversible inhibition occurred in wheat. The obtained results may indicate a more active use of ascorbic acid as an antioxidant in rice, which is the result of the effective work

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of enzymes of the ascorbate-glutathione cycle. Plants that are resistant to oxygen deficiency are better able to resist oxidative stress, which occurs when leaving an anaerobic environment under conditions of aeration [Emelyanov et al., 2009].

It is known that the composition of antioxidant enzymes of mitochondria includes superoxide dismutase, which reacts with their primary ROS - superoxide oxygen radical. It was shown that the activity of superoxide dismutase in sugar beet root mitochondria was 5-15 units/mg protein, that is, it corresponded to or was slightly lower than in mitochondria isolated from other plant tissues. On the other hand, high activity of hydrogen peroxide detoxification enzymes, specifically enzymes of the ascorbate-glutathione cycle, was detected in the mitochondria of the root crop. The activity of the key enzyme of this cycle, ascorbate peroxidase, was 400-500 nmol of ascorbic acid (mg of protein per minute), and the activity of glutathione reductase was 160-220 nmol of NADPH (mg of protein per minute) [Shugaev et al., 2009]. Shown. that the enzymatic system of antioxidant protection of mitochondria changes in the process of ontogenesis, probably responding to changes in the intensity of energy exchange. In the growing root crop, the mitochondria are quite strongly energized, and therefore a high activity of superoxide dismutase is necessary for the activation of superoxide generated by the respiratory chain. The energization of the mitochondria of the stored root crop is significantly reduced, which implies a decrease in the effectiveness of antioxidant enzymes, specifically, superoxide dismutase. It is likely that the activation of ascorbate peroxidase and glutathione reductase during this period, as well as high activity of catalase, are necessary to protect the mitochondria of the stored root crop from extramitochondrial hydrogen peroxide [Lashtabega, Shugaev, 2010]. It is believed that during the transition of this root crop to a state of rest, mitochondria can play the role of ROS acceptor rather than generator, and their antioxidant enzymes protect mitochondria and the cell as a whole from exogenous, that is, extracellular ROS.

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Consequently, some regularities of changes in the activity of mitochondrial antioxidant enzymes during ontogenesis were revealed, which respond, quite likely, both to a change in the intensity of energy exchange in the cells of the sugar beet root, and to a change in the conditions of the surrounding environment [Lashtabega et al., 2011].

Also, a glutathione-dependent enzyme is methyltransferase, which catalyzes the reverse reactions of the transfer of methyl groups. So, for example, selenocysteine methyltransferase contributes to the methylation of selenocysteine with subsequent formation of methylselenocysteine [LeDuc et al., 2004]. There is currently no direct evidence of the participation of methyltransferase in the protective mechanisms of plants against the effects of heavy metals. At the same time, however, it was shown that overexpression of the SMTAt and SMTBj genes isolated from the selenium hyperaccumulator *Astragalus bisulcatus* contributed to the increase of selenium resistance in *Arabidopsis thaliana* and Indian mustard [Zhu et al., 2009]. In addition, transgenic plants had a greater ability to accumulate selenium than wild-type plants [LeDuc et al., 2004].

According to the principle of action, antioxidants can be chain-breaking - reducing the level of ROS by interrupting the chain reactions of oxidation of organic substances, as well as preventive - reacting with metal ions of variable valence, inhibiting their participation in Fenton reactions that stimulate the formation of ROS. It also shows the perspective of using an electrochemical approach to determine the mechanism of action and determine the preventive antioxidant activity of biologically active compounds [Kruglyak et al., 2014].

It was shown that in *Triticum timopheevii* plants resistant to the *Septoria nodorum* fungus, a different increase in the level of ROS was detected depending on the degree of aggressiveness of the pathogen, which correlates with the degree of development of septoriosiis. If in a resistant pathosystem, the generation of ROS occurs due to the expression of NADPH oxidase, superoxide dismutase, and

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peroxidase genes and a decrease in the transcriptional activity of catalase, then in a susceptible pathosystem, the increase in the activity and expression of the peroxidase enzyme is insufficient for a hypersensitivity reaction, which ultimately leads to the development of the disease [Burkhanova, Maksimov, 2016]. A hypothesis was proposed, which states that the resistance of wheat plants against septoriosiis is regulated through the antagonistic interaction of the signaling pathways of salicylic acid and ethylene with the participation of hydrogen peroxide and cytokinins [Veselova et al., 2016]. At the same time, it was shown that treatment of pea plants with a solution of salicylic acid (500  $\mu$ M) increased the resistance of their prooxidant-antioxidant system to the next heat shock [Kurganova et al., 2007].

It is well known that the rate of peroxidation is regulated by natural antioxidants, the action of which is based on the inhibition of free radical reactions. A change in the concentration of ascorbate and glutathione, that is, the number of SH-groups, can determine the rate of peroxidation both at the initiation stage and at the stage of breaking the chains of free radical reactions [Jimenez et al., 1996, Grishko, Syshchikov, 1999, 2000, Taran et al. , 2004].

Glutathione plays a key role in maintaining the redox status in the cell, which is determined by the ratio of concentrations of oxidizing and reducing equivalents [Forman, Dickinson, 2003]. It exists in two redox forms - reduced and oxidized (glutathione disulfide). Most of the biological functions of glutathione are carried out by converting reduced glutathione into an oxidized form with the help of the enzyme glutathione peroxidase and subsequent return to the reduced form with the participation of NADPH-dependent glutathione reductase, which uses this cofactor from the pentose phosphate shunt [Dickinson, Forman, 2002, Jones, 2002]. The ratio of reduced and oxidized glutathione determines the oxidant status of cells and is quite clearly regulated by these two above-mentioned enzymes [Schafer, Buettner, 2001]. Normally, a relatively low level of the

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oxidized form of glutathione is maintained. All this is related to the need to limit the formation of disulfides mixed with proteins, since oxidized glutathione can react with sulfhydryl groups of proteins, forming mixed disulfides. Thiol-disulfide balance within the cell is regulated by various metabolic processes, which include the activity of enzymes and transport systems, signal transduction, and gene expression through changes in redox-sensitive transcription factors such as activator protein-1 (AP-1), factors kappa B (NFκB) and p53 [Lu, 1999, Townsend et al., 2003].

The main antioxidant effect of glutathione is realized through its participation in the work of enzymatic antioxidants; being a substrate for glutathione peroxidase and glutathione transferase, glutathione also serves as a donor for hydrogen atoms, hydrogen peroxide, and lipid peroxides. At the same time, reduced glutathione, like other SH-containing proteins, is an inhibitor of ROS and a membrane stabilizer. Intracellular glutathione effectively binds copper cations, thereby preventing their involvement in the Fenton-type peroxide decomposition reaction. It is believed that this compound has a protective effect in relation to the replicative system of the cell: a deficiency of reduced glutathione in conditions of increased generation of ROS or the toxic effect of heavy metal cations leads to a decrease in the synthesis of DNA and proteins [Chupakhina et al., 2016]. To eliminate the excess of free radicals, the plant organism uses protective mechanisms aimed at detoxifying ROS or blocking their formation, as well as at the launch of enzymatic and non-enzymatic antioxidant protection [Masella et al., 2005]. The ability of glutathione to intercept free radicals determines its resistance to oxidative stress [May et al., 1998, Lallement et al., 2014]. In addition, under conditions of stress caused by heavy metals, plants synthesize metal-binding proteins, such as phytochelatins [Borisova et al., 2012]. Thus, in cell cultures of *Rubia tinctorum*, the synthesis of phytochelatins was induced by many metals, but metal-phytochelatin complexes were detected only

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for silver, cadmium, and copper [Grill et al., 1989]. However, judging by everything, the regulation of phytochelatin synthesis occurs at different levels in different plant species.

Glutathione is the main redox buffer because it is present in the cell mainly in reduced form and in high concentrations up to 10 mM [Fahey et al., 1978, Romero, Canada, 1991]. It is known that glutathione takes an active part in the processes of detoxification, transport and metabolism [Colleman et al., 1997, Foyer et al., 1997, Leustek et al., 2000]. It was also shown that it is a donor of reducing equivalents in the glutathione-ascorbate cycle (Halliwell-Asada cycle). During this process, the reduced form of glutathione is oxidized to regenerate dehydroascorbate, which is then converted to ascorbate [Noctor et al., 2002]. It was shown that under the combined effect of low temperature and excess moisture in green barley seedlings, the content of ascorbate, reduced glutathione, as well as the activity of ascorbate peroxidase and glutathione reductase on the first day increased in relation to the control level by 2.0, 1.61, 1.72 and 1.63 times, respectively, which indicates active work of the ascorbate-glutathione cycle [Dremchuk, Shalygo, 2011].

Glutathione reduces the formation of disulfide bonds in cytoplasmic proteins with cysteines, serving as an electron donor. During this process, glutathione is transformed into its oxidized form - glutathione disulfide, which is also called L-(-)-glutathione. After oxidation, glutathione can be reduced again with the help of glutathione reductase, using NADPH as an electron donor. Among the functions performed by glutathione in the cell, first of all, it is necessary to note its participation in the protection of cells from the products of oxidative stress. Oxidative stress, or a change in cellular redox status, can affect the state of nuclear chromatin and cause changes in gene expression. The development of oxidative stress leads to single- or double-strand breaks in DNA. Damage to mitochondria caused by oxidative stress is accompanied by a decrease

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in the transmembrane potential, a change in membrane permeability, and an accelerated release of apoptotic factors, which stimulates cell death [Grill et al., 1988, Tausz et al., 2004, Gill, Tuteja, 2010, Ortega et al., 2011, Han et al., 2013, Tyulenev, 2015, Caverzan et al., 2016]. Glutathione is the most significant thiol compound that takes an active part in maintaining intracellular homeostasis in plants and is considered the strongest antioxidant and the best means of cell protection [Smirnova, 2005]. In fact, glutathione not only protects the cell from such toxic agents as free radicals, but also generally determines the redox status of the intracellular environment [Struznka et al., 2005]. Normally, 95% of the glutathiol pool is represented by the reduced form and only 5% is its oxidized form, and this balance is maintained in the cell due to the functioning of the enzyme glutathione reductase, which is one of the most important parameters that determines the cellular redox status and serves as an indicator of plant resistance against the action of various adverse environmental factors. At the same time, the basis of cellular redox homeostasis, with the help of which the redox state –SH of protein groups can be maintained, is determined by the ratio of reduced glutathione to oxidized glutathione, in which the norm in leaves is on average 20:1 and the total content of glutathione, which in various organelles, tissues and plant organs varies in the range of 0.1-10.0 mM [Meister, Anderson, 1983; Schroder, 2001, Yuan, Kaplowitz, 2009, Noctor et al., 2012]. Thus, maintaining the optimal ratio of reduced glutathione to oxidized glutathione and its total content is a rather important condition for the viability of plants, and their decrease below the physiological norm serves as an indicator of a violation of the cellular redox status and changes in redox-dependent gene regulation [Noctor et al., 2012; Kalinina et al., 2014]. Glutathione is essential for effective plant defense against pathogens such as *Pseudomonas* and *Phytophthora*. Reduced glutathione is a tripeptide consisting of L-glutamate amino acids, L-cysteine and glycine are less susceptible to oxidation than cysteine, which makes it the most suitable for maintaining the

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intracellular redox potential. The importance of reduced glutathione in redox-dependent processes is determined by its participation in the regulation of cellular redox-dependent signaling and the activity of transcription factors, as well as the fact that it is an intracellular antioxidant, playing the role of a "trap" of free radicals, a co-substrate in the detoxification reactions catalyzed by peroxides glutathione peroxidase and glutathione transferase, and acts as an agent that reduces oxidized glutaredoxin, necessary for the reduction of disulfides [Franco, Cidlowski, 2009, Deponte, 2013, Lu, 2013].

Maintaining the ratio of reduced glutathione to oxidized glutathione optimal for the cell is a very important condition for its viability. A decrease in the level of reduced glutathione below normal levels can serve as an indicator of a violation of the cellular redox status and changes in redox-dependent gene regulation [Gill et al., 2013, Townsend et al., 2003].

It has been shown that glutathione not only protects the cell from such toxic agents as free radicals, but also generally determines the redox status of the intracellular environment [Strużńska et al., 2005]. Glutathione interacts quite effectively with metal ions of variable valence, protecting cells from their toxic effects. Experiments on the study of the content of glutathione in cyanobacterial cells showed that the ratio of reduced to oxidized thiol in the studied culture allows us to assess the possibility of its use for soil and water contaminated with pollutants, including heavy metals. The increase in the amount of this peptide suggests that cyanobacteria are able to withstand adverse conditions, which is provided by binding to glutathione. This reaction, respectively, leads to a decrease in the toxicity of the object [Lyalyna, Hudyna, 2013]. It has been proven that under stressful conditions, changes in the content of glutathione and other low-molecular-weight thiols in the extracellular space are so significant that they can significantly affect the redox potential of microorganisms. As a rule, the effects of stress factors cause noticeable changes in the content of intracellular



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glutathione [Apontoweil, Berends, 1975, Adams et al., 1984, Beutler, 1989, Deneke, Fanburg, 1989, De Vos et al., 1992, May, Leaver, 1993, Wellburn, 1994, Ding, Demple, 1996, Foyer et al., 1997, May et al., 1998, Noctor et al., 1998, Tommasini et al., 1998, Creissen et al., 1999, Zhu et al., 1999, Foyer et al., 2001, Rauser, 2001, Dickinson, Forman, 2002, Smirnova, 2005, Smirnova, Oktyabrsky, 2006 Trukhan et al., 2013, Maslennikova, Shakirova, 2015, Mateikovich, 2016, Smirnova et al., 2016, Berezhneva et al., 2017].

Numerous scientific studies have shown that antioxidant properties are decisive in many aspects of the biological activity of substances [Deineka et al., 2010]. Currently, world science has achieved significant progress in understanding metabolic processes and molecular mechanisms of regulation of the growth of plant resistance against the negative effects of stress [Nakano et al., 1981, Buwalda et al., 1988, Kunert et al., 1989, Zhang, Klessig, 1997, Lenton, 1998, Pilet, 1998, Maxwell et al., 1999, Lachman et al., 2000, Shakirova, 2001, Sahu et al., 2002, Fu, Harberd, 2003, Slupphaug et al., 2003, Singh et al., 2005, Teslyuk et al., 2008, 2010a, 2010b, 2015, 2017, 2018, Kovbasenko et al., 2008, Ryabushkina, 2005, Dmitriev et al., 2010, Dulnev et al., 2010, Kovbasenko et al., 2008, 2010, 2011, 2011a, 2013a, 2013 b, 2013 c, 2014 a, 2014 b, 2016, 2017, 2019, Lapa et al., 2011, 2012, 2013, Kalinina et al., 2014, Kaminsky et al., 2015, Yashchuk et al., 2015, Kotlyarov, 2017, Oliynyk et al., 2017, Hryhoryuk et al., 2019, Kovbasenko, Kovbasenko, 2019].

Copper is a redox-active metal capable of directly participating in the generation of ROS. However, under the influence of a high concentration of copper ions, the development of symptoms of oxidative stress was not noted; an increase in the level of peroxidation of membrane lipids, an increase in the content of superoxide radicals in root tissues and the degree of oxidation of the intracellular pool of glutathione. The probable reason for this can be found against the background of high concentration of copper, a deficiency of reduced

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glutathione. Conversely, under the influence of a moderate excess of copper ions, in which a significant pool of reduced glutathione was found in rapeseed root cells, the processes of peroxidation of membrane lipids and superoxide radical generation were significantly enhanced [Zlobyn, 2013, 2015].

The following tasks were set for the study: 1) to find out how the size of the intracellular fraction of these metals bound to low molecular weight ligands changes under the conditions of an excess of copper and zinc ions in the environment; 2) to reveal the role of reduced glutathione in the binding of excess copper and zinc in rapeseed root cells. At the same time, it was established that with an excess content of copper and zinc ions in the environment, a significant increase in the content of the forms of these metals in root cells associated with low-molecular compounds was observed. Reduced glutathione binds metal ions through the SH-group of cysteine, and therefore complexes of metals with glutathione lose the specific reactivity characteristic of free glutathione. Thus, if copper or zinc ions do bind to reduced glutathione molecules *in vivo*, this should reduce monochlorobimane fluorescence in tissues [Ortega-Villasante et al., 2005]. However, the content of reduced glutathione under the action of an excess of metals can change for other reasons: 1) synthesis of reduced glutathione *de novo*, 2) oxidation of reduced glutathione to oxidized; 3) consumption of reduced glutathione for the synthesis of phytochelatins. It was found that the content of reduced glutathione in the root cells under the influence of 50  $\mu\text{M}$   $\text{CuSO}_4$  significantly decreased already during the first hours, compared to the control, and by the end of the experiment, reduced glutathione was practically absent in rapeseed roots. The content of total glutathione decreased by almost 25-30% in the first hours of exposure to excess copper, but by the end of the first day of the experiment, it significantly increased, by about 50%, compared to the control, and by the end of the experiment, it returned to the initial level. With the effect of excess zinc already in the first hours, the content of total glutathione decreased by

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approximately 25% of the control and remained at the same level for the first 24 hours of the experiment, and by the end of the experiment, the content of total glutathione returned to the initial level. At the same time, the share of oxidized glutathione decreased under the influence of excess copper and zinc. If under control conditions the content of oxidized glutathione was approximately 12-14% of the total, then after 24 hours of exposure it was already lower than 10%. The greatest decrease in the share of oxidized glutathione was found under the influence of excess zinc - up to 3% of the total before the end of the experiment. Thus, a sharp decrease in the content of free glutathione in the cells already in the first hours of the experiment, which was observed under the action of an excess of copper ions, was not explained by a decrease in the content of total glutathione, nor by an increase in the fate of its oxidized form. From this it can be concluded that with a high concentration of copper in the medium, the main part of reduced glutathione molecules was spent on the formation of complexes with copper ions. As for zinc, judging by everything, reduced glutathione practically did not bind ions of this metal, and therefore the content of free molecules of reduced glutathione practically did not change in conditions of excess zinc [Zlobyn et al., 2015].

The mechanism of counteracting oxidative stress, which develops in triticale stems due to the appearance of sodium chloride in the environment, primarily includes the "glutathione - glutathione reductase" system, followed by the connection of other components of antioxidant protection [Ivanishchev, Zhukov, 2017]. The rather important role of the glutathione-dependent system of horse chestnut seeds in the adaptation of plants to the conditions of urban coenoses is also shown [Chromykh, 2012].

The use of LED lighting with a narrow red or blue band of the radiation spectrum leads to the development of oxidative stress in cucumber leaves, which is accompanied by a change in the activity of a fairly important protective system

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of the plant cell - the ascorbate-glutathione cycle. The combined use of red and blue LEDs is not enough to create full-fledged lighting when growing cucumber plants. It is assumed that expanding the emission bands of the LED illuminator due to the use of LEDs with emission maxima in the yellow-orange and blue region of the spectrum will allow the construction of more efficient sources of photosynthetically active light. When designing such lighting sources, determining the activity of the ascorbate-glutathione cycle can provide quite valuable information, namely: the normalization of the activity of the ascorbate-glutathione cycle will testify to the effectiveness of the created lamps [Vyazov et al., 2014].

Exo- and endogenous metabolites oxidized by vacuolar phenol peroxidase, changing their reactivity, must enter conjugation reactions. The main role in this process in plants and animals is played by the glutathione system. Glutathione and glutathione-S-transferase activity were detected in vacuoles. Thus, metabolites with an oxidized modification inside the vacuole can conjugate with glutathione. The level of reduced glutathione is maintained mainly by glutathione reductase, which was detected in the vacuolar content. The identified enzymes and glutathione directly or indirectly participate in the antioxidant protection of beet plants. In general, within the framework of detoxification, three levels of antioxidant protection are distinguished, at which they function quite actively: 1) superoxide dismutase; 2) catalase and peroxidase; 3) glutathione peroxidase and glutathione [Pradedova et al., 2013].

Glutathione not only protects the cell against free radicals, but also determines the redox status of the intracellular environment [Oktyabrsky, Smirnova, 2007, 2012].

The dynamics of accumulation of glutathione synthetase gene transcripts was analyzed. It was established that at a temperature of 40 C, the accumulation of GS1 gene transcripts occurs already in the initial period of its action (15 min. -

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1 h.), and in the subsequent period (5 h. - 7 dobp), the mRNA content remained at an elevated level. In the initial period of exposure to cadmium (100  $\mu$ M), a slight increase in GS1 gene transcripts was observed, which increased significantly after 2 days. And with an even longer exposure to the metal, a significant decrease in the level of gene transcripts was noted. The obtained results allow us to conclude that the increase in resistance of wheat against the action of low temperature and cadmium is associated with a change in the expression of the glutathione synthetase gene, which confirms the rather important value of glutathione in nonspecific adaptive reactions to the action of stress factors of various nature [Repkina et al., 2014] . Similar studies were conducted on wheat germplasm to determine the expression of Wrab15 and Wrab18 genes under the influence of low temperature and cadmium sulfate (100  $\mu$ M) both individually and when used together, and similar results were obtained [Repkina et al., 2012].

The adaptive response of organisms, including bacteria, to oxidative stress occurs through the activation of numerous genes, which allows the cell to maintain homeostasis in constantly changing conditions. *Escherichia coli* has two global transcriptional regulators, OxyR and SoxRS, which are activated during oxidative stress [Pomposiello, Demple, 2001; Chiang, Shellhorn, 2012]. When hydrogen peroxide acts on wild-type *Escherichia coli* bacteria in concentrations that slow cell growth, no decrease in the intracellular levels of low-molecular-weight thiols and glutathione was noted. Treatment of growing cells with menadione leads to a drop in the level of total glutathione and a decrease in the ratio of reduced to oxidized glutathione. At the same time, the relationship between the catalase and glutathione antioxidant systems was revealed: the effect of hydrogen peroxide on cells of the bacterium *Escherichia coli*, deficient in catalase-hydroperoxidase I (HPI), leads to a drop in the level of total glutathione, an increase in the amount of oxidized glutathione, and a decrease in the ratio of reduced to oxidized glutathione ; in strains deficient in glutathione synthesis, HPI

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catalase activity increases markedly. HPI catalase plays a dominant role in the protection of growing *Escherichia coli* cells against oxidative stress caused by hydrogen peroxide. During the first phase of the response of *Escherichia coli* bacteria to the action of hydrogen peroxide, a direct relationship between changes in the levels of protein thiols and potassium and an inverse relationship between changes in the levels of low-molecular thiols and potassium is observed. An increase in resistance to menadione in growing cells of the bacterium *Escherichia coli* 821 was also found, which is associated with reduced activity of NADPH:menadione diaphorase [Muzyka, 2000]. It has also been shown that the main regulation of the SOS response in *Escherichia coli* is carried out by two proteins - the recombinase RecA and the transcriptional repressor LexA, which are also SOS-induced. The accumulation of damage in DNA leads to the activation of the coprotease activity of the RecA protein. In the cultured state, RecA induces autocatalytic cleavage of the LexA protein. As a result, the concentration of the functionally active LexA dimer is sharply reduced and the SOS regulon gene operators are released and their transcription rapidly increases. DNA damage does not induce the SOS response directly. It is assumed that single-stranded DNA, which accumulates in the cell when DNA replication is inhibited by damage, gives the metabolic signal that activates the coprotease activity of RecA. In the activated state, RecA forms helical nucleoprotein filaments on single-stranded DNA [Nikolaichyk, 2002]. It was shown on suspension culture that when *Escherichia coli* interacted with potato cells, the maximum level of hydrogen peroxide after 40 minutes was 2 times higher than when infected with *Clavibacter michiganensis* subsp. *sepedonicus*. Co-infection with two types of bacteria induced a level of ROS similar to single infection with *Escherichia coli*. A similar phenomenon is most likely connected with the fact that for *Clavibacter michiganensis* subsp. *sepedonicus* potato is the host plant, and this bacterium is able to suppress the plant's defense responses, which is quite characteristic of a

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specific interaction. *Escherichia coli*, on the contrary, not being a typical stressor for this plant, caused a more violent non-specific reaction [Alekseenko et al., 2011].

To study the influence of the redox status of thiols on the expression of the *sulA* gene when acting on *Escherichia coli* UF254, we used strains that simultaneously carry a mutation in one of the thiols of the redox system and a fusion of the *sulA* gene with the structural gene of  $\beta$ -galactosidase. In *Escherichia coli*, the *sulA* (*sfiA*) gene is part of the SOS regulon, which participates in the bacterial response to DNA damage. The measurements showed that the highest induction of *sulA* was possessed by the strains lacking thioredoxin (*trxA*) and thioredoxin reductase (*trxB*) (induction index in relation to the control – 9.7), and the lowest by strains defective in glutathione (*gshA*) and glutaredoxin (*grxA*) (induction index – 4.0). It is known that the content of glutathione in *Escherichia coli* is many times higher than the concentration of other intracellular thiols, and this tripeptide plays a rather important role in adaptation against extreme environmental conditions. In unirradiated cells, the highest level of intracellular glutathione was observed in *trxB* mutants (11 mM/g dry matter), the lowest in *trxA* (3.2 mM/g dry matter) and *grxA* (6 mM/g dry matter) mutants. dry thing.). After UV254 irradiation for 6 minutes, the concentration of total intracellular glutathione increased in all strains. The level of glutathione increased to the greatest extent in *trxA* mutants: 6.2 times. In *trxB* and parental cells, the level of intracellular reduced glutathione increased 4-fold and 2-fold, respectively, after irradiation. Measurements of intracellular glutathione showed that in *trxB* and parental cells, the amount of glutathione decreased by an average of 29% and 34%, respectively. All this indicates that the increase in the concentration of glutathione after ultraviolet irradiation may be the result of a decrease in its export to the environment. It should also be noted that in irradiated cells a direct correlation ( $r = 0.93$ ) was observed between the fold increase in glutathione and

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the expression level of *sulA::lacZ*. It was also shown that a change in the redox status of thiols leads to changes in the expression of SOS response genes [Ushakov, Smirnova, 2009].

The response of *Arabidopsis thaliana* (L.) Heynh plants (Colombia ecotype) to an excess of copper with a changed intracellular content of reduced glutathione was investigated. Addition of buthionine-sulfoximine in the amount of 500  $\mu\text{M}$  to the nutrient solution decreased the content of intracellular reduced glutathione, and exogenous reduced glutathione in the amount of 500  $\mu\text{M}$  increased it. An increase in the concentration of copper from 5 to 50  $\mu\text{M}$  led to a decrease in the content of reduced glutathione in the roots, but increased it in the stems. Buthionine-sulfoximine did not suppress plant growth, in contrast to exogenous reduced glutathione and copper, which strongly reduced the accumulation of crude matter. Treatment with copper in combination with buthionine-sulfoximine or reduced glutathione induced changes in root structure and leaf chloroplast ultrastructure. At the same time, copper treatment did not lead to the accumulation of phytochelatins. Treatment of plants with buthionine-sulfoximine or reduced glutathione practically did not affect the level of reduced glutathione in plants treated with copper, except for copper/reduced glutathione 50/50 and 50/500  $\mu\text{M}/\mu\text{M}$ , when the intracellular content of reduced glutathione increased in the roots, nor on toxicity copper for plants. The obtained results showed that reduced glutathione was not directly involved in copper detoxification and resistance of *Arabidopsis thaliana* (L.) Heynh (Colombia ecotype) against copper, but influenced the anatomical structure of plants [Wojchyk et al., 2009].

The effect of negative temperature ( $-5^{\circ}\text{C}$ , 5 h) on the activity of antioxidant enzymes: ascorbate peroxidase (APR), glutathione reductase (GR), superoxide dismutase (SOD), catalase (KT) in spring barley seedlings was studied. It was shown that the activity of all studied enzymes increased under conditions of cold



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stress. After the elimination of the stress factor (post-stress period), the activity of APR, GR, SOD and CT continued to increase, reached a maximum, and then decreased almost to the initial values. At the same time, the activity of APR increased to a greater extent than that of other enzymes. The obtained data showed that under the influence of negative temperature, oxidative processes begin primarily in the cytosol and occur most intensively in this cellular compartment [Radyuk et al., 2013]. The participation of the antioxidant system in the formation of cross-resistance of plants against cold and other stresses has also been demonstrated. Thus, it was shown that the previous exposure of barley seedlings to a low temperature (about 00 C) caused an increase in their resistance to hyperthermia, which was accompanied by an increase in the activity of superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase in the conditions of the subsequent exposure to high temperatures [Mei, Song, 2010].

Drought resistance of winter wheat plants is provided by reducing the intensity of oxidizing processes, stabilizing the activity of antioxidant enzymes, the content of low-molecular antioxidants, and stimulating the release of ethylene from leaves. Such adaptive changes in the activity of antioxidant processes in winter wheat plants are accompanied by the stabilization of their water status, as a result of which drought resistance increases and grain productivity losses in drought conditions decrease [Mamenko et al., 2014].

The functional state of the glutathione (GR-GSH) system in *Mimulus guttatus* Fischer ex DC. plants was studied under conditions of separate and combined action of salts of heavy metals zinc (ZnSO<sub>4</sub>) and nickel (NiSO<sub>4</sub>). The state of this system was evaluated, on the one hand, by the change in the redox status of the ratio of reduced to oxidized glutathione, the total content of glutathione, the activity of glutathione reductase and peroxidase glutathione-S-transferase, on the other hand. Plants after 4 weeks of germination in perlite and 2 weeks of acclimatization in modified Rorizon medium were grown for the next

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4 weeks under phytotron conditions on the same culture medium in the presence or absence of heavy metal salts of zinc ( $\text{ZnSO}_4$ ) and nickel ( $\text{NiSO}_4$ ). As a result, a direct correlation was established between the total concentration of  $\text{ZnSO}_4$  and  $\text{NiSO}_4$  in the nutrient medium and the content of zinc and nickel in the roots and leaves. It was shown that  $\text{ZnSO}_4$  (50, 100 and 200  $\mu\text{M}$ ) and  $\text{NiSO}_4$  (20 and 80  $\mu\text{M}$ ) had no significant effect on the accumulation of dry biological mass of plant organs. The content of malondialdehyde in both roots and leaves was higher under the influence of  $\text{ZnSO}_4$  than under the influence of  $\text{NiSO}_4$ . Nickel sulfate reduced the intensity of lipid peroxidation induced by  $\text{ZnSO}_4$ . Under the action of these heavy metals, an inverse correlation was found between the content of malondialdehyde and the total content of chlorophylls and carotenoids. The roots responded to the combined effect of zinc and nickel by increasing the content of glutathione, decreasing the activity of glutathione reductase and glutathione-S-transferase with a slight decrease in the ratio of reduced to oxidized glutathione. Leaves, unlike roots, responded to the action of zinc and nickel with a significant increase in the activity of glutathione reductase, a decrease in the activity of reduced glutathione, and a slight decrease in the level of oxidized glutathione, resulting in an increase in the ratio of reduced to oxidized glutathione. These changes in the glutathione system under the combined action of zinc and nickel were aimed at maintaining a more restored state of the intracellular environment, which, quite likely, ensured a decrease in the intensity of oxidative stress under the conditions of the damaging effect of the salts of these heavy metals. The obtained results indicate that at the level of functioning of the glutathione system, the combined effect of zinc and nickel is antagonistic in nature [Bashmakova et al., 2016].

Differences in the activity of glutathione reductase in tartar buckwheat calli with different morphogenesis ability and its role in the protection of calli cells during the induction of oxidative stress caused by a non-specific pro-oxidant -

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paraquat were studied. It was established that during the passage in morphogenic calli the activity of glutathione reductase reaches higher values than in non-morphogenic calli. At the same time, glutathione reductase activity did not differ significantly in hormone-dependent and hormone-independent non-morphogenic cultures. It was shown that paraquat at a concentration of 10  $\mu\text{M}$  caused a significant decrease in the viability and growth of both morphogenic and non-morphogenic calli. At the same time, the lowest resistance against oxidative stress in both types of calli was shown by actively dividing cultures, and the highest by cultures at the aging stage. However, morphogenic calli, regardless of age, were more resistant to the action of paraquat compared to non-morphogenic calli. It was shown that morphogenic calli not only activate glutathione reductase more strongly in response to oxidative stress caused by paraquat, but also perform this activation faster in time compared to non-morphogenic calli. Thus, cultivation on a medium with paraquat after 6 hours increased glutathione reductase activity in morphogenic callus cells by 7 times, while in non-morphogenic callus glutathione reductase activity, both in the control and in the experiment, practically did not change and remained low [Nigmatullina, Romyantseva, 2010].

Wild-type bacteria and deletion mutants of *Escherichia coli* (Keio collection) for the genes *gshA* (the first enzyme of glutathione synthesis), *gor* (glutathione reductase), *trxA* (thioredoxin I), *trxB* (thioredoxin reductase), *grxA*, *grxB* (glutaredoxins A and IN). At the same time, it was shown that under conditions optimal for growth (37°C), *gor*, *trxA*, and *trxB* mutants grew at a lower rate, and the glutathione-deficient *gshA* mutant grew at a higher rate than wild-type cells. With a sharp drop in temperature to 20°C. or increasing the cultivation temperature to 46°C, the growth rate significantly slowed down in all studied strains. The greatest effect was observed at 46°C in double mutants *gshAtrxA* and *gortrxB*, where growth stopped almost completely. The presence of *gshAtrxA*, *gortrxB* mutations led to a decrease in the survival of bacteria at all growth

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temperatures. At temperatures of 24 and 44°C, i.e. near the limits of the temperature range of growth of *Escherichia coli*, the ability of wild-type cells to form biofilms increased dramatically. Mutants of the *gor*, *trxA*, *gshAtrxA*, *gortrxB*, *grxA*, and *grxB* genes showed an increased ability to form biofilms in the temperature range of 30-40°C, but lost the maxima characteristic of the wild type at 24 and 44°C. Absence of glutathione in *gshA* mutants significantly reduced biofilm formation at all tested temperatures. When studying the dependence of the sensitivity of wild-type bacteria to antibiotics of different classes (ciprofloxacin, ampicillin, and streptomycin) on the cultivation temperature, a V-shaped curve with a maximum sensitivity at 40°C was found. Survival of bacteria was inversely proportional to their specific growth rate, the maximum of which was also reached at 40°C. The correlation coefficient between specific growth rate and log CFU (number of colony-forming units) was -0.97 for ciprofloxacin, -0.93 for ampicillin and -0.89 for streptomycin [Lepekhina, Smirnova, 2014].

The role of calcium in the implementation of the biological action of exogenous 24-epibrasinolide (EBL) in the metabolism of cells of transgenic plants *Nicotiana tabacum* ecotype KY-160 and transgenic tobacco plants *cax1* created on their basis, which express the coding part of the H<sup>+</sup>/Ca<sup>2+</sup> vacuolar antiporter gene of *Arabidopsis*, was investigated SAH1. It was established that EBL activates the systems of antioxidant protection - superoxide dismutase, ascorbate peroxidase and glutathione reductase, and also causes an increase in the level of superoxide anion radical and reduced glutathione. It is shown that disruption of the intracellular homeostasis of calcium ions leads to a decrease in the reaction of plants to the action of exogenous EBL. Under the conditions of action of the glucose-6-phosphate dehydrogenase inhibitor, the activity of glutathione-ascorbate cycle enzymes — ascorbate peroxidase and glutathione reductase — decreases [Kretinin et al., 2015].

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The processes induced by a sharp change in temperature, antibiotics of different classes, and the combined action of suboptimal growth temperatures and antibiotics in *Escherichia coli* bacteria mutant in the components of the glutathione (gshA, gor, grxA, grxB) and thioredoxin (trxA, trxB) redox systems were studied. It is shown that the studied mutations significantly change the number of SS bonds in proteins, the levels of oxidants (H<sub>2</sub>O<sub>2</sub>) and reductants (glutathione), the expression of antioxidant genes, and the sensitivity of bacteria to the action of antibiotics and extreme temperatures. The direction of action (increased or decreased sensitivity) depends on the type of mutation and has a different character for antibiotics of different classes. In all studied situations (the effect of mutations on the effect of antibiotics at the optimal growth temperature, the effect of combined stresses and the effect of additives that change the redox status), inverse correlations were found between lgCFU (colloid-forming units) under the action of ciprofloxacin and ampicillin and the specific growth rate of bacteria [Smirnova et al., 2017].

Changes in the status of glutathione in the studied strains of *Escherichia coli* under far ultraviolet irradiation showed that the concentration of intracellular glutathione increased in all strains after exposure to UV254. The maximum increase in concentration was found in redoxin mutants (trxA). Correlation analysis showed that there is a relationship between intracellular glutathione status and *sulA::lacZ* induction ( $r = 0.93$ ). A similar correlation between intracellular glutathione status and *sulA* induction was not found in experiments using hydrogen peroxide. The use of intracellular thiol compounds showed that a change in the redox state of the cytoplasm can lead to modulation of the SOS response. Thus, treatment of bacteria of the parent type with thiosalicylic acid led to a 1.7-fold increase in the level of *sulA* induction. Incubation of cells with  $\alpha$ -monothioglycerol led to a significant decrease in the expression of *sulA* both in the case of UV irradiation - by 3.0 times, and in its absence - by 2.2 times.

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Addition of dithiothreitol to the parent-type growing bacteria reduced the UV-induced SOS response by 3.7 times. When the cells were exposed to the parent type of diamide, which promotes the formation of disulfide bonds, ultraviolet-induced expression of *sulA* significantly decreased at a high reagent concentration of 0.8 mM (3-fold), and increased at a concentration of 0.08 mM (2-fold) [Ushakov, 2009].

The different sensitivity of plants to fluorine compounds determined the study of the specifics of the functioning of antioxidant systems of glutathione and ascorbic acid. At the minimum concentration of fluoride, a feature of the stress response of oat seedlings was the exhaustion of the available pool of ascorbic acid. As the concentration of fluorine increased, its level increased both in the roots and in the leaves of oats and corn. With the growth of fluorine supply in oat and corn plants (at 10 mg of fluorine/l), it is quite likely that the mechanisms of glutathione synthesis or its restoration by the glutathione reductase enzyme system are "started". In pea leaves, as a less stable crop, with increasing fluoride concentration, a decrease in the level of antioxidants, both ascorbic acid and glutathione, was noted, while in roots this trend is characteristic only for glutathione [Gryshko, 2010].

Over the past decades, quite a few gene families have been isolated and characterized, the activation of whose expression contributes to the increase of metal resistance in various plant species [Repkina et al., 2013].

Transcription factors are proteins that control the process of mRNA synthesis on the DNA matrix by binding to specific DNA regions, which is why they play a rather important role in initiating the program of increasing or decreasing the level of gene transcription [Patrushev, 2000]. A characteristic feature of transcription factors is the presence in their composition of one or more DNA-binding domains, which, in turn, bind to specific DNA regions located in the regulatory regions of genes [Vaahtera, Brosche, 2011]. Currently, several

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hundred transcription factors in plants have been isolated and described [Weber et al., 2006].

It was found that in the presence of cadmium in the roots of barley in all variants of the experiment, the expression of the HbPCS gene, as well as the total content of phytochelatins, increases significantly. At the same time, the level of gene expression in the root of 3-day-old seedlings, despite the lower amount of metal in them, was almost 2 times higher, and the amount of phytochelatins was 1.5 times higher than in 7-day-old seedlings. At the same time, the content of glutathione in the roots of younger plants decreased significantly, while in more mature plants, on the contrary, it increased. The effect of cadmium on the studied parameters in the leaves was expressed to a much lesser extent than in the roots, and their dependence on the age of the plants was manifested less clearly. Specifically, no significant increase in the expression of the HbPCS gene was found in the leaves. The content of phytochelatins increased slightly. The amount of glutathione changed to a somewhat greater extent, and as in the roots, it decreased in 3-day-old seedlings, and increased in 7-day-old seedlings. In general, the research results showed that in the presence of cadmium in the roots of 3-day-old barley plants, the expression of the HbPCS gene increases, the content of phytochelatins increases, and at the same time the level of glutathione decreases. In contrast, in the roots of 7-day-old plants with lower values of the expression level of the HbPCS gene and phytochelatins, an increase in the amount of glutathione is observed. In the leaves, changes in the studied indicators, as well as the influence of age differences on them, were insignificant [Kaznina et al., 2010].

The effect of lead acetate on the development of oxidative stress in isolated chloroplasts of the moss *Fontinalis antipyretica* Hedw was studied. The introduction of a heavy metal into the incubation medium of chloroplasts caused the degradation of chlorophylls, induced the process of lipoperoxidation and oxidative modification of proteins. A decrease in the activity of superoxide

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dismutase and a decrease in the amount of the reduced form of glutathione were also established. The antioxidant  $\alpha$ -tocopherol significantly reduced the negative effects of lead, inhibiting the growth of the lipoperoxidation process and stabilizing the functional state of nuclear DNA, which confirms its protective properties under conditions of oxidative stress [Kiyak, 2010].

A coordinated response of two types of genes encoding high molecular weight chelators (metallotioneins, MT1 and MT2 genes) and small polypeptides (phytochelatins, phytochelatase gene PCS) to copper ions in high concentrations was demonstrated. This coordination is manifested in the dominant expression of the PCS gene compared to the MT1 and MT2 genes in the first hours of the action of an excess of copper ions, which at a later stage of adaptation is replaced by the predominant transcription of the MT1 and MT2 genes when the intensity of the PCS gene decreases [Ivanova, 2011].

The influence of anoxia and subsequent reaeration on the activity of guaiacol peroxidase, catalase, enzymes of the ascorbate-glutathione cycle - ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase, as well as the content of low-molecular components of the ascorbate-glutathione cycle were studied in wheat and rice plants contrasting in resistance to hypoxia. Moreover, the influence of anoxia and subsequent reaeration on the expression of genes encoding catalase and enzymes of the ascorbate-glutathione cycle was studied in rice plants. The analysis of low-molecular components of the ascorbate-glutathione cycle revealed the accumulation of oxidized forms of ascorbate and glutathione, specific for an unstable plant (wheat) under the action of anoxia and subsequent aeration, which indicates the development of oxidative stress in this plant. In the resistant object (rice), no significant differences in the level of low-molecular-weight antioxidants were found in the control and under stressor action, which may indicate their intensive recovery. Activity studies revealed similar changes in most enzymes. In



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wheat, the activity of enzymes was most often significantly suppressed under the influence of anoxia, and if it was restored, then only after a short reaeration. In rice, on the contrary, the activity of antioxidant enzymes was preserved in the absence of oxygen and increased during post-anoxic reaeration. Growth or maintenance of enzyme activity in rice seedlings under conditions of anoxia and reoxygenation was removed as a result of the action of cycloheximide and, to a lesser extent, actinomycin D. In rice under the influence of anoxia and reoxygenation, a rapid increase in the activity of extracellular guaiacol peroxidase was detected, which was blocked by inhibitors of protein synthesis, as well as an inhibitor of vesicular transport (brefeldin A). Thus, the activation of antioxidant protection enzymes in rice seedlings occurred at the transcriptional, translational, and for the apoplast form of guaiacol peroxidase, at the secretory level. The study of the expression of genes encoding guaiacol peroxidase was not carried out, since at least 160 loci with presumed peroxidase activity were found in the rice genome. The total expression of catalase genes increased under the influence of anoxia and especially reaeration. The greatest contribution to the expression was made by OsCAT2, which encodes an enzyme in perisomes and cytoplasm, and OsCAT3, whose product functions in mitochondria. Expression of genes encoding enzymes of the ascorbate-glutathione cycle had similar dynamics: under the influence of anoxia, it was maintained at a more or less constant level, and increased during post-anoxic reaeration. It is also quite important to preferentially activate the expression of genes encoding antioxidant enzymes of energy organoids - plastids and mitochondria (OsCAT3, OsAPx5 and OsAPx6, OsGR3) during post-anoxic oxidative stress, which should prevent their oxidative damage. The results of this study indicate in favor of mainly transcriptional regulation of the work of the antioxidant system of rice under conditions of anoxia and subsequent oxidative stress. The effective work of antioxidant enzymes during anoxia and post-anoxic aeration of a resistant plant ensures the restoration of ascorbate and glutathione,

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prevents the accumulation of ROS and is a rather important adaptive mechanism, both against oxygen deficiency and against the coming oxidative stress [Emelyanov et al., 2015].

Analysis of the database on the rice genome showed that 8 genes encoding ascorbate peroxidase OsAPx are known; 5 genes encoding monodehydroascorbate reductase OsMDAR; 3 genes encoding dehydroascorbate reductase OsDHAR and 3 genes encoding glutathione reductase OsGR. In addition, 3 more genes whose products have ascorbate peroxidase activity are known, and they were also included in the study. All genes are nuclear, gene products are localized in different compartments of the cell: cytoplasm, mitochondria, chloroplasts and peroxisomes. It was shown that the total expression of genes of the OsAPx family and those similar to them changed in a similar way: it slightly decreased during anoxia, and increased during reaeration, especially after 12-24 hours of anoxia. Among the stem ascorbate peroxidases, the contribution of peroxisomal isoforms OsAPx increased during reaeration. In the roots, the mitochondrial and plastid isoforms were most actively transcribed, and the contribution of the mitochondrial to the total expression increased with the extension of the reaeration period. Among the genes of the OsDHAR and OsMDAR families, expression changes were similar to OsAPx, although organ specificity of isoforms was observed. For glutathione reductases, a marked inhibition of expression was observed in the roots, while the expression was maintained in the stems. Regarding the isoform composition, the isoform with cytoplasmic localization (OsGR2) prevailed in the roots, and the plastid-mitochondrial (OsGR3) in the stems. Thus, the work of the ascorbate-glutathione cycle under oxidative stress was regulated by the expression of genes encoding the key enzymes of the ascorbate-peroxidase cycle and glutathione-reductase, respectively. At the same time, the organ specificity of the expression of different isoforms and the growth of the contribution to the expression of the predominant

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isoform during the stress response were observed. This research will help identify adaptations of rice against hypoxia at the level of regulation of expression of genes encoding enzymes of the ascorbate-glutathione cycle, and can be used to improve the resistance of existing varieties of cereal plants [Prykazyuk et al., 2015].

The expression of genes encoding glutathione and homoglutathione synthesis enzymes in symbiotic pea nodules was investigated: GSH1 ( $\gamma$ -glutamylcysteine synthetase), GSHS (glutathione synthetase) and hGSHS (homoglutathione synthetase). At the same time, a significant increase in the expression of the GSH1 gene compared to the original form was observed in all mutant lines. At the same time, it was shown that the highest level of expression of the GSHS gene is characteristic of the sym40 gene mutant, and the hGSHS gene is characteristic of the sym33 gene mutants. We previously showed that mutants of the sym33 and sym40 genes are characterized by increased production of ROS in nodules, which most likely causes increased activity of glutathione synthesis enzymes. In addition, glutathione probably plays a rather important role in maintaining the redox balance in an efficient symbiotic pea nodule [Ivanova, Tsyganov, 2015a].

In addition to mycorrhizae, multicomponent symbiotic associations in the rhizosphere include endophytic microorganisms whose main functions are biological control of pathogens and pests [Ryan et al., 2008; Rosenblueth, Martinez-Romero, 2006]. For the rhizosphere of corn, a reminder of the endophytic species *Klebsiella pneumoniae* is often found [Chelius, Triplett, 2000]. The mechanisms of the existence of endophytic microbial associations of the rhizosphere have not yet been sufficiently studied, although it has been shown that regulation by the Quorum Sensing (QS) mechanism, which controls the expression of genes that determine the density of the bacterial population, can play a rather important role here [Sanchez-Contreras et al., 2007]. QS is a form of intra-population intercellular communication between bacteria, which is

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carried out through low-molecular autoinducers. Plants themselves can intervene in this regulation by synthesizing signaling factors that mimic the action of autoregulators of population density. In the formation of endophytic microbocenoses in the rhizosphere, the vertical transmission of microorganisms through seeds, as well as their introduction by vector organisms penetrating into the plant - invertebrates, such as nematodes, is of great importance [Riley, Reardon, 1995].

The high resistance of barley seedlings against cadmium ions is ensured by the presence and active functioning of various molecular genetic mechanisms, a rather important place among which is the ability of cells to quickly start the synthesis of molecules that bind ions of this metal in the cytoplasm (glutathione, phytochelatins, metallothioneins), as well as stimulation of the transport of the remaining free cadmium ions from the cytoplasm to the vacuole due to increased activity transport proteins, specifically SAH-proteins and vacuolar H<sup>+</sup>-ATPase [Kaznina et al., 2015]. With the combined effect of cadmium and low temperature, the effects of these actions are summed up, which leads to a significant increase in the expression of the studied genes. On the basis of the obtained results on increased expression of Wrab15 and Wrab18 genes in the leaves of wheat seedlings under the influence of low temperature and cadmium, as well as their combined action, it is possible to predict their participation in nonspecific protective reactions of plants to the action of stress factors of various nature [Repkina et al., 2012].

Low temperatures and heavy metals can lead to the development of oxidative stress in plant cells. One of the indicators of resistance of plants against oxidative stress is the content of the final product of lipid peroxidation - malondialdehyde. In the course of the conducted research, significant changes in the content of malondialdehyde in relation to the control in the roots of wheat seedlings under the influence of cadmium and low hardening temperature were

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not detected. A certain increase in the level of malondialdehyde in the leaves of wheat seedlings occurred mainly with prolonged exposure to 4°C and cadmium (3-7 days). In general, the nature of the change in the content of malondialdehyde in the leaves and roots of wheat under the influence of cadmium and low temperature turned out to be similar. Thus, the totality of the obtained results allows us to claim that increased expression of GS1, GS3, and PCS1 genes, which encode enzymes for the synthesis of glutathione and phytochelatins, contribute to the adaptation of plants against the effects of cadmium ions and low temperature [Repkina et al., 2015]. However, expression of the WCS120 dehydrin gene can be induced by cold, while cadmium does not affect it [Repkina et al., 2016].

Since the vacuole has a fairly high activity of glutathione transferase, it is quite likely that it should make a rather significant contribution to the process of intracellular detoxification. The very presence of enzymes of the glutathione transferase family indicates that the main function of the vacuole is not limited to the deposition and degradation of glutathione conjugates, which are redirected to it for isolation and utilization, as previously thought. Thanks to vacuolar glutathione transferases, conjugates of compounds of endogenous and exogenous origin can be formed inside the vacuole. The second function of glutathione transferases localized in the vacuole is most likely related to antioxidant protection, which is indirectly evidenced by the weak association of enzymes with membranes [Nimaeva et al., 2015].

It is believed that redox reactions take place in the central vacuole, as well as in any point of the cellular space, with the participation of redox proteins capable of forming redox chains, within which redox pathways of electron transfer occur. Redox chains are united by mobile electron donors into a single redox system. The study of redox elements and modeling of vacuolar redox chains will reveal redox processes characteristic of the vacuole, which will expand our

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understanding of the role of this compartment in redox metabolism, and possibly redox signaling of the plant cell [Pradedova et al., 2015a].

The growth of plant resistance against adverse environmental factors is a rather complex multicomponent process, which involves both non-specific and specific reactions. At the same time, non-specific reactions are most likely included at the very early stages of plant adaptation. After a certain period of time, and in some cases, perhaps immediately, they are supplemented, and in the future to a large extent replaced by more specific reactions. The existence of formed general non-specific plant resistance mechanisms is probably aimed at reducing the number of simultaneously functioning mechanisms and allows the plant organism to avoid significant expenditure of energy and structural resources associated with the need to form specific adaptation mechanisms in response to any deviation of plant vegetation conditions from normal [Shevyakova and others, 1994; Kuznetsov, 2001]. Be that as it may, namely, the combination of general, i.e. non-specific, and specific reactions provides the possibility of sufficiently effective adaptation of plants against adverse factors of the external environment. The relative role of specific and non-specific reactions in the process of adaptation is most likely not constant and depends on many factors and circumstances - biological features of the species and variety of plants, the type of stressor, intensity and duration of its action, accompanying conditions, etc. Moreover, as the analysis of the world literature shows, along with others, among the most important non-specific reactions to adverse environmental factors, a change in the content of individual phytohormones and their balance can be included, since they, performing a regulatory function, ensure, among other things, a coordinated response of plants to levels of the whole organism [Tytov et al., 2007, Talanova, 2009, Tytov, Talanova, 2009].

The key role in the regulation of the redox balance, as well as the NADP-glutathione-ascorbate system and thioredoxin-related processes localized in

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chloroplasts, mitochondria and cytoplasm, is shown. Photorespiration has a special coordinating role in the energy exchange regulation system. The oxidative pentose-phosphate cycle and alternative respiratory pathways also take an active part in this regulation [Rakhmankulova, 2011].

It was found that the osmotic water permeability of the plasmolemma of pea roots is sensitive to the redox potential of the environment. The results of the analysis of the state of aquaporins made it possible to predict that in pea seedlings grown in optimal conditions, it is due to the reverse oxidation-reduction transition of the SH-groups of a pair of cysteines located in the loop facing the apoplast. According to the results of the action of phosphatase inhibitors in seedlings that have undergone cooling, such sensitivity may be mediated through the effect on the activity of tyrosine protein phosphatase aquaporins as a component of the endogenous NADPH oxidase regulatory system [Ampylogova et al., 2007].

When growing non-morphogenic callus of Tatar buckwheat on a medium with the catalase inhibitor 3-amino-1,2,4-triazole (2 mM) and the inhibitor of glutathione synthesis - buthionine sulfoximine (0.1 mM), a change in the localization of hydrogen peroxide was shown: on the 4th day during cultivation, hydrogen peroxide was detected in the vacuole and on the tonoplast, but not on the cell walls. At the same time, the productivity of the culture decreased by 51%, and the increase in biological mass - by 3 times. Compared to the control, a significant number of small vacuoles was noted in the cytoplasm of cells of the experimental variant, and a significant increase in the number of nuclear vacuoles in the nucleolus. Numerous oval mitochondria 0.6-1.0  $\mu\text{m}$  in length with a swollen matrix were observed in the invasions of the lobular nucleus. The appearance of giant mitochondria (up to 4  $\mu\text{m}$  in length) that had both an elongated oval and a dumbbell-like shape was also noted. Accumulation of hydrogen peroxide in the vacuole can probably be considered as an indicator of oxidative stress, or a

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cytological marker of cells entering the path of programmed cell death [Kostyukova et al., 2011].

The nature of changes in the dynamics of glutathione reductase activity in pine needles during the growing season is similar in all studied variants, but for cedar, all changes are much weaker and the differences between trees from plantings of different densities are small. In the functioning of the glutathione system, the role of the enzyme glutathione reductase is to replenish the pool of reduced glutathione due to its oxidized form. In pine needles in dense plantings, the activity of this enzyme is higher, but its concentration is quite low. One of the most important tasks of the reduced form of glutathione in plants is to counteract oxidation of protein SH-groups by free radicals, which ensures the normal functioning of enzymatic reactions. The lack of accumulation of reduced glutathione may be a consequence of its rapid and intensive involvement in maintaining the normal status of the redox system of cells [Milyutyna et al., 2013]. There is a prediction that a significant decrease in the concentration of the reduced form of glutathione, noted during severe stress, indicates a weakening of the defense system and may serve as a symptom of the beginning of a violation of cellular organization [Tausz, 2001].

The participation of the key enzymes of the ascorbate-glutathione cycle, ascorbate peroxidase, dihydroascorbate reductase, glutathione reductase, and low molecular weight antioxidants - glutathione and ascorbic acid in the protective response of potato suspension cells upon infection with the fungus *Fusarium solani* was investigated. We analyzed 2 strains of suspension cells obtained from potato plants with contrasting resistance against the fungus *Fusarium solani*. It was found that in both strains, the change in enzyme activity upon infection has a biphasic nature: a quick response (0.5-6 hours) and a later response stage (24-48 hours). An increase in the activity of ascorbate peroxidase and glutathione reductase (by 35-52%) and inhibition of the activity of dihydroascorbate reductase



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(by 20-25%) was noted in the suspension cells obtained from a relatively resistant variety at the stage of rapid response. At a later stage of the response, with an increase in the intracellular content of hydrogen peroxide (by 2.8-3.7 times) and a significant decrease in cell viability (necrosis), an induction of the activity of ascorbate peroxidase by 3.2-3.5 times and glutathione reductase by 1.9 times was observed -2.3 times. Cells of the sensitive variety, on the contrary, reacted more intensively in the fast response phase by inducing the activity of all enzymes of the ascorbate-glutathione cycle: ascorbate peroxidase – 2.2 times, glutathione reductase – 2.7 times, and dihydroascorbate reductase – 1.8 times. At the late stage of the response, there was an increase in the activity of ascorbate peroxidase by 1.4-1.6 times, the activity of dihydroascorbate reductase at the control level, and the inhibition of glutathione reductase activity by 1.7-2 times, compared to the initial level. The role of ascorbic acid and glutathione in maintaining the redox balance of potato suspension cells was also investigated. The total content of ascorbic acid in the cells of the suspension (Tamash variety) varies from 200 to 400  $\mu\text{M/g}$  of raw mass, depending on the stage of growth, and the share of its reduced form does not exceed 24%. When suspension cells were infected with conidia of the fungus, a drop in the total (total) level and the level of oxidized forms of ascorbic acid by 55-79% of the initial level and an increase in the relative content of the reduced form of ascorbic acid were observed. At the same time, two phases of growth of the fate of the reduced form of ascorbic acid were noted: in the early stage of the response, with a maximum after 1 hour in 2.1-2.4 times, and in the later stage of the response, in 24 hours - in 3.5-3, 7 times. During infection of suspension cells, biphasic induction of biological synthesis of glutathione was observed. In the fast phase of the response, a slight increase in the activity of oxidized and reduced forms (18-35%, 0.5-3 hours) was observed, and later, after 24 hours, a significant reverse increase in the total level and the level of oxidized forms of glutathione was observed. The ratio of oxidized and

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reduced forms of glutathione plays a rather important role in maintaining the redox status, and accordingly, the ability to adapt plant cells. Along with the increase in the total level of glutathione, the dynamics of the redistribution of the content of oxidized and reduced forms was analyzed. When cells are infected with a suspension, a biphasic accumulation of glutathione is observed - after 3 and 24 hours. The significant reversal of glutathione levels after 24 hours refers to a specific response and change in glutathione metabolism to infection. Along with the increase in the total level of glutathione, there is a predominant accumulation of the oxidized form, more than 3 times, compared to control cells. Thus, the specific response of *Solanum tuberosum* potato cells to infection with the fungus *Fusarium solani* is a drop in the total level of ascorbic acid (3-24 hours) and a biphasic reverse increase relative to the content of the reduced form, and a reverse biphasic induction of the biological synthesis of glutathione with active transformation of the reduced form into oxidized [Sapko et al., 2017].

It has been shown that *Escherichia coli* strains deficient in glutathione and catalase-1 synthesis are more sensitive to the action of cumene hydroperoxide, which indicates the important role of antioxidants in the response of bacterial cells to oxidative stress [Baiderin, 2005].

It has been shown that treatment with salicylic acid causes significant shifts in the balance of reduced and oxidized forms of glutathione in plants, and this leads to activation of the transcription of genes encoding glutathione S-transferase, glutathione reductase, and monodehydroascorbate reductase, which make an important contribution to reducing the degree of damaging effects of drought on growth processes [Kang et al., 2013]. It was also found that the formation of antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase, was restored thanks to the application of salicylic acid solution [Tariq et al., 2011].

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The stability of plant vacuolar membranes (tonoplast) that were exposed to two types of osmotic stress, the action of redox agents, in particular oxidized and reduced glutathione, and nitric oxide, as well as at different pH values, was studied. The fatty acid composition of tonoplast lipids was also determined. It was found that under hyperosmotic stress, in contrast to hypoosmotic stress, the stability of vacuoles decreased. These effects are quite likely not related to the fatty acid composition of vacuolar membranes. The stability of vacuoles changed under different redox conditions, and more intensively under hypoosmotic stress in experiments with oxidized glutathione. The effect of nitric oxide differed under different types of stress: under hypoosmotic stress, the stability of vacuoles significantly decreased, while under hyperosmotic stress, nitric oxide increased the stability of vacuolar membranes [Nurminsky et al., 2011]. It was shown that the activity of peroxidase, catalase and glutathione reductase increased during hypoxic stress [Kumutha et al, 2009, Tuanhui Bai et al, 2010].

In a field experiment, it was established the ability of ragwort, ragweed and white quinoa to significantly increase the activity level of reduced glutathione under the action of the harness, and thus to detoxify the herbicide by conjugation with glutathione. The obtained results explain the insufficient effectiveness of weed control by means of pre-emergence herbicide treatment itself. The study of the reaction of black-leaved sedge to the action of the harness showed the existence of peculiarities in the functioning of the glutathione-dependent system of various types of dicotyledonous weedy plants [Chromykh, 2010].

It is known that high concentrations of trinitrotoluene lead to a decrease in the level of reduced equivalents, and glutathione can also act as an additional hydrogen donor that participates in the process of reducing the nitro groups of the aromatic ring [Kurynenko et al., 2003].

It was found that insufficient nitrogen moistening in the soil leads to an increase in the activity of enzymes of the ascorbate-glutathione cycle in winter

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wheat leaves. At the same time, the activity of ascorbate peroxidase and glutathione reductase is particularly intensively increased in the varieties *Kyivska ostista* and *Malynyvka*, in comparison with *Astarta*. Treatment of plants with urea on a low background of nitrogen nutrition induced an increase in the activity of ascorbate peroxidase and glutathione reductase in the leaves of winter wheat varieties, compared to untreated plants. Special mention should be made of the *Malynyvka* variety, which has the highest activity of these enzymes. During the treatment of plants with urea against the background of optimal provision of plants in the soil, a slight intensification of the work of antioxidant enzymes was observed, in comparison with untreated plants. Under such growing conditions, the variety *Kyivska ostista* stood out, with the highest activity of ascorbate peroxidase and glutathione reductase. Therefore, insufficient supply of winter wheat with nitrogen in the soil induces the development of stress-protective reactions in plants, which is evidenced by an increase in the level of hydrogen peroxide and the activity of antioxidant enzymes in the leaves [Mamenko, 2017].

The rather important role of glutathione in the detoxification of mercury and silver ions, which can form stable complexes with metals, has been shown [Howe, Merchant, 1992]. In addition, glutathione as one of the most effective low-molecular antioxidants protects plants against the damaging effects of cadmium [Kaplan et al., 1995, Zhu et al., 1999; Pietrini et al., 2003].

The effect of low temperature on plants can lead to the accumulation of ROS, and in response to this, the activity of the antioxidant enzymes catalase, peroxidase, superoxide dismutase and glutathione content increases [Koshkin, 2010; Kreslavsky et al., 2012]. It was shown that even a slight increase in the concentration of the products of oxidation processes under the influence of high temperature can also be a trigger for the subsequent activation of the plant's defense mechanisms [Wang et al., 2014]. At the same time, the opinion was expressed that the peroxidation products formed at high temperature switch the

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gene to a new mode of expression [Kurganova, Veselov, 1999]. In the case of intense high-temperature exposure, membranes are irreversibly damaged and lipid peroxidation products accumulate [Kong et al., 2016]. The accumulation of lipid peroxidation products, including malondialdehyde, is a consequence of the destruction of membrane structures. Products of lipid peroxidation can cause denaturation of proteins and nucleic acids [Gill, Tuteja, 2010]. As a result, oxidation products toxic to the cell accumulate, such as oxylipins and oxidized peptides [Chmielowska-Bąk et al., 2015]. According to some predictions, the accumulation of malondialdehyde along with the increased generation of hydrogen peroxide is one of the important mechanisms of the aging process of plants, and the action of high temperatures only accelerates this process [Sung, Jeng, 1994].

It was found that in lentils, temperatures in the range from 300C to 400C lead to the activation of superoxide dismutase, ascorbate peroxidase and catalase, while higher temperatures (400C - 500C) reduce their activity. The activity of peroxidase and glutathione reductase, on the contrary, decreases under the influence of all the studied temperatures [Chakraborty, Pradhan, 2011].

After 28 days of drought, *Trifolium repens* seedlings showed a decrease in growth and an increase in lignin synthesis in leaves, which was accompanied by an increase in the activity of guaiacol peroxidase, syringaldazine peroxidase, and coniferol alcohol peroxidase [Bok-Rye et al., 2007]. It is shown that the mechanisms of formation of heat resistance during local heating of the stem, as well as of the whole plant, are connected with induced synthesis, i.e. the neoformation of certain types of mRNA and their corresponding proteins. This conclusion is consistent with the modern idea of the formation under the influence of adverse temperatures in plant cells of stress proteins and their protective role [Voinikov et al., 2004; Vinocur, Altman, 2005; Kuznetsov, Dmitrieva, 2006; Titov et al., 2006; Trunova, 2007; Zhu et al., 2007; Kuznetsov, 2009; Koshkin,

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2010]. And it is also known that high temperature induces the expression of some genes and stops or reduces the expression of others. As a result of this, new mRNAs are detected in the cells of heated tissues within the first 5 minutes, and within 15 minutes - stress proteins, some of which are specific for this stressor [Kulaeva, 1997]. Their accumulation is accompanied by an increase in heat resistance of cells [Lin et al., 1984; Vierling, 1991; Pareek et al., 1995; Timperio et al., 2008; Zou et al., 2009], and vice versa, inhibition of de novo protein synthesis with the help of specific inhibitors leads to cell death during thermal exposure [Nover et al., 1984]. As the results of numerous studies show, the increase in heat resistance of tissues is associated with certain changes in the work of the genetic apparatus of cells and the synthesis of stress proteins [Key et al., 1981; Nover et al., 1984; Blechman, 1987; Guy, Haskell, 1987; Voinikov, 1989; Kulaeva, 1997; Jin et al., 1997; Downs et al., 1998; Burke et al., 2000; Lobov, 2001; Fowler, Thomashow, 2002; Kuznetsov, Dmytryeva, 2006]. It has also been established that under these conditions, as a result of changes in the expression of the genome in cells, the synthesis of proteins characteristic for normal temperature is inhibited, and at the same time, there is a fairly active neoplasm of thermal shock proteins [Ristic et al., 1991; Trofimova et al., 1999; Keeler et al., 2000; Titov et al., 2006].

Under the influence of cadmium ions on plants, the content of reduced glutathione initially decreases, which is associated with the consumption of its molecules for the formation of phytochelatins. As the exposure time of this metal increases, the concentration of reduced glutathione may increase, indicating its enhanced synthesis in the cell. Similar effects were observed in root and leaf cells of corn, wheat, rice and barley. The increase in the concentration of reduced glutathione is a rather important mechanism of protection of cultivated plants against the toxic effect of cadmium ions. In addition to the chelating function, glutathione provides antioxidant protection of cells and increases the

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concentration of thiols not only in the cytoplasm, but also in chloroplasts, which contributes to the preservation of the activity of the photosynthetic apparatus in conditions of increased cadmium content in the environment. Despite the fact that reduced glutathione can independently bind cadmium ions in the cytoplasm and transport them to the vacuole, it is almost 1000 times less effective in protecting against this metal than phytochelatins. In the cell cytoplasm, cadmium ions form low-molecular complexes with phytochelatins that are transported into the vacuole. An increase in the content of these peptides under the influence of cadmium was found in the roots and leaves of corn, rice, and barley [Tytov et al., 2012]. Phytochelatins also take an active part in the homeostasis of metals necessary for the normal passage of physiological processes, for example, zinc and copper [Repkina, 2014].

The protective effect of selenium in barley plants is associated with an increase in the activity of a number of enzymes of the antioxidant system, in particular catalase, superoxide dismutase, glutathione peroxidase, dehydroascorbate peroxidase, ascorbate peroxidase and an increase in the pool of some low-molecular antioxidants: carotenoids, ascorbic acid, glutathione. In Chinese cabbage plants grown under conditions of oxidative stress under the influence of exogenous sodium selenate, there is a decrease in the concentration of malondialdehyde, an increase in the stability of chlorophylls and the accumulation of biological mass, as well as an increase in the activity of glutathione peroxidase, dehydroascorbate reductase, and catalase [Skrypnyk, 2009].

A high level of oxidative stress can exceed the ability of the cell to restore oxidized glutathione to reduced glutathione, which leads to the accumulation of oxidized glutathione. To protect the cell from a shift in the redox balance, oxidized glutathione can be actively exported from cells or react with sulfhydryl groups of

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proteins, which leads to the formation of mixed disulfides that deplete the reserves of cellular reduced glutathione [Lu, 1999].

The ability of plants to remove unwanted ions is associated with a decrease in the oxygen content in the roots. Effectiveness of immobilization of metals in the rhizosphere, localization in the cell wall, binding with –COOH groups of uronic acids of mucus and deposition in the form of oxalates; while detoxification mechanisms include sequestration of lead ions in vacuoles by complexation, binding of lead by glutathione, amino acids and phytochelatins. The synthesis of phytochelatins occurs simultaneously with a reduction in the amount of glutathione in cells and an increase in the activity of glutamyl-cysteine synthetase, glutathione synthetase, and glutathione reductase. Increased activity of glutamyl-cysteine synthetase, glutathione synthetase, and glutathione reductase correlates with increased expression of *gsh1*, *gsh2*, *gr1*, and *gr2* genes. Non-specific protective mechanisms are expressed in the fact that the presence of lead ions leads to the accumulation of osmolytes, antioxidants, callose and suberin, amino acids and a change in the hormonal balance [Dykarev, 2015].

The antioxidant system is represented in the symbiotic nodule by both non-enzymatic elements, primarily ascorbic acid and homoglutathione, present in millimolar concentrations, and various enzymes, including superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, peroxiredoxins and a number of others [Becana et al., 2010]. Quite high activity of antioxidants is also observed in symbiosomes [Ribeiro et al., 2015]. In addition, the functioning of the antioxidant system in the nodule is considered in detail in the review [Ivanova, Tsyganov, 2017].

The influence of low hardening temperatures (4, 8 and 12° C) on cold resistance, the influence of proline and glutathione on the level of gene transcripts of enzymes involved in their synthesis in wheat (*Triticum aestivum* L.) variety Moskovskaya 39 was studied. Glutathione content under the influence of



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temperature 40 °C increased after 1 hour, then gradually decreased, but even by the end of the experiment it remained quite tall. The content of transcripts of the GS3 gene, which encodes the glutathione synthesis enzyme, at temperatures of 4 and 8 °C initially increased in the first 5 hours, but subsequently decreased somewhat, but even on the 7th day it exceeded the initial level, and at a temperature of 12 °C it increased throughout the entire action. The obtained results indicate that the dynamics of the content of low-molecular antioxidants and the accumulation of transcripts of genes encoding enzymes for their synthesis depends on the intensity of hardening: the higher it is, the greater the degree of manifestation of allative changes aimed at the formation of increased resistance of wheat plants [Fenko et al., 2015]. It has been established that the induction by low temperatures of the expression of Cor genes (cold-regulated), which encode COR proteins, correlates with the increase in frost resistance of plants [Thomashow, 1999; Ohno et al., 2001; Kume et al., 2005; Ishibashi et al., 2007]. In addition, it is also known that low temperatures induce the expression of CBF/DREB (C-repeated binding factor/dehydration response elements binding protein) transcription factor genes [Kume et al., 2005; Chinnusamy et al., 2006]. In turn, CBF factors activate gene expression of a number of COR proteins, which in the promoter regions contain CRT/DREB cis-elements [Thomashow, 1999; Chinnusamy et al., 2006; Heidarvand, Amiri, 2010].

Mechanisms of plant resistance against cadmium can be presented in various combinations. This can be extracellular binding of cadmium on the fungal cover or free mycelium through excreted ligands, metal binding on the surface of hyphae of the cover or the cell wall of free mycelium, increased outflow from the fungal cell, intracellular chelation with the participation of glutathione, intracellular isolation inside the vacuole in reactions with glutathione, compartmentalization in the vacuole or plant cytosol cells, interaction with metallothiothein. Isolation of cadmium in fungal symbiotic structures prevents the

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penetration of the metal into plants, and thus helps them to avoid intoxication. Free mycelium, thus, serves as a rather important obstacle on the way of cadmium into the plant. There is also information that fungi that form a significant amount of free mycelium provide the best resistance of the plant component of the symbiosis against cadmium and other heavy metals [Yang et al., 2005, Yadav, 2010]. Two mechanisms were found that ensured the binding of zinc and cadmium ions. Cadmium formed extracellular complexes in Hartig's network, as well as inside the cell walls of the root cortex. This indicates that transport from the fungus to the plant is active, mainly via the apoplast pathway. In contrast to cadmium, zinc accumulation occurred where the transport towards the plant is not so intense. This process occurred mainly in the cell walls, as well as in the cytoplasm of the hyphae of the cover [Frey et al., 2000, Tribys, 2015]. The proteome of cell walls includes several hundred different proteins and peptides. More than 50% of them have a signal N-peptide, and thus enter the cell wall through classical vesicular secretion, passing through the Golgi apparatus [Krause et al., 2013].

14-day-old pea plants of the Albumen variety were treated with ionizing radiation from a  $\gamma$ -Co60 source in doses of 0.1 Gy and 1.0 Gy. After irradiation at a dose of 1.0 Gy, the activity of glutathione reductase decreased by 20%, and against this background there was also a decrease in the content of reduced glutathione. Irradiation at a dose of 1.0 Gy caused a 35% increase in glutathione reductase activity and maintenance of the reduced glutathione concentration at the control level [Synitsyna et al., 2008].

The toxic effect of 2,4,6-trinitrotoluene on *Bacillus subtilis* SK1 consists, to a lesser extent, of two components - the toxic xenobiotic or its transformation products, as well as oxidative stress, which is manifested primarily in the inhibition of culture growth. The introduction of glutathione at a concentration of 200 mg/l did not lead to significant changes in the growth dynamics of *Bacillus*

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*subtilis* SK1 on a synthetic medium without 2,4,6-trinitrotoluene. High concentrations of glutathione (200-270 mg/l) stimulated the acceleration of the transition of *Bacillus subtilis* SK1 cells into the dying phase. In the presence of the xenobiotic, glutathione stimulated the growth of *Bacillus subtilis* SK1. The reduction of the toxic effect of 2,4,6-trinitrotoluene is caused by the neutralization of ROS produced at the initial stages of transformation by glutathione, as well as its possible use by the cell as a reducing agent. At the same time, glutathione stimulated the process of transformation of 2,4,6-trinitrotoluene (100 mg/l); and with an increase in the concentration of the xenobiotic, this effect was no longer observed. Both nitroreduction products and nitrites were found in the transformation products of 2,4,6-trinitrotoluene, and the proportion of nitroreduction products increased in the presence of glutathione. The decrease in the share of nitrites among transformation products indicates that glutathione does not take part in the removal of the nitro group from the 2,4,6-trinitrotoluene molecule [Yakovleva et al., 2018].

It was established that in more drought-resistant rice genotypes, the decrease in the content of reduced glutathione during drying was less noticeable than in non-resistant ones [Wang et al., 2012]. A positive relationship was also found between the heat resistance of wheat varieties and their content of reduced glutathione [Sairam et al., 2000; Dash and Mohanty, 2002]. Studies of the participation of glutathione in the cold adaptation of the bioenergy plant *Jatropha curcas* showed that under the conditions of hardening (at 12° C) there was an increase in the content of reduced glutathione, and with the subsequent effect of stress temperature (1° C) the content of glutathione in hardened plants was significantly higher than in not tempered [Ao et al., 2013]. The rather important role of glutathione and enzymes that catalyze its synthesis and transformation in various types of plants under salt stress was also shown [Waskiewicz et al., 2014]. In *Arabidopsis* plants, it was shown that cadmium ions induced an increase in the

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mRNA level of genes of enzymes involved in the synthesis of glutathione and the phytochelatin synthase gene [Semane et al., 2007].

The chronic effect of pollutants in the seeds of the sharp-leaved maple lion showed a genetically determined tendency to decrease metabolic activity during ripening and desiccation, while in the seeds of pseudo-sycamore maple it caused an increase in the genetically determined high metabolic status during ripening. At the same time, for the seeds of both species, a significant activation of the processes of their metabolic neutralization should be considered a general reaction to the effect of pollutants. The species specificity of the functioning of the metabolic cycle of glutathione in seeds indicates the involvement of the glutathione defense system in the implementation of various adaptation strategies of *Acer platanoides* L. and *Acer pseudoplatanus* L. to the conditions of complex anthropogenic pollution of the environment [Chromykh, 2013]. The increase in the intracellular pool of reduced glutathione can be caused by the induction of its biosynthesis process or by increasing the intensity of reduction reactions of oxidized glutathione molecules with the participation of glutathione reductase [Pukacka, Ratajczak, 2006, Gryshko, Syshchikov, 2012].

The proposed system of multilevel regulation of the energy balance in the plant cell. It also shows the key role in the regulation of the balance system: pro/antioxidant and redox, as well as the NADP-glutathione-ascorbate system and thioredoxin-related processes localized in chloroplasts, mitochondria and cytoplasm. A special coordinating role in the regulation of energy exchange belongs to photorespiration, which ensures the coordination of this regulatory system through peroxisomes, provides communication and interaction between chloroplasts and mitochondria, participates in the formation of redox homeostasis, pro/antioxidant and energy balances, i.e. controls the entire chain of processes. So, metabolic pathways are the biochemical framework of the integral organization of the gut and the body. The organization of metabolic pathways in

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plants is characterized by the presence of two different compartments that generate reducing equivalents and ATP: chloroplasts and mitochondria, while in animal cells these processes occur only in mitochondria. The interaction of these two functionally opposite types of energy organelles in a plant cell involves the organization of metabolite flows into a single regulated system specific only to a plant organism [Rakhmankulova, 2009].

Since antioxidant enzymes in plants can be inhibited under the action of certain stressors, for example, superoxide dismutase during salinity, it can be predicted that the activation or induction of biological synthesis of low molecular weight antioxidants under changed conditions becomes a more effective protective mechanism. Not much is known about the competition and mutual regulation of metabolic reactions in the biological synthesis of low molecular weight compounds. Although it becomes obvious that such a system should be under strict regulation. Until then, there are almost no comprehensive studies that would allow us to assess the contribution of various antioxidant reactions to the plant's protective response. Changes in the functioning of antioxidant enzymes or low-molecular-weight antioxidants, the expression of genes for their metabolism against the background of strengthening one of the metabolic pathways are not analyzed. Available literature information shows that low-molecular antioxidants are not passive markers of the functioning of the defense system. They can be quite active participants, and possibly key signaling molecules that interact between the metabolism changed under stressors and the defense response [Shao et al., 2008]. Thus, questions remain open about the existence of competition between the activation of biological synthesis of specific low-molecular metabolites, about the functioning of antioxidant enzymes in conditions of an increased level of specific low-molecular metabolism, as well as questions about the general and specific features of the functioning of the antioxidant system in plants that do not belong to model structures.

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In the literature, there is a point of view that low-molecular organic antioxidants in a number of cases more effectively protect metabolism from ROS than antioxidant enzymes [Radyukina et al., 2008].

Summing up, it is necessary to state that the protection of the plant cell and its components against the action of ROS occurs by various mechanisms, which significantly increases the reliability of biological systems. Antioxidant mechanisms prevent the development of free radical processes and lipid peroxidation in children. And the mechanisms of participation of various antioxidants contribute to a quick reaction and prevent excessive accumulation of ROS and cell destruction.

### **2.3 The role of glutathione-s-transferase in redox-dependent processes**

Complex antioxidant protection systems play a rather important role in maintaining intracellular homeostasis and stress resistance of plants. The key component of the plant's antioxidant defense system is glutathione and numerous enzymes related to it, the best known of which are glutathione-S-transferases [Habig et al., 1974, Tommasini et al., 1993, Creissen et al., 1994; Marrs, 1996, Cnubben et al., 2001, Seth et al., 2001, Scarponi et al., 2006, Basantani, Srivastava, 2007, Oztetik, 2008, Chronopoulou, Labrou, 2009, Ahmad et al., 2010, Tolpygina, 2012, Rojas-Loria et al., 2014, Sharma et al., 2014, Baimukhametova et al., 2016]. These enzymes were first discovered in the 60s of the last century in the body of mammals and their rather important role in drug detoxification was noted [Dixon et al., 2002]. The extraordinary importance of this family of enzymes is also explained by the fact that their content in the plant organism is at least 1% of the total number of all cell proteins [Salinas, Wong, 1999]. And the potential possibility of using enzymes that stimulate conjugation reactions of synthetic components to protect plants against the damaging effects

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of oxidative stress, and accordingly, against adverse abiotic factors of the environment, fuels the scientific interest of researchers in this family of enzymes.

A rather significant role in cellular redox-dependent processes belongs to glutathione-S-transferase, which forms a superfamily of isoforms that catalyze the conjugation of glutathione with a wide range of nonpolar compounds of endogenous and exogenous origin that contain electrophilic atoms of carbon, nitrogen, sulfur, and phosphorus, and this contributes a significant contribution to cell protection against the possible toxic effects of these compounds [Ezaki et al., 2004, Hayes et al., 2005, Tew, Townsend, 2012, Wu, Dong, 2012]. Each molecule of glutathione-S-transferase is a dimer consisting of subunits with a mass of approximately 20-26 kDa, which form a hydrophobic protein with a mass of 50 kDa with an isoelectric point in the pH range of 4-5 [Sheehan et al., 2001]. They can be either homodimers, that is, products of the expression of one gene, or heterodimers controlled by different genes [Chronopoulou, Labrou, 2009]. In the case of phi and tau GSTs, only subunits of the same class can dimerize. Despite significant differences in the amino acid sequence between classes of reduced glutathione, in general, their structure is quite similar. Within one class, proteins can be encoded by several genes, which leads to a significant variety of variants of expressed subunits [Borvinskaya et al., 2013].

Glutathione-S-transferases are characterized by a rather large structural diversity and form a multifunctional superfamily. Based on the similarity of polypeptide sequences and the exon/intron structure of genes, several classes of glutathione-S-transferases were identified, which were designated by letters of the Greek alphabet [Droog et al., 1995, . Edwards et al., 2000].

Glutathione-S-transferases are fairly common multifunctional enzymes that take an active part in the processes of cell detoxification from various endo- and xenobiotics and use the tripeptide glutathione ( $\gamma$ -Glu-Cys-Gly) [Cummins et al., 2011]. Based on the predicted amino acid sequences, soluble plant glutathione-S-

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transferases were divided into several classes:  $\phi$  (phi),  $\tau$  (tau),  $\lambda$  (lambda), dehydroas-corbate reductases (DHAR),  $\theta$  (theta),  $\zeta$  (zeta), elongation factor 1- $\gamma$  (EF1G) and tetrachlorohydroquinone dehalogenase (TCHQD). Classes  $\phi$ ,  $\tau$ ,  $\lambda$  and DHAR are specific for plants [Edwards, Dixon, 2005]. Plant glutathione-S-transferases were the first to be discovered and their ability to detoxify exogenous toxins, especially herbicides, is well known [Edwards et al., 2005]. The activity of glutathione-S-transferase is estimated by the enzyme's formation of a conjugate of reduced glutathione with 2,4-dinitrobenzene [Habig et al., 1974].

Glutathione-S-transferases are a family of enzymes with a molecular weight of 50 kDa, which play a rather important role in protecting cellular macromolecules from a number of toxic compounds of endogenous and exogenous origin, such as xenobiotics, herbicides, and free radicals [Ezaki et al., 2004 ].

It is known that the isozyme composition of glutathione-S-transferases varies greatly in different plant species, as well as in different tissues of the same plant at different stages of development and after their treatment with chemicals [Edwards, Cole, 1996, Cummins et al., 1997, Dixon et al., 1998, Pascal, Scalla, 1999].

Glutathione-S-transferases bind quite a lot of hydrophobic substances and inactivate them, but only those with a polar group undergo chemical modification with the participation of glutathione. That is, substances that, on the one hand, have an electrophilic center, such as an OH group, and on the other hand, hydrophobic zones, serve as substrates. These molecules are lipid peroxidation products. Decontamination of lipid peroxidation products with the participation of glutathione-S-transferase can be carried out in three different ways: by conjugation of the substrate with glutathione; as a result of nucleophilic substitution; and by reducing organic peroxides to alcohols [Polovynkina et al., 2010].



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In many organisms, glutathione-S-transferases are integral components of detoxification and antioxidant defense systems. Enzymes of this group are characterized by polyfunctionality, they catalyze not only the conjugation reactions of glutathione with electrophilic compounds, but also glutathione-dependent reactions, similar to the reactions of glutathione-peroxidases, in which glutathione is a peroxide reducer. In plants, glutathione-S-transferases also perform a transortal function, acting as carrier proteins of endogenous substrates and secondary compounds and carrying out transport from the cytosol to the vacuole [Riechers et al., 2003, Mohsenzadeh et al., 2011].

It has been proven that glutathione-S-transferases appeared as a result of the transformation of a thioredox-like precursor in response to the emergence of aerobic conditions in the environment [Borvynskaya et al., 2013]. Currently, isoforms of glutathione-S-transferases have been found both in prokaryotic cells, as well as in the cells of fungi, animals, and plants [Kalinina et al., 2014a]. The importance of this family of enzymes is confirmed by the fact that their share is at least 1% of the total amount of cell protein [Salinas, Wong, 1999]. And therefore, the potential possibility of using enzymes that stimulate conjugation reactions of synthetic components to protect plants against the damaging effects of oxidative stress, and, accordingly, against adverse abiotic factors of the environment, provoked an increase in interest in this family enzymes

Glutathione-S-transferases catalyze the interaction reaction of glutathione tripeptide GSH and cosubstrate (RX) containing an electrophilic center with the formation of a polar S-glutathionylated reaction product. For example, GSH can reduce hydroperoxides to alcohols:  $ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$  [Kalinina et al., 2014a]. Glutathione-S-transferase, concentrated mainly in the cytosol, also performs antioxidant functions, disinfecting hydrophobic products of lipid peroxidation by reducing them, adding a molecule of reduced glutathione

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or nucleophilic substitution of hydrophobic groups:  $2\text{GSH} + \text{ROOH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O}$ ;  $\text{R} + \text{GSH} \rightarrow \text{HRSG}$ ;  $\text{RX} + \text{GSH} \rightarrow \text{RSG} + \text{HX}$  [Lukatkin, 2002].→

It is known that glutathione-S-transferase is a rather important enzyme for the detoxification of cell membrane lipids and plays an important role in the antioxidant protection of the cell under stressful conditions. Analysis of glutathione-S-transferase activity in the roots of wheat seedlings exposed to salinity revealed a more than two-fold increase in the activity of this enzyme [Maslennikova, Shakirova, 2015a].

In addition to neutralizing herbicides, glutathione-S-transferases of plants play a rather important role in reactions to abiotic and biotic stresses, such as salinity [Yang et al., 2014], dehydration [Chen et al., 2012], hydrogen peroxide [Yu et al. , 2003], metal ions [Kumar et al., 2013], cold [Seppanen et al., 2000], UV radiation [Loyall et al., 2000] and the action of pathogens [Mauch, Dudler, 1993].

Studies of the glutathione-S-transferase of corn plants have shown that the action of the enzymes ensured the resistance of this crop to such herbicides as chlorotriazine, chloroacetanilide, and thiocarbamate. However, there is a possibility that these enzymes are involved in herbicide resistance of weeds [Cummins et al., 2010].

The antagonistic effect of zinc and nickel cations on the activity of glutathione-S-transferase peroxidases indicates a possible reduction in the role of these enzymes in the detoxification of hydrogen peroxide and ROOH [Bashmakova, 2017].

It was shown that with the participation of glutathione-S-transferase, resistance to low temperatures increased in transgenic plants of tobacco and rice with overexpression of the GST gene [Roxas et al., 1997 Toshikazu et al., 2002].

The positive effect of glutathione-S-transferases on plant growth has been shown not only under conditions of environmental pollution with heavy metals

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and xenobiotics, but also under the action of such abiotic stress factors as drought, salinity, and low positive temperatures [Kuluev et al., 2017].

With the help of the genetic engineering construct 35S::AtGST, 22 lines of transgenic plants were created. Based on the results of quantitative RT-PCR in real time, 9 lines of transgenic plants characterized by a high level of AtGST gene expression were selected. Under normal conditions of germination, transgenic plants were characterized by an increase in raw and dry weight of stems, compared to the wild type. On vertically oriented Petri dishes with agar medium according to the prescription of Murashige and Skoog, the improvement of root growth parameters in transgenic plants under the influence of sodium chloride (50 and 100 mM) and low positive temperatures (+12 and +15° C) was found. When grown on soil in conditions of drought (watering once a week), salinity (100 mM NaCl) and exposure to low positive temperatures (+12 and +15° C), transgenic plants differed from wild-type plants in growth of raw and dry mass of the stem. The results of this study allow us to draw conclusions about the positive influence of glutathione-S-transferases on plant growth not only in conditions of environmental pollution with heavy metals and xenobiotics, which is quite well covered in the literature, but also under the influence of such abiotic stress factors as drought, salinity and hypothermia. The 35S::AtGST genetic engineering construct tested on tobacco plants is planned to be used in the future for the transformation of economically valuable plants [Kuluev et al., 2017a].

The conducted studies showed that the expression of  $\lambda$ -GST class glutathione-S-transferase from rice (OsGSTL2) in Arabidopsis was accompanied by accelerated root growth, faster seed germination at the exit of cotyledons under stress caused by heavy metals, and also accelerated root growth under conditions salt and osmotic stress [Kumar et al., 2013]. Similar phenotypic features were noted when studying the role of the ThGSTZ1 gene from *Tamarix hispida* in the formation of drought resistance to salinity in plants [Yang et al., 2014]. Exposure

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of plants to conditions of salt stress and dehydration stimulates the formation of ROS and leads to oxidative stress [Chen et al., 2012]. Therefore, maintenance of ROS homeostasis is carried out through a complex complex of detoxification mechanisms with the participation of not only non-enzymatic components (ascorbate, glutathione), but also enzymes of the antioxidant system. The most important of these enzymes are superoxide dismutase, glutathione, catalase, peroxidase, and glutathione peroxidase [Jiang et al., 2007].

Changes in the endogenous level of total and oxidized glutathione and the activity of antioxidant enzymes - glutathione reductase, glutathione-S-transferase in pea plants (*Pisum sativum* L., Skinado variety) after its treatment with glyphosate were studied. Plants were treated with this herbicide in two ways: 1) the leaves were sprayed with a 10 mM solution and 2) it was added to the nutrient medium to a final concentration of the drug of 0.01 mM. Measurements of the above-mentioned indicators were carried out both in leaves and in roots. Treatment of roots and leaves with glyphosate led to an increase in the content of both total and oxidized glutathione. With both methods of herbicide treatment, an increase in glutathione reductase activity was observed in the treated plant parts. A slight increase in glutathione reductase activity was also found in untreated roots. It was also shown that glyphosate treatment of leaves caused a significant increase in glutathione S-transferase activity in leaves, while root treatment stimulated enzymatic activity in roots. The highest activity of glutathione-S-transferase was noted in the organs of plants directly treated with the herbicide. In addition, the authors assume that activated isoforms of glutathione-S-transferase take an active part in the detoxification of hydrogen peroxide and lipid peroxides [Miteva et al., 2010].

The activity of glutathione-S-transferase is directly related to the detoxification processes of xenobiotics and their metabolites, the role of which is mainly based on catalyzing the conjugation reactions of compounds toxic to the

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plant organism with glutathione with the formation of  $\gamma$ -glutamyl derivatives. Quite often, these processes are considered as the second phase of biological transformation of xenobiotics after their recovery. The functional meaning of this phase is based on giving xenobiotic conjugates greater hydrophilicity. In the future, such compounds are subjected to the action of  $\gamma$ -glutamyl transpeptitase, which separates the  $\gamma$ -glutamyl residue, facilitating the subsequent detoxification of these substances [Gryshko, Syshchikov, 2012].

To increase the resistance of plants against heavy metals, it is necessary to create individuals carrying additional glutathione-S-transferase genes. These enzymes are responsible for the conjugation of the sulfhydryl SH<sub>2</sub> group with electrophilic C, N, S, O atoms of xenobiotic molecules. Glutathione-S-transferases catalyze the reaction of glutathione with various aliphatic, aromatic, epoxy and heterocyclic radicals of exogenous harmful substances. Thus, the catalytic activity of glutathione-S-transferases provides the cell with a mechanism of protection against the harmful effects of such substances. In this regard, the genes AtGST of Arabidopsis and BnGST of rapeseed were identified, and model transgenic tobacco plants were created on their basis under the control of the 35S viral promoter as part of the genetic constructs of the vector pCAMBIA 1301. Then, morphometric experiments were conducted with reliably confirmed lines of such plants. According to the obtained information, the model plants were characterized by increased growth characteristics relative to the control, non-transgenic plants. Specifically, differences were observed in stem length and leaf area. Then leaf plates of control and transgenic plants were exposed to different concentrations of sodium chloride. Concentrations from 100  $\mu$ M to 500  $\mu$ M were selected and the experiment was carried out for 3 days. As a result, a protective effect was shown as a result of the activity of the studied genes, compared to the control due to a more reduced change in the raw mass of leaf plates. A more intense change in the raw mass of leaf plates was observed in the control variants.

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The least contrasting results were obtained for variants with concentrations from 200 to 300  $\mu\text{M}$ . In the experiment with 500  $\mu\text{M}$ , no significant differences were obtained - as well as between transgenes at the most significant salt concentrations. In the second experiment, control and transgenic plants were grown for one and a half months on soils with different contents of cadmium ions, as the most typical representative of heavy metals - concentrations from 100 to 400  $\mu\text{M}$  cadmium were selected. The purpose of this experiment was to simulate the conditions of germination on soils with extremely high concentrations of cadmium, when the plant will react quite acutely to the stress caused by the action of the heavy metal. The survival and viability of plants were evaluated through their morphological parameters, as well as their accumulative properties. At all cadmium concentrations, all lines of transgenic plants showed good growth and development, reliably little different from such parameters of the control group. At the same time, the growth and development of the control group were inhibited, especially at concentrations of 300 and 400  $\mu\text{M}$  cadmium. It is also worth noting that in almost all groups and lines of transgenic plants, no differences in the shape and color of leaves were observed for all studied genes. In the control, especially at high concentrations, yellowing of the leaves was observed, and some of them dried up. However, quantitatively, according to the accumulation of cadmium, transgenic plants, carrying the AtGSH and BnGSH genes differed little from the control in all concentrations, and therefore such plants do not show the properties of hyperaccumulators [Postrygan, Knyazev, 2015].

Glutathione-dependent enzymes – glutathione peroxidase, glutathione-S-transferase and others – play a rather important role in recovery reactions involving glutathione. Glutathione-S-transferase in plant cells performs conjugation and thiolase functions. Thanks to the latter function, it is able to show glutathione peroxidase activity. Since the thiolase function is aimed at the restoration of peroxides of the remains of unsaturated fatty acids of lipids, and the

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mechanisms of antioxidant protection of lipids of the vacuolar membrane have not been fully investigated, the possibility of its localization in vacuoles is relevant. As a result of the study, this activity was detected in the fractions of isolated vacuoles and it was established that its level in the presence of different substrates varied quite significantly. At the same time, the activity of vacuolar glutathione-S-transferase was 1.5-2 times lower than the activity of this enzyme from the tissue extract. It is known that the transport of glutathione through cell membranes is somewhat difficult. In this connection, research was conducted to identify the possibility of reducing oxidized glutathione to its reduced form inside the vacuole. The results obtained in the process of spectrophotometric measurements demonstrated a sufficiently high level of activated glutathione reductase in the fractions of isolated vacuoles, which can be compared with the activity of this enzyme from tissue extracts. Using the zymographic method, glutathione reductase activity was detected in the localization zones of vacuolar proteins. Based on the obtained results, it was concluded that the glutathione redox system functions in the vacuoles of plant cells, as well as in other cellular structures, an integral part of which is glutathione and glutathione-dependent elements that regulate the optimal ratio of the reduced form of glutathione to its oxidized form. forms [Pradedova et al., 2011].

Glutathione and glutathione-S-transferase activity were detected in the vacuoles of beetroot cells. Thus, intracellular metabolites that have undergone oxidative modification can conjugate with glutathione. The level of reduced glutathione is maintained mainly by glutathione reductase, which was detected in the vacuolar content. The enzymes and glutathione listed by the authors are directly and indirectly quite actively involved in antioxidant protection. In general, within the framework of detoxification, three levels of antioxidant protection are distinguished, which are actively functioning: 1) superoxide dismutase; 2) peroxidase and catalase; 3) glutathione peroxidase and glutathione.

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Among the listed enzymes in the vacuoles of beet root cells, along with peroxidase and glutathione, the authors discovered and investigated Cu, Zn-superoxide dismutase. The authors of this study believe that the detoxification systems formed in the process of evolution in animals and plants are quite close, and therefore the enzymes investigated in the course of this work, taking into account the functional features of vacuoles, should be considered within the framework of these systems [Pradedova et al., 2013].

Conducted studies of glutathione-S-transferase of corn showed that the action of enzymes ensured resistance of plants to such herbicides as chlorotriazine, chloroacetanilide and thiocarbamate. It was also established that much less attention was paid to the study of glutathione-S-transferases of weeds, although the possibility of the participation of these enzymes in the resistance of weeds to the action of herbicides cannot be rejected [Cummins et al., 2010].

The large variety of isoforms of these enzymes is the result of gene duplications and divisions, along with single polymorphisms [Kolesov et al., 2016]. The genome of *Arabidopsis thaliana* contains 48 glutathione-like genes: 28 tau, 13 phi, 3 theta, 2 zeta, 2 lambda. In *Arabidopsis thaliana*, 6 families of glutathione genes are distinguished: GST 1cDNA, which is induced by pathogenic infection; GST 2cDNA, which regulates the action of ethylene; GST 2cDNA, inducible by dehydration; PM 239x14 cDNA encoding a protein with glutathione peroxidase activity that does not use hydrogen peroxide as a substrate; GST 5cDNA capable of binding auxin; GST6, the expression of which is under tissue-specific control and is induced by auxin, salicylic acid, and hydrogen peroxide [Yang et al., 1998].

It was shown that tobacco plants into which the GST-cr1 cotton glutathione-S-transferase gene was introduced by the method of agrobacterial transformation showed increased resistance against oxidative stress induced by methyl violet [Yu et al., 2003]. The corn ZmGSTU1 gene, which was expressed



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in cells of transgenic tobacco and rice, gave them resistance to herbicides [Milligan et al., 2001; Chronopoulou, Labrou, 2009]. There is also information in the literature that overexpression of glutathione-S-transferase genes contributes to the improvement of the antioxidant status of the cell, which is not directly related to their ability to stimulate the formation of glutathione conjugates with electrophilic components [Moons, 2015]. The expression of the PpGST gene – an inducer of glutathione-S-transferase synthesis from the fruits of *Pyrus pyrifolia* Nakai cv Huobali and its transfer and inoculation into tobacco plants (*Nicotiana tabacum* L. cv Xanthi) made it possible to confirm PCR transcription by Southern blotting methods [Liu et al., 2013].

It is shown that overexpression of one enzyme of the antioxidant system affects the expression of genes of other enzymes of this system. For example, seedlings of transgenic plants expressing glutathione-S-transferase show increased activity of ascorbate peroxidase and monodehydroascorbate peroxidase compared to "wild-type" plants [Roxas et al., 2000].

The conducted studies showed that the expression of  $\lambda$ -GST class glutathione-S-transferase from rice (OsGSTL2) in *Arabidopsis* was accompanied by accelerated root growth, faster seed germination and cotyledon emergence from heavy metal stress, as well as accelerated root growth under salt and osmotic stress [Kumar et al., 2013]. Similar phenotypic features were noted when studying the role of ThGSTZ1 from *Tamarix hispida* in the formation of resistance to drought and salinity in plants [Yang et al., 2014]. Exposure of plants to conditions of salt stress and dehydration stimulates the formation of ROS and leads to oxidative stress [Chen et al., 2012]. Therefore, maintenance of ROS homeostasis is carried out through a complex complex of detoxification mechanisms with the participation of not only non-enzymatic components (ascorbate, glutathione), but also enzymes of the antioxidant system. The most important of these enzymes are superoxide dismutase, peroxidase, glutathione reductase, catalase, and

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glutathione peroxidase [Jiang et al., 2007]. It was shown that overexpression of one gene of the antioxidant system affects the expression of genes of other enzymes of this system. For example, seedlings of transgenic plants expressing glutathione peroxidase show increased activity of ascorbate peroxidase and monodehydroascorbate reductase compared to "wild-type" plants [Roxas et al., 2000]. Tobacco plants with transformed chloroplasts expressing dehydroascorbate peroxidase are characterized by increased activity of glutathione reductase [Kwon et al., 2001].

Therefore, analyzing the obtained data of the vegetation experiment regarding the passage of lipid peroxidation reactions and the activity of the enzyme glutathione-S-transferase in the leaves of common spelled wheat, it can be concluded that the combined use of the herbicide "Prima Forte 195" in rates of 0.5, 0.6 and 0.7 l/ha with plant growth regulator "Vuksal BIO Vita" at a rate of 1.0 l/ha against the background of pre-sowing treatment of seeds with the same plant growth regulator at a rate of 1.0 l/t leads to faster rates of xenobiotic detoxification in plants, which is manifested in an increase in the activity of the enzyme glutathione-S-transferase with a significant content of malondialdehyde [Karpenko et al. , 2013].

Genetic analysis of Arabidopsis cell cultures treated with phytoprostanoids B1 showed that these compounds trigger a number of detoxification processes. At the same time, it was found that under the action of phytoprostanoids, the expression of several genes - glutathione-S-transferase, glycosyltransferase, and ATP-binding transporters - can increase the ability of plants to inactivate lipid oxidation products. It is known that glutathione-S-transferase is a trigger in oxidative stress [Loeffler et al., 2005]. The enzyme ATP synthase has a fairly universal importance as the main source of ATP in all types of organisms [Kabaleeswaran et al., 2006].

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The intracellular content of reduced and oxidized forms of glutathione, the activity of glutathione reductase, glutathione-S-transferase, and ascorbate peroxidase in morphogenic and non-morphogenic calli of Tatar buckwheat during the cultural cycle, as well as under the action of D,L-buthionine-S,R-sulfoximine (BSO) were studied. Inhibitor of the first enzyme of biological synthesis of glutathione - glutamylcysteine synthase. It was found that during passage the cultures slightly differed in the content of total glutathione, but the content of reduced glutathione was higher in the morphogenic culture, and the content of oxidized glutathione was higher in the non-morphogenic culture. In morphogenic callus, glutathione-S-transferase activity was 10-20 times higher, and glutathione reductase activity was 2-2.5 times lower than in non-morphogenic callus. Under the influence of BSO, the decrease in the content of reduced glutathione in the morphogenic callus was temporary - on day 6-8 of the passage, while in the non-morphogenic callus it decreased already after a day and remained lower than in the control throughout the entire passage. BSO did not affect the content of oxidized glutathione in morphogenic callus, but caused its accumulation in non-morphogenic callus. These differences are most likely due to the fact that glutathione reductase is activated in the morphogenic callus on the medium with BSO, and glutathione reductase is inhibited in the non-morphogenic callus, on the contrary. Despite the fact that BSO caused a decrease in the total content of glutathione only in the non-morphogenic culture, the cytostatic effect of BSO was more pronounced in the morphogenic callus. BSO also had a negative effect on the differentiation of proembryonic cell complexes in the morphogenic callus. At the same time, the role of the redox status of glutathione in maintaining the embryogenic activity of cultured plant cells is quite actively discussed [Nigmatullina et al., 2014]. It is known that an effective way of detoxifying herbicides in cultivated plants is the process of conjugation with glutathione, which is catalyzed by glutathione-S-transferases [Gronwald et al., 1987, 1998].

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The methods of two-dimensional electrophoresis and matrix laser desorption ionization mass spectroscopy (MALDI-TOF MS) showed that treatment of plants with methyl jasmonate promoted the growth of a number of protective proteins. Including polypeptides with antioxidant properties, such as L-ascorbate peroxidase, glutathione-S-transferase, and chloroplast 2-cis-peroxiredoxin [Avalbaev et al., 2017].

Organic peroxides can be reduced by glutathione peroxidase and glutathione-S-transferase, hydrogen peroxide is also reduced by catalase, which is present in peroxisomes. Since catalase is absent in mitochondria, the importance of reduced glutathione for mitochondria is beyond doubt. Therefore, the level of mitochondrial reduced glutathione is critical for protection against oxidative stress, both physiological and biotic stress [Garcia-Ruiz, Fernández-Checa, 2006].

The expression of OsDHAR genes in rice (*Oryza sativa* var. japonica) under oxygen deficiency and subsequent oxidative stress was studied. The products of these genes - dehydroascorbate reductases - play a rather important role in the detoxification of ROS, connecting the ascorbate and glutathione halves of the ascorbate-glutathione cycle (Halliwell Asada pathway). The enzyme reduces dehydroascorbic acid, oxidizing glutathione. Dehydroascorbate is formed in the cell by the action of the enzyme monodehydroascorbate reductase or by non-enzymatic disproportionation of two monodehydroascorbate molecules. Although dehydroascorbic acid can be regenerated by other compounds, not necessarily glutathione, as part of the ascorbate-glutathione cycle, glutathione S-transferases are interesting. At the same time, the activity of DHAR did not change significantly in the roots and stems of rice after a whole day of anaerobic action. The effect of three-day anaerobiosis led to a decrease in the activity of this enzyme, which leveled off after a day of reaeration, and in the stems of rice seedlings, DHAR was even activated under the effect of reaeration. Previous transcriptional analysis results showed similar results. The total expression of

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OsDHAR genes in rice stems was maintained at a constant level under anoxia and increased under reaeration, especially after short-term anoxia, 12 and 24 h, while in roots the expression level of OsDHAR decreased under the influence of anoxia and increased under reaeration. Of the two putative OsDHAR genes, OsDHAR1 was the most active in both stems and roots [Prykazyuk, Emelyanov, 2016].

When the VI gene is expressed in *Escherichia coli* cells, it was found that the Bax inhibitory protein has glutathione S-transferase and minor glutathione peroxidase activity, it causes a decrease in the number of phospholipids, reduces the membrane potential of mitochondria and changes the intracellular redox potential. With the co-expression of BI\$GST/GPX protein genes, the content of glutathione and the membrane potential of mitochondria in yeast cells is normalized and the resistance of cells against hydrogen peroxide increases sharply. Based on this information, it is concluded that oxidative stress plays an extremely important role in Bax-induced death of yeast and plant cells [Kampranis et al., 2000].

The activity of glutathione-S-transferases detected in the vacuoles of beet root cells was investigated in comparison with the activity of glutathione-S-transferases of plastids and tissue extracts. The activity level of glutathione-S-transferases, determined using the spectrophotometric method, turned out to be sufficiently high in aqueous extracts and membrane fractions of isolated vacuoles and plastids, as well as in aqueous tissue extracts. The pH dependence of glutathione-S-transferases of the studied objects was slightly different. Optimum activity of glutathione-S-transferases of vacuoles was at pH 7.0-7.5, and glutathione-S-transferases of plastids and tissue extracts were at 7.5. Glutathione-S-transferase differed in specificity in relation to the substrates - fluorodifen and ethacrynic acid (a derivative of phenylacetic acid). The activity of glutathione-S-transferases of vacuoles and tissue extracts with fluorodifen was several times higher than that of glutathione-S-transferases of plastids. Ethacrynic acid, which

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is quite often used as a competitive inhibitor, inhibited the activity of glutathione-S-transferases in all studied objects, detected with 1-chloro-2,4-dinitrobenzene as the main substrate. However, ethacrynic acid served as a substrate only for glutathione-S-transferases of vacuoles and tissue extracts, but not for plastids [Pradedova et al., 2016]. It was also shown that vacuolar glutathione-S-transferases had a wider pH-optimum (7.0 and 7.5), while plastid enzymes have it at 7.5. It is quite likely that vacuolar isoforms are able to function at a wider range of pH values, which is caused by the peculiarities of the compartment in which they are localized. The intravacuolar environment of beet root cells is mainly weakly acidic, the pH values in it vary between 5.5-6.8. depending on the stage of development [Nimaeva et al., 2014]. With the help of the zymographic method, which allows determining the activity of glutathione-S-transferases in the gel after protein electrophoresis, several zones of enzymatic activity were found in all the studied objects, which could correspond to different isozymes. At the same time, it was established that the composition of isoforms of glutathione-S-transferases of vacuoles and their substrate specificity may differ from glutathione-S-transferases of other cellular structures. The obtained results clarify a number of functional features of vacuolar and plastid glutathione-S-transferases of beet root cells [Pradedova et al., 2016]. Researchers predict that the vacuole, which has a fairly high activity of glutathione-S-transferases, should make a significant contribution to the processes of intracellular detoxification. It is also noted that with the use of spectrophotometry and electrophoresis methods, the authors obtained new facts confirming the presence of glutathione-S-transferases in the vacuolar compartment of plant cells, which were previously obtained using immunological and proteomic analysis methods [Dixon et al., 2002, 2009, Pradedova et al., 2016].

It is known that in *Arabidopsis* the *gst* gene family encodes glutathione-S-transferase of the U class, representatives of this family in rice are the *osgTu3* and

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osgtu4 genes. At the same time, it was shown that zinc (30  $\mu\text{M}$ ) and cadmium (20  $\mu\text{M}$ ) induce the expression of osgtu3 and osgtu4 genes in the roots of rice seedlings already 2 hours after the start of their action [Moons, 2003].

When plants germinate on nutrient solutions with lead acetate, reduced glutathione is intensively used, the content of which in the germ axes of seeds that germinate at a low concentration of lead ions (10<sup>-8</sup> M) in the nutrient solution is several times lower than when germinated at a high concentration (10<sup>-3</sup> M). The increased pool of reduced glutathione in the cotyledons when selenite (10<sup>-9</sup> M) is added to the nutrient solution with lead (10<sup>-8</sup> M) contributes to the normalization of metabolism in the germ axis and the passage of plants to the next phases of ontogenesis. The high activity of glutathione peroxidase and glutathione-S-transferase in germ axes, which is observed under the action of lead ions, promotes germination and initial growth of plants. Sodium selenite (10<sup>-9</sup> M) activates glutathione peroxidase and glutathione-S-transferase of plants due to the action of lead ions on them [Vorobets, 2004]. It has also been shown that glucose can induce many stress response genes in Arabidopsis, specifically glutathione-S-transferase genes and glutathione conjugate transporters [Couee et al., 2006].

In the roots of peas, salicylic acid caused an increase in the content of proteins that do not have a direct antipathogenic effect, but increase the resistance of cells. They take an active part in the detoxification of toxic products formed in the process of ROS accumulation (glutathione-S-transferase, ascorbate peroxidase, thioredoxin peroxidase), in energy metabolism (malate dehydrogenase, enolase), in cell signaling (NBS-LRR, 14-3-3 protein, lipoxygenase, 10,11-reductase 12-oxophytodienoic acid), in the processes of DNA duplication (nuclear antigen of proliferating cells), translation (translation elongation factor), protein refolding (chaperonin 60) and their proteolysis (polyubiquitin). In the leaves, protective responses are aimed at direct protection

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against pathogens, while in the roots - at the development of cell resistance and the activation of various signaling systems [Egorova, Yakovleva, 2010].

It has been shown that oxygen stimulates the development of oxidative stress, which exceeds the range of physiological values, and the response of the antioxidant defense system, the important components of which are glutathione and the phase II enzyme of biotransformation of xenobiotics - glutathione-S-transferase [Hayes, Strange 1995; Lesser, 2006].

### **2.4 Thioredoxins and their role in redox-dependent regulation**

One of the conditions for the normal life of cells is the maintenance of a certain redox state in the cytoplasm due to the fact that a significant number of reactions that occur in cells are related to the transfer of redox equivalents. Shifts in intracellular redox status induced by external or internal stimuli are modulated by the glutathione redox buffer system, which includes glutathione itself, glutaredoxins, and glutathione oxidoreductase. Along with the glutathione redox system, the thioredoxin redox system, which includes thioredoxins 1 and 2 and thioredoxin reductase, plays a rather important role in maintaining the thiol disulfide status of proteins. Isoforms of thioredoxin Trx1 and Trx2 are capable of restoring intra- and intermolecular disulfide bonds in proteins, as well as in oxidized peroxiredoxins that decompose organic hydroperoxides, hydrogen peroxide, and peroxynitrite. NADPH-dependent thioredoxin reductase, which reduces a number of substrates, including the oxidized form of thioredoxin, can also directly reduce lipid hydroperoxides, hydrogen peroxide, dehydroascorbic and lipoic acids. Redox systems of glutathione and thioredoxin function in cells at the same time, complementing each other [Lepekhina, 2014]. A significant block in the development of the destructive effect of oxidative stress is reduced glutathione, which has a fairly high activity in relation to DNA and lipid peroxidation products. Disulfides and mixed disulfides are substrates of



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glutaredoxin, which plays a rather important role in thiol-disulfide exchange, regulating, for example, the activity of transcription factors and the process of apoptosis. Isoforms of reduced glutathione and thioredoxin are important in the regulation of cell signaling through protein-protein interactions with key kinases that control the cellular response to stress, proliferation, and the development of apoptosis [Kalinina et al., 2014].

Thioredoxins are proteins with a mass of about 12 kDa. The feature that distinguishes them is the presence of two adjacent cysteine residues, combined into a motif of the SCHS type, where C is cysteine, and X is any, as a rule, hydrophobic amino acid. Another distinguishing feature of all thioredoxins is a specific tertiary structure called thioredoxin stacking. The main part of the protein is a disulfide bond. With the help of this connection, it can restore the disulfide bonds of other proteins, destroying their disulfide bridges. Thus, it regulates the activity of some enzymes. In addition, by restoring disulfide bonds, thioredoxin supplies electrons, which are then used in many biochemical processes of the cell. For example, together with glutathione, it supplies electrons for ribonucleotide reductase, that is, it participates in the synthesis of deoxynucleotides and FAPS reductase. In this respect, its function is similar to that of glutathione and partially overlaps with it. Thus, thioredoxin is a rather strong antioxidant - together with the glutathione system, it actively participates in the decontamination of ROS, transferring its electrons to various peroxidases. In plants, thioredoxins regulate a whole range of vital functions, from photosynthesis and growth to flowering, development and seed germination. In addition, it turned out that they also take an active part in intercellular interaction and information exchange between plant cells [Arnér, Holmgren, 2000, Wang et al., 2014]. Restoration of thioredoxin is carried out by a special flavoprotein-thioredoxin reductase, which removes one molecule of NADPH for this purpose [Mustacich, Powis, 2000, Dietzel et al., 2015]. At least 20 isoforms of thioredoxins have been found in Arabidopsis,

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which are localized in different cellular compartments: in chloroplasts, mitochondria, and cytosol [Meyer et al., 2005].

Prevention of oxidative stress in plant cells is provided by a complex mechanism. Sufficiently numerous enzymes such as ascorbate peroxidase, glutathione peroxidase, catalase, peroxiredoxin and superoxide dismutase protect against prooxidants. Redox control of protein activity is carried out by thioredoxin and glutaredoxin [Meyer et al., 2009, 2012].

In plants, there is a rather complex system of thioredoxins, consisting of six well-differentiated thiols (thioredoxins f, m, x, y, h, o). They are located in different parts of the cell and take part in many different processes. It is the action of thioredoxins that underlies the light-dependent activation of enzymes. In the light, as a result of the combined action of photosystem-1 and photosystem-2, a significant amount of reducing equivalents - ferredoxins - is formed. When a certain concentration of ferredoxin is reached due to the action of the ferredoxin-thioredoxin reductase enzyme, thioredoxin is restored, which in turn activates enzymes, restoring disulfide bonds. In this way, at least 5 key enzymes of the Calvin cycle are activated, as well as Rubisco protein activase, alternative oxidase of mitochondria and terminal oxidase of chloroplasts. The mechanism of activation through thioredoxin makes it possible to regulate the activity of enzymes not only depending on the NADPH/NADP<sup>+</sup> ratio, but also simultaneously on the light intensity [Ermakov et al., 2005].

Depending on the localization, thioredoxins are able to accept electrons from a significant number of reducing agents from various electron transport systems. Thus, thioredoxins localized in mitochondria and cytosol are reduced by NADPH-thioredoxin reductase, and chloroplast thioredoxins by ferredoxin-alkaline reductase, which receives an electron from ferredoxin, a component of the electron transport chain of chloroplasts [Schurmann, Jacquot, 2000]. Thioredoxins can control the accumulation of ROS because they compete with

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molecular oxygen for accepting an electron from the electron transport chain of chloroplasts [Baier, Dietz, 2005].

A new form of thioredoxin-like bioc z-form (TRX z) has been discovered. TRX z takes an active part in the regulation of chloroplast gene expression, which determines the relationship between redox signals and transcription of plastid genes [Arsova et al., 2010]. Mutations in the z-form thioredoxin gene (TRXZ) lead to discoloration of plants, which indicates the uniqueness of this form of the protein [Arsova et al., 2010; Schroter et al., 2010]. Also, *trxz*-mutants have reduced expression of chloroplast genes, which are transported with the help of plastid-encoded RNA polymerase. Based on this, it was proposed that TRX z regulates the activity of protein kinases, which in turn regulate RNA polymerase when plants are transferred from darkness to light [Barajas-Lopez et al., 2013]. Thus, chloroplast thioredoxins can participate in the transmission of chloroplast-nuclear signals that occur when the redox state of the organelle as a whole changes.

Redox-dependent processes significantly affect the functional activity of many proteins, which take an active part in the regulation of the most important processes for the life of cells, such as proliferation, differentiation, and apoptosis. Recently, special attention of researchers has been drawn to the study of thiol-disulfide regulation carried out by redox proteins, the activity of which is due to a redox-active site in the form of an amino acid sequence with two or one active thiols. Among these proteins, two thiol disulfide reductases stand out - thioredoxin and glutaredoxin, which are part of the superfamily of thioredoxins. These enzymes are multifunctional and form thioredoxin- and glutaredoxin-dependent systems, which play a rather important role in maintaining intracellular redox homeostasis. The first system contains, in addition to thioredoxin, NADPH-dependent thioredoxin eductase, which restores the oxidized form of thioredoxin. The second system includes glutathione as an agent that reduces oxidized

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glutaredoxin and glutathione reductase, which reduces glutathione from its oxidized form. Both of these systems make a significant contribution to the antioxidant protection of cells against the destructive effects of oxidative stress, which causes the formation of intra- and intermolecular disulfide bonds in proteins, the oxidation of functional SH-groups with the formation of sulfonic acid, and the subsequent proteasomal degradation of proteins. Peroxiredoxins, which decompose hydrogen peroxide, organic hydroperoxides and peroxynitrite, also participate in antioxidant protection [Fernandes, Holmgren, 2004].

Glutaredoxin is one of the most important enzymes in the processes of disulfide reduction and deglutathionylation, and also takes an active part in redox-dependent processes in cells. Isoforms of glutaredoxins have a molecular weight of 9-14 kDa and belong to the superfamily of thioredoxins by their structure and play a rather important role in cellular redox-dependent processes. Their structure is characterized by the presence of thioredoxin stacking, as well as glutathione transferase enzymes. They are found in almost all taxonomic groups, including prokaryotes, plants and eukaryotes, from yeast to humans. Isoforms of glutaredoxins are heat-resistant low-molecular-weight proteins (10–16 kDa) that function as glutathione-dependent oxidoreductases, which by their structure belong to the Trx superfamily and, along with Trx, play a rather important role in cellular redox-dependent processes. In the N-terminal region of the Grx amino acid sequence, there is a Cys–X–X–Cys/Ser active site, while a conserved glutathione-binding domain is located at the C-end of the molecule. Grx isoforms are found in almost all living organisms, with the exception of some types of bacteria and archaea [Alves et al., 2009]. There is also an additional division of the family of glutaredoxin isoforms on the basis of the presence of a cysteine residue in the active center in the second position Cys–Cys–X–Cys/Ser, such isoforms were named CC-type glutaredoxin. The primary function of glutaredoxin was considered to be its ability to restore disulfide bonds and carry

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out deglutathione. However, it was later discovered that certain isoforms of glutaredoxin preferentially serve as carrier proteins for iron-sulfur [FeS] clusters, using reduced glutathione as a ligand [Rouhier, 2010].

Glutaredoxins are found in almost all taxonomic groups, including prokaryotes, plants, and eukaryotes, from yeast to mammals. They have a fairly high degree of amino acid sequence homology, especially in the region of the active center [Noctor et al., 2012]. One of the main functions of glutaredoxins is the restoration of disulfide bonds in a system connected with reduced glutathione, NADPH and glutathione reductase [Holmgren, Aslund, 1995]. This process uses two catalytic mechanisms. The adjacent cysteine residues of the active center of glutaredoxin take part in the dithiol mechanism [Bushweller et al., 1992]. According to the alternative monothiol mechanism, only mixed disulfides can be reduced [Bushweller et al., 1992]. Oxidized glutaredoxin, which is formed after glutathioneation of thiols and restoration of protein disulfide bonds, is itself restored, as a rule, with the participation of reduced glutathione, although phrodoxin- or NADPH-dependent thioredoxin reductase takes part in the restoration of some isoforms of glutaredoxin [Johansson et al., 2004].

An additional division of the Grx isoform family was also proposed based on the presence of a cysteine residue in the active site in the second position Cys–Cys–X–Cys/Ser, such isoforms were named Grx CC-type. The primary function of Grx was considered to be its ability to repair disulfide bonds and carry out deglutathione. However, it was later discovered that certain Grx isoforms more often serve as carrier proteins for iron-sulfur [FeS] clusters, using glutathione as a ligand [Rouhier, 2010]. Binding of Fe–S clusters can lead to the formation of dimers and tetramers. At the same time, the formation of various alternative protein-protein contrast sites in the mono- and dithiol isoforms of Grx is possible, which ensures the existence of Grx in the form of both mono- and multidomain forms [Lillig et al., 2008]. Glutaredoxins of photosynthetic organisms are

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characterized by considerable diversity - they are divided into 6 classes, depending on the similarity of the amino acid sequence [Couturier et al., 2009]. Among the monothiol isoforms of glutaredoxin, there are representatives of the 1st and 2nd classes, dithiols belong to the 1st class, and SS-type glutaredoxins belong to the 3rd class. In fact, class 1 and class 2 glutaredoxin isoforms can be found in all organisms, class 3 isoforms in higher plants, these isoforms control, for example, plant flowering and some other functional activities [Deponete, 2013]. Class 4 isoforms are found in photosynthetic eukaryotes, class 6 in cyanobacteria, and class 5 glutaredoxins are found in proteobacteria and cyanobacteria [Benyamina et al., 2013]. The process of catalysis of the glutaredoxin isoform is carried out either by a monothiol or by a dithiol mechanism. It can be used by both mono- and dithiol isoforms [Deponete, 2013]. Numerous studies show that all known dithiol isoforms of glutaredoxin have the ability to function according to the monothiol mechanism, but at the same time, not all dithiol isoforms have been shown to be capable of dithiol catalysis.

It is shown that not only photorespiration, but also almost any metabolic pathway can be controlled on the basis of thiol redox regulation. Biochemical and redox-proteomic evidence has been published that the activity of all enzymes of the Calvin-Bateson cycle [Michelet et al., 2013], some enzymes of the tricarboxylic acid cycle that are targets of thioredoxin or glutaredoxin [Daloso et al., 2015] are regulated in this way.

It was noted that glutaredoxin takes part in maintaining the normal thiol-disulfide balance in the cell, as well as in the regeneration of proteins damaged by oxygen radicals. To study these important regulatory proteins in cyanobacteria, the first cyanobacterial glutaredoxin gene *ssr2061* was cloned and the corresponding protein expressed in *Escherichia coli*. The second glutaredoxin gene *slr1562* was also cloned and the corresponding protein was expressed. It was found that 90% of the Grx2061 protein encoded by the *ssr2061* gene is

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synthesized in a soluble form, while 90% of the Grx1562 protein encoded by the *slr1562* gene accumulates in inclusion bodies. To obtain a soluble form of the Grx1562 protein, 2 mutant forms were created: Grx1562NC with replacement of cysteines in the conservative region by serines and Grx1562M with deletion of the N-terminal hydrophobic region. At the same time, it was shown that only the mutant protein Grx1562M was synthesized in a soluble form and was characterized by increased glutaredoxin activity and reduced sensitivity against oxidative stress. Spectroscopic analysis showed that the structure of Grx1562M is less hydrophobic, which may contribute to obtaining a soluble form of the protein and improve its catalytic activity. The results of this study indicate that the hydrophobic N-terminal region determines the insolubility of the Grx1562 protein, and may also contribute to the development of a new strategy for increasing the expression level of heterolytic proteins in *Escherichia coli* in a soluble form [Li et al., 2007].

In the *in vitro* system, it was shown that disulfide bonds that support the oligomeric state of the NPR1 protein are broken under the influence of thioredoxin reductant. Its participation in the transition of NPR1 into a monomeric form has been proven by genetic methods. *Arabidopsis* thioredoxin mutants (*trx-h5*), unlike wild-type plants, did not form the monomeric form of NPR1 and were unable to synthesize the PR1 protein in response to treatment with exogenous salicylic acid [Tada et al., 2008]. Although it is possible that thioredoxin is not the only reducing agent involved in the transformation of NPR1 from oligomeric to monomeric form. At the same time, it was established that this transformation depends on the redox status, and a decrease in the content of reduced forms of glutathione and ascorbate in cells prevents the transmission of salicylic acid signals [Brosche, Kangasja, 2012].

Grx isoforms use two mechanisms of catalysis: monothiol and dithiol. The monothiol mechanism is typical for substrate deglutathione reactions, in which

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case only the catalytic cysteine residue (the first of the two cysteines of the active center at the N-terminus) takes part in the reaction. Recovery of the glutathione substrate begins with the nucleophilic attack of the thiol group CysA of glutaredoxin. The substrate is released with the formation of the glutathione intermediate Grx-SSG. Subsequently, with the help of reduced glutathione, glutaredoxin is regenerated to Grx(SH)<sub>2</sub> with the formation of oxidized glutathione. The monothiol mechanism is used by both mono- and dithiol isoforms of Grx [Deponte, 2013].

In addition to the catalytic cysteine residue, the dithiol mechanism also requires a recycling cysteine, which can be either the second cysteine residue from the glutaredoxin active site or an additional cysteine residue not from the active site. If the substrate undergoes deglutathination, then the first step proceeds according to the monothiol mechanism, but the glutathione intermediate Grx-SSG further donates reduced glutathione with the formation of an intramolecular disulfide bond, which is formed between the catalytic cysteine and one of the recycling cysteines. In the future, the disulfide bond is restored either with the participation of two molecules of reduced glutathione, or with the help of thioredoxin reductase. If the oxidized glutaredoxin substrate requires the reduction of an intra- or intermolecular disulfide bond, the thiol group of thioredoxin binds temporarily to one of the cysteines of the substrate, and then the reduced substrate is released and a disulfide bond is formed between the thiol group of thioredoxin and the second cysteine residue of the active site or an additional cysteine residue not from the active center; at the final stage, the disulfide bond is restored with the help of two molecules of reduced glutathione or with the participation of thioredoxin reductase [Xing et al., 2006].

All dithiol isoforms of glutaredoxin studied so far are capable of catalysis by the monothiol mechanism, but not all of them have yet been tested for dithiol



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catalysis. However, all dithiol isoforms of glutaredoxin capable of dithiol catalysis can also carry out monothiol catalysis.

Despite the fact that the amino acid sequence of glutaredoxin-1 and glutaredoxin-2 is only 34% identical, these isoforms use the same mechanism of catalysis [Gallogly et al., 2009, Stroher, Millar, 2012].

Glutaredoxin-1 and monomeric glutaredoxin-2 are able to catalyze both the deglutathione reaction and the reverse S-glutathione reaction. At the same time, the direction of the reaction depends on the relative concentrations of "protein-reduced glutathione", "protein-SN", reduced glutathione and oxidized glutathione. The redox potential of the reduced glutathione and oxidized glutathione pair is the most important for determining the cellular redox potential. The magnitude of the redox potential of reduced glutathione and oxidized glutathione largely depends on the functional state of the cell. During proliferation, this value is approximately equal to -240 mV, and in the state of differentiation -200 mV, during the development of apoptosis, it continues to grow to approximately -170 mV [Watson et al., 2003].

Glutaredoxin 1 consists of 85 amino acid residues [Hoog et al., 1983]. The tertiary structures of glutaredoxin 1 and thioredoxins are similar, but differ significantly in their amino acid composition [Xia et al., 1992]. Based on immunochemical analysis, glutaredoxin occupies the periphery of the cell [Nygren et al., 1981]. Expression of the *grxA* gene is under the control of the OxyR transcription factor [Zheng et al., 2001]. Glutaredoxin 1 is a fairly effective hydrogen donor for ribonucleotide reductase and PAPS reductase [Holmgren, 1979]. Glutaredoxin 2 and glutaredoxin 3 were isolated from double mutants of the *grxA* and *trxA* genes [Aslund et al., 1994]. Glutaredoxin 2 consists of 215 amino acids, and its structure resembles the structure of glutathione-S-transferases [Vlamiš-Gardikas et al., 1997]. It was shown that the intracellular pool of glutaredoxin 2 is 1% of the total protein content [Shi et al., 2000]. The

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concentration of glutaredoxin 2 is 3 times higher in the stationary phase than in the growing culture, and the expression level of the *grxB* gene is regulated by the  $\sigma$ -subunit of RNA polymerase and guanosine-3',5'-tetraphosphate (ppGpp) [Potamitou et al., 2002]. The activity of glutaredoxin 2 as a donor of reducing equivalents is 100 times higher than that of other glutaredoxins [Shi et al., 1999]. Glutaredoxin 3 has a similar tertiary structure to glutaredoxin 1 and 33% identical amino acids. Glutaredoxin 3 transfers reducing equivalents to ribonucleotide reductase in vitro [Aslund et al., 1996]. Although the amino acid sequences of glutaredoxin 1 and glutaredoxin 2 are only 34% identical, these isoforms share the same mechanism of catalysis [Gallogly et al., 2009, Stroher, Millar, 2012]. The activity of glutaredoxin 2 is almost 10 times lower than the activity of glutaredoxin 1, but its content in the intermembrane space of mitochondria is higher than that of glutaredoxin 1, which can compensate for differences in their enzymatic activity [Cross, Templeton, 2004]. Only reduced glutathione is involved in the reduction of glutaredoxin 1. Conversely, glutaredoxin 2 can be restored even with the participation of thioredoxin reductase, which characterizes it as a protein with the functions of both glutaredoxin and thioredoxin and testifies in favor of the interconnection of metabolic pathways in mitochondria that are controlled by thioredoxin and glutaredoxin [Johansson et al., 2004]. The possibility of reactivation of glutaredoxin 2 by thioredoxin reductase also opens up the possibility for it to maintain functional activity at different values of the ratio of reduced to oxidized glutathione and in a situation when oxidative stress in mitochondria reaches a sufficiently high level [Beer et al., 2004]. Monothiol glutaredoxin 3 is localized in the nucleus and cytoplasm and is a multidomain protein, while monothiol glutaredoxin 5 is present in mitochondria [Kalinina et al., 2014, Hanschmann et al., 2013]. Almost all monothiol glutaredoxins and some isoforms of dithiol glutaredoxins of trypanosomes, plants and humans can form complexes with iron-sulfur clusters with the participation of sulfhydryl residues

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from active centers [Lillig et al., 2008, Riondet et al., 2012]. Such complexes are present, as a rule, in mitochondria. Two monomers of glutaredoxin tear apart the  $[2\text{Fe}-2\text{S}]^{2+}$  cluster between themselves using coordination bonds with two thiol residues from the active centers on the side of the N-terminus, and two more coordination bonds are formed with two thiol residues of two non-covalently bound glutathione molecules. Reduced glutathione comes from the pool of free glutathione, which indicates its rather important contribution to the stabilization of Fe-S clusters [Berhane et al., 1994]. During the existence of such a complex, glutaredoxin molecules lose their enzymatic activity, since the cysteine residues of the active centers are bound. Glutaredoxin activity is restored upon degradation of the cluster and dissociation of the holocomplex. Under aerobic conditions, reduced glutathione effectively protects against slow degradation of the complex, while oxidized glutathione, on the contrary, stimulates cluster degradation and glutaredoxin activation [Lillig et al., 2008]. Glutathione molecules shield iron atoms of  $[2\text{Fe}-2\text{S}]^{2+}$  from the environment, and therefore iron has no possibility of interaction with oxidants that require direct contact, for example, with hydrogen peroxide. It was shown that glutaredoxin monomers are released under the influence of  $\text{O}_2^-$  [Mitra, Elliott, 2009].

Thiols of the active center of some isoforms of glutaredoxin are able to form complexes with iron-sulfur clusters. Such enzymes include a limited number of dithiol isoforms of glutaredoxin from humans, plants, trypanosomes and almost all monothiol isoforms of glutaredoxin [Lillig et al., 2005, Feng et al., 2006, Riondet et al., 2012].

Most of such clusters were found in mitochondria. The  $[2\text{Fe}2\text{S}]^{2+}$  cluster is located between two monomers of glutaredoxin due to coordination bonds with two cysteine residues from the active centers from the N-ends with two non-covalently bound molecules of reduced glutathione. Reduced glutathione comes from the pool of free glutathione, which indicates a rather important role of

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glutathione in the stabilization of Fe–S clusters [Berndt et al., 2007]. Since cysteine residues involved in catalysis are involved in the binding of the  $[2Fe_2S]^{2+}$  cofactor by the holo-Grx complex, the glutaredoxin complex is enzymatically inactive during the existence of such a complex. Degradation of the cluster and dissociation of the holo-complex restores glutaredoxin activity. Slow degradation of the complex under aerobic conditions is effectively prevented by reduced glutathione. On the contrary, oxidized glutathione promotes cluster degradation and glutaredoxin activation [Lillig et al., 2005]. Two molecules of reduced glutathione in the complex successfully shield iron atoms from the surrounding environment. Thus, iron with  $[2Fe_2S]^{2+}$  cannot interact with oxidants that require direct molecular interaction, specifically hydrogen peroxide. It was shown that the release of glutaredoxin monomers causes the action of  $O\bullet-2$  [Mitra, Elliott, 2009].

It was found that thiol-oxygen oxidoreductase and glutathione-dependent protein disulfide oxidoreductase are simultaneously present in the ripening grain of wheat, which catalyze the formation and decomposition of disulfide bonds in reserve proteins, respectively. The synchronicity of changes in the ratio of SS/SH redox status and thiol-oxygen oxidoreductase and glutathione-dependent protein disulfide oxidoreductase in ripening grain indicates that the enzymes regulate SS/SH redox status of grain proteins. The activity level of thioloxidase, disulfide reductase and lipoxygenase in ripening and mature grain, as well as the physical properties of gluten, which is characterized by high modification variability, which is shown on varieties and intervarietal substituted lines of soft wheat grown in different soil and climatic conditions. Wheat grain disulfide reductase is a glutaredoxin-like protein and functions in the NADPH/glutathione reductase reducing system /OZN/ glutathione-dependent protein disulfide oxidoreductase. The high activity of disulfide reductase in germinating grains

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indicates the participation of this enzyme in the preparation of proteolytic degradation of glutenin aggregates during germination [Osypova, 2011].

It was established that the maintenance of a high level of the ratio of reduced to oxidized glutathione and the activities of glutathione transferase and glutaredoxin contributes to the protection of resistant cells from apoptosis induced by oxidative stress under the action of hydrogen peroxide, which is accompanied by the absence or low growth of ROS and the absence of a change in the ratio of the anti-apoptotic protein of the Bcl-2 family of proteins to the pro-apoptotic a protein of the Bcl-2 protein family and antiapoptotic protein of the Bcl-2 protein family to proapoptotic protein of the Bcl-2 protein family [Novichkova, 2018].

It is known that reduced glutathione modulates DNA synthesis by maintaining the level of reduced glutaredoxin or thioredoxin, which are necessary for the activation of ribonucleotide reductase, the enzyme that limits the rate of DNA synthesis [Holmgren, 1981].

Among the redox-dependent processes occurring in bacterial cells, regulation with the participation of two systems - glutaredoxin and thioredoxin - is of particular interest. The first system includes glutaredoxins, glutathione, which non-enzymatically reduces oxidized glutaredoxins, and glutathione reductase, which in turn reduces oxidized glutathione using NADPH. The second system, in addition to thioredoxins, includes NADPH-dependent thioredoxin reductase, which reduces the oxidized form of thioredoxin [Holmgren, 1985; Holmgren and Aslund, 1995]. In *Escherichia coli*, glutaredoxins and thioredoxins are small proteins that contain a structure characteristic of the thioredoxin superfamily (Trx fold) and catalyze redox reactions using the redox chemistry of cysteine residues [Tyulenev, 2015]. L-cysteine is a thiol-containing amino acid that is part of proteins and is necessary for the biological synthesis of a significant number of sulfur-containing compounds (methionine, thiamine, biotin, and coenzymes). In addition, cysteine plays a key role in the assembly of protein

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molecules, their folding and stabilization through the formation of disulfide bonds. L-cysteine-containing proteins, such as thioredoxin and glutaredoxin, take an active part in the protection of cells against oxidative stress. It was shown that periplasmic cysteine protects *Escherichia coli* cells from hydrogen peroxide produced by phagocytes [Ohtsu et al., 2010]. At the same time, under certain conditions, cysteine can induce oxidative stress. When the intracellular concentration increases, it restores free iron, promoting the production of hydroxyl radicals that damage DNA during the Fenton reaction [Park, Imlay, 2003].

*Escherichia coli* contains three glutaredoxins Grx1, Grx2 and Grx3, which are encoded by the genes *grxA*, *grxB*, *grxC*, [Aslund et al., 1994] and two Grx-like proteins (Grx4, encoded by *grxD*, and NrdH, encoded by *nrdH*) [Vlami-Gardikas 2008; Meyer et al. 2009]. Unlike other glutaredoxins, oxidized NrdH is reduced by TrxR, whereas Grx4 is reduced by TrxR or Grx1. Glutaredoxins use two catalytic mechanisms, mono- and dithiol, with the participation, respectively, of one or two cysteine residues in the active center. According to the monothiol mechanism, only mixed disulfides can be reduced, and according to the dithiol mechanism, disulfides in proteins and mixed disulfides can be reduced [Bushweller et al., 1992].

Regulation by glutathione of essential SH groups is common in eukaryotes and is also possible in prokaryotic cells. One of the prokaryotic proteins, for which the possibility of regulation by the formation of a mixed disulfide with glutathione is shown, is PAPS-reductase of *Escherichia coli* [Vlami-Gardikas et al., 2002]. It was also shown that in *Escherichia coli* cells, thioredoxin is able to bind to 80 proteins that participate in various cellular processes. The presence of transcriptional regulators among these proteins indicates the potential participation of thioredoxin in the redox regulation of bacterial cell activity [Kumar et al., 2004].

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Glutathione and thioredoxin systems are considered parallel redox systems of the cell, which do not exchange reducing equivalents [Das, White, 2002]. However, if there are defects in one or another system, they may duplicate each other in terms of functions. In this regard, the deletion of components of one of the systems quite often does not manifest itself phenotypically, and only numerous mutations affecting both systems lead to significant metabolic disorders. Glutaredoxin or thioredoxin is necessary for the reduction of sulfate to sulfite with the help of PAPS reductase, and therefore *grxAtrxA* double mutants lose the ability to assimilate sulfate. In connection with this, in this strain there is a sharp decrease in the level of glutathione with a shift of the redox status towards the oxidized form. The induction of ribonucleotide reductase and glutaredoxin 1 is also increased in *gshAtrxA* double mutants, 55 times higher than in wild-type cells [Miranda-Vizuete et al., 1997].

An inverse correlation between the levels of glutaredoxin and thioredoxin with a relatively stable content of other redoxins is also shown. Most likely, glutaredoxin 1 and thioredoxin 1 have specific overlapping fractions associated with ribonucleotide reductase repair [Potamitou et al., 2002a]. Along with glutathione, thioredoxins and glutaredoxins participate in the creation of general reducing conditions in the cytoplasm, which was confirmed in experiments with the remaining signal sequence of alkaline phosphatase [Derman et al., 1993]. The expression of glutaredoxins and thioredoxins is induced under conditions of oxidative stress when the redox balance in the cell is disturbed [Prieto-Alamo et al., 2000, Potamitou et al., 2002a]. At the same time, the resistance of the strains left with all thioredoxins against oxidative stress was shown. It is likely that in the absence of thioredoxins, the formation of disulfide bonds occurs in the OxyR protein, which leads to the activation of other antioxidant systems of the cell, such as AhpC and catalase, which have a higher ability to remove hydrogen peroxide [Ritz et al., 2000]. Thus, the thioredoxin system is not necessary for protection

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against oxidative stress in *Escherichia coli* cells, but is critical for maintaining cellular protein disulfide/dithiol redox control [Lu, Holmgren, 2014].

To clarify the influence of the level of oxidation of ferredoxin on the functioning of the entire electron transport chain of chloroplasts, the influence of infiltration of dichlorophenolindophenol on the change in the level of reduction of the plastoquinone pool, the flow of electrons to photosystem 2 during thermal shock was evaluated. It was shown that pretreatment of leaves with dichlorophenolindophenol increased the level of recovery of photoactive plastoquinone molecules. That is, during the activation of cyclic ferredoxin-dependent electronic transport, there was no heat-induced decrease in the level of reduction of photoactive plastoquinone molecules, which indicates that the inhibition of this particular transport during thermal action was the reason for the increase in the oxidation level of these molecules. Analysis of the dark kinetics of chlorophyll a fluorescence relaxation showed that the addition of dichlorophenolindophenol practically did not affect the oxidation phases of QA- in control barley seedlings. At the same time, the presence of dichlorophenolindophenol, which reduces the level of reduction of ferredoxin during thermal inactivation, leads to an increase in the oxidation level of plastoquinones, and this, in turn, prevents the inactivation of photosystem 2 on the donor side. Most likely, the excess flow of electrons passing through photosystem 1 during thermal shock leads to the restoration of the ferredoxin pool. All this can lead to inactivation of ferredoxin: NADPH-oxidase. It is known that the activity of this enzyme is quickly regulated by binding to membrane proteins Tic62 and TROL, which protect this flavoenzyme from inactivation. Therefore, it can be argued that one of the main causes of heat-induced disruption of the electron flow in chloroplasts is the complication of the outflow of electrons from P700 via the ferredoxin-dependent pathway [Pshybytko, 2016].



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The content of ROS and the activity of peroxiredoxin and thioredoxin in *Arabidopsis thaliana* tissue culture under the development of osmotic and oxidative stress were investigated. It was established that in the first 30-180 minutes, there was an increase in the content of ROS, which led to an increase in the activity of peroxiredoxin and thioredoxin. It is suggested that ROS molecules, in particular H<sub>2</sub>O<sub>2</sub>, as secondary messengers, cause an ROS-dependent increase in the activity of peroxiredoxin and thioredoxin. In the tissue culture we studied, which grew in the dark, such processes probably occur in mitochondria [Zhadko et al., 2015].

In the algae genus *Chlamydomonas*, a calcium-binding type of thioredoxin, called calredoxin, has been identified, which may be a connecting chain between the redox state of plastids and Ca<sup>2+</sup>-dependent regulation. Homologs of this protein have not been found in higher plants [Kmieciak et al., 2016].

It is also necessary to note the ability of glutaredoxin for protein-protein interaction. Thus, glutaredoxin takes an active part in the regulation of protein kinase ASK1, which activates JNK1- and p38-dependent signaling pathways of apoptosis activation [Ichijo et al., 1997]. In many cell lines, it has been shown that ASK1 is activated by ROS, and specifically, by hydrogen peroxide, as a result of the breakdown of the complex with glutaredoxin-1. The reconstituted glutaredoxin-1 binds to the C-terminal domain of ASK1, which leads to inactivation of the kinase. Conversely, oxidation of glutaredoxin-1 leads to disintegration of the complex, activation of ASK1 and induction of apoptosis. This decay is prevented, for example, by the action of catalase or N-acetylcysteine. Reduction of reduced glutathione with the help of buthionine sulfoximine leads to inhibition of binding of glutaredoxin-1 to ASK1. It is likely that reduced glutathione is required to repair the intramolecular disulfide bonds of adjacent cysteine residues in glutaredoxin-1, which creates the ability of ASK1 to bind to glutaredoxin-1. The obtained results allow us to assert the ability of

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glutaredoxin-1 to act as a sensory factor regulating signaling cascades of MAP kinases JNK1 and p38, sensitive to the redox status of the microenvironment [Song et al., 2002].

It is known that wheat grains contain enzymes that catalyze thiol-disulfide redox reactions. Protein disulfide isomerase functions mainly as a dithiol oxidase in protein folding, thioredoxin reductase catalyzes the destruction of disulfide bonds of reserve proteins in the process of grain germination. At the same time, the glutathione-dependent protein-disulfide oxidoreductase enzyme was isolated from wheat grains and studied. This enzyme is a heterodimer with a molecular weight of the native protein of 167 kDa and molecular weights of subunits of 73 and 77 kDa, and requires reduced glutathione as a cofactor, the pool of which in the cell depends on the activity of glutathione reductase. The glutathione reductase/reduced glutathione/protein-disulfide oxidoreductase enzyme system most likely performs the function of protecting protein SH-groups from oxidation under various stresses. The activity of protein-disulfide oxidoreductase in the ripening grain of wheat increases in the third week after flowering during the period of maximum synthesis of reserve proteins and decreases until the phase of full maturity of the grain. The results of the inhibitory analysis allow predicting the belonging of the protein-disulfide oxidoreductase to the superfamily of thioredoxins. Protein-disulfide oxidoreductase is able to destroy disulfide bonds of vinegar-soluble gluten proteins and reduce the aggregating ability of these proteins. Addition of exogenous protein-disulfide oxidoreductase preparation to flour increases dough extensibility in different wheat varieties from 17 to 49%. All this makes it possible to consider protein-disulfide oxidoreductase as a potential bakery improver that can be used to correct the rheological properties of gluten that is too strong and breaks quickly [Osypova et al., 2008].

It is well known that artificially changing the redox situation in *Escherichia coli* cells by treating bacteria with thiol-containing reagents can modify the

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expression of the *recA* gene, which encodes the SOS response regulator, and affect the resistance of bacteria against ultraviolet irradiation. In the conducted research, *Escherichia coli* bacteria were used, the redox status of which was changed due to mutations in the genes encoding the components of the redox systems of reduced glutathione and thioredoxin (*gshA*, *grxA*, *trxA* and *trxB*). Bacteria with various defects in the above-mentioned redox systems responded differently to 254 nm ultraviolet irradiation. In the absence of irradiation, bacteria lacking glutathione (*gshA*) and glutaredoxin (*grxA*) had the lowest levels of survival:  $27.7 \pm 0.6$  and  $32.3 \pm 2.5$ , respectively. *Escherichia coli* strain deficient in thioredoxin-1 (*trxA*) turned out to be the most viable ( $49.5 \pm 3.1$ ). After short-term irradiation (6 minutes), survival decreased in all strains, excluding thioredoxin reductase (*trxB*) and glutaredoxin (*gshA*) mutants. The frequency of mutations after irradiation (6 minutes) increased slightly in all bacteria. With a fifteen-minute exposure to ultraviolet radiation of 254 nm, survival significantly decreased, and the frequency of mutations increased. The lowest degree of survival, which was 28% in relation to the control, was found in the *Escherichia coli* strain deficient in thioredoxin reductase. And the maximum increase in the frequency of mutations, which exceeded the control indicators by 16 times, was observed in mutants that did not have glutathione and thioredoxin reductase. The lowest frequency of mutations after 15 minutes of 254 nm irradiation was found in cells of the parental type. The low level of survival and the high frequency of mutations in cells lacking glutathione and thioredoxin reductase indicate a rather important role of donors of reducing equivalents in the protection of *Escherichia coli* bacteria from ultraviolet radiation and indicate the need for coordinated work of the redox systems of glutathione and thioredoxin [Ushakov, Brysova, 2007].

The main components of the mitochondrial electron transport chain of plants were structurally characterized: the structure of cDNA and genes of adrenonedoxin-like ferredoxins of tobacco, tomato, potato of the Solanaceae

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family and *digitalis purpurea* was cloned and established family Scrophulariaceae. At the same time, it was established that plants of the Solanaceae family have two different mitochondrial-type ferredoxin genes (MFDX1 and MFDX2), which have a similar exon-intron structure (containing 8 exons each) and encode precursors of adrenonoxin-like proteins, differing in the structure of the N-terminal part of the protein (encoded by the first and second exons), which correspond to the leader peptide. The primary structure of the cDNA and the gene encoding the *Nicotiana tabacum* MFDXR protein, which is structurally related to mammalian ADXR, was determined. The results obtained by the researchers indicate the absolute similarity of the electron transport chains of P450-dependent monooxygenases in the mitochondria of plants and mammals [Shpakovsky et al., 2013].

The effect of mutations in glutathione, glutaredoxin, thioredoxin and thioredoxin reductase in oxidative stress of growing *Escherichia coli* bacteria was studied. The lowest resistance against high doses of hydrogen peroxide was shown by *gshA* mutants defective in glutathione synthesis, and the highest by *trxB* mutants defective in thioredoxin reductase synthesis, they were even more resistant to the action of the oxidant than cells of the parent type. Of the studied mutants of this bacterium, *trxB* had higher basal levels of catalase activity and intracellular glutathione and possessed the ability to quickly restore normal glutathione levels after oxidative stress. At the same time, an increased frequency of induced mutations was found in these bacteria. The results of measuring the expression of the *katG* and *sulA* genes allow us to predict that the differences in sensitivity against the action of high oxidant concentrations in the studied mutants are determined, first of all, by the ability to induce antioxidant genes that are part of the OxyR and SOS regulons [Oktyabrskii et al. 2007].

**CHAPTER 3. ECOLOGICAL MANAGEMENT OF  
INTERACTION OF GLUTATHIONE WITH SIGNALING MOLECULES**

Modern studies of the physiological functions of plant organisms are quite closely connected with the study of the mechanisms of perception of intracellular signal transmission. Knowledge of the mechanisms of formation of the response of cells to the action of extracellular signals is fundamentally important for the development of an understanding of the regulation of functional and metabolic activity of cells. And this, in turn, is necessary for a deeper understanding of the essence of ontogenesis, the peculiarities of the interaction of organisms with the environment, and the nature of various biological functions of living objects [Dzhameev, 2015].

The life of any cell, including the global processes of its development, division and even death, depends on the external regulatory signals it perceives. Such signals can be physical actions (temperature, ionizing and other electromagnetic radiation) or numerous chemical compounds. Well-studied substances that the plant uses to regulate the vital activity of cells are, for example, steroid hormones, cytokinins or growth factors, which, reaching the target cells, cause specific metabolic changes in them, including changes in the expression of large groups of genes. An equally strong and often specific response is caused by various physiologically active substances of exogenous origin, such as pheromones and toxins. All these signals transmitted through the corresponding signal molecules are primary in relation to those cascades of biochemical reactions that are triggered in cells in response to their action [Patrushev, 2000, Shakirova, Sakhabutdinova, 2005, Sharma, Dietz, 2006, Suzuki, Mittler, 2006, Yurina, Odintsova, 2007]. Also, the signal can be transmitted by replication of spin-polarization portraits when the signal molecules collide with each other

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almost without moving the molecules themselves, and water molecules and organic molecules located in the interorganismal space can also be involved in information replication [Vorobyov et al., 2009]. In the transmission of signals, proteins and small messenger molecules (salicylic and jasmonic acids, hydrogen peroxide, nitric oxide) also play a significant role [Karpun et al., 2015]. It is also believed that the component of the signaling network during stress caused by heavy metals may include have receptors for the perception of signal and non-protein messengers used for signal transmission [Belyavskaya et al., 2018]. It has been shown that ROS also function as signaling molecules, taking an active part in the regulation of plant development and adaptation processes [Ivanov et al., 2007]. Plant factors produce various ROS, for example, hydrogen peroxide and superoxide anion radical are produced in response to pathogens [Overmyer et al., 2003]. It is assumed that ROS, including those formed by NADPH oxidase, may be involved in cold signal transmission [Awasthi et al., 2015]. and many other transcription factors, including bZIP (basic leucine zipper), MYB (Myeblastosis), MYC (Myelocytomatosis) [Christmann et al., 2006; Shinozaki, Yamaguchi-Shinozaki, 2007; Jan et al., 2009; Weltmeier et al., 2009; Agarwal, Jha, 2010].

It is known that the accumulation of hydrogen peroxide in cells can lead to changes in the balance between sulfhydryl and disulfide groups of proteins (specifically, protein kinases and transcription regulatory factors) and, as a result, to the transmission of a stress signal to the genome. Thus, histidine kinases are considered as possible sensors of hydrogen peroxide [Szalai et al., 2009]. Oxidative modification of OMTK1, a specific kinase, MAP kinase, by hydrogen peroxide was also shown [Gechev, Hille, 2005]. The beginning of the formation of a persistent response begins with the recognition of the elicitor of the pathogen and the transmission of the signal to the signaling system. A feature of signaling systems is not only the transmission of the signal to the cell genome, but also its significant amplification. Using the example of the cycloadenal system, it is

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shown that the recognition of one elicitor molecule can lead to the synthesis of a million molecules that determine the response of cells [Grechkin, Tarchevskii, 2000, Tarchevskii, 2000, Dmitriev et al., 2015].

Under stress, during the transition of metabolism to a new mode, the body's reserve capabilities are combined thanks to intracellular and intercellular regulation systems. Under the influence of the stimulus, the structure of the receptor molecules changes. These structural changes are transmitted to neighboring molecules: as a result, enzymes are activated and intermediate substances (secondary messengers) are formed, with the help of which the signal is amplified many times over. Perception of the signal is accompanied by its "translation" into biological language. Then the signal is transmitted to the components of intracellular signaling pathways, the final of which are genes. As a result of the change in protein conformation, membrane permeability may increase and the substrate will combine with the enzyme. And in this case, secondary messengers are also formed [Yakovets, 2009].

Localization of signaling molecules near their sites of action is a central principle of cellular signal transduction. Colocalization of multicomponent signaling complexes is carried out through protein scaffolds, which allow to achieve greater specificity than in the case of simple diffusion of the same set of participants in this process [Belousov et al., 2012]. In the case of local actions on plants, a non-specific system of resistance against adverse factors becomes possible thanks to the remote transmission of a signal from the organ that has undergone the action of the stressor to spatially distant organs and parts of plants, which allows them to quickly and efficiently start and coordinate the physiological processes that are involved to the process of increasing resistance [Polevoy et al., 1997; Pyatigin, Opryotov, 2004; Pyatigin, 2008; Vodeneev, 2009].

Signaling in plants is a sequence of events that ensure the transformation of external and internal signals into such functional reactions as dynamic changes in

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enzyme activity, ion transport, or gene expression [Conde et al., 2011; Rout, Panigrahi, 2015; Soni et al., 2015; Sewelam et al., 2016].

To external signals, in particular, stress cells react mainly by perceiving the influence of these factors with certain receptors of the plasma membrane, which recognize the signals and start intracellular information transduction pathways to the nucleus and other organelles, which leads to the emergence of a physiological response. There are three main mechanisms of transmembrane signal transmission: ligand-regulated ion transport, ligand-regulated receptor-enzymes, and ligand-regulated activation of the receptor-G-protein chain [Krutetskaya, Lebedev, 2000]. At the same time, the starting enzymes of signaling systems are activated indirectly or directly. The product of the starter enzyme reaction serves as a messenger whose signal can be transformed by other secondary messengers. Signal sensors are mainly protein kinases and protein phosphatases that phosphorylate or dephosphorylate proteins - factors of transcription regulation that change the expression of the corresponding genes [Tarchevsky, 2002].

The redox-signaling system of plant mitochondria is based on the use of numerous signaling pathways, which provides differential regulation of separate groups of mitochondrial and nuclear redox-sensitive genes in response to changes in the redox homeostasis of mitochondria [Garnyk, 2009].

It was shown that the presence of a signal peptide is a necessary but not sufficient condition for transmembrane translocation. At the same time, there are no arguments to predict the presence of some discrete elements of the primary structure responsible for translocation in the composition of the mature protein. It should be noted that for the successful transfer of the protein through the plasma membrane of *Escherichia coli* K-12, some compatibility of the protein and the signal peptide is necessary [Manuvera, Veiko, 2008].

Currently, the study of retrograde signaling in chloroplasts is developing quite rapidly. Traditionally, retrograde signaling is defined as the process of



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transmitting information from plastids to the nucleus, which leads to changes in the transcription of nuclear genes and the biosynthesis of proteins necessary for the chloroplast. In a broader sense, retrograde signaling is understood as a process in which an external "stimulus" disrupts plastid homeostasis and gives rise to one or a whole series of retrograde signals that exert multilevel control over the transcription of nuclear genes of plastid proteins and ultimately change the functioning of plastids [Chi et al., 2015, Chan et al., 2016]. Retrograde signaling has been found in representatives of all eukaryotic kingdoms: mammals, fungi, and plants, but each kingdom most likely has its own retrograde systems [Van Aken, Pogson, 2017]. Further studies of retrograde signaling using the latest advances in proteomics, metabolomics and high-throughput sequencing of the new generation will eventually allow us to get a fairly detailed idea of how changes in the environment regulate the state of the intracellular compartments of the cell and what role retrograde signals play in this. At the same time, the participation of retrograde signals of plastids in inter- and intracellular signaling pathways, their role in the processes of growth and development of plants is emphasized. Attention is also focused on the fact that quite a few metabolic processes can serve as potential sources of retrograde signals at various stages of plant development or during responses to various stresses [Yuryna, Odintsova, 2019]. Based on the sources of signals, the presence of four retrograde pathways in chloroplasts was initially predicted: tetrapyrrole biosynthesis, chloroplast gene expression, chloroplast redox state, and ROS formation. But recently it was discovered that in addition to "classic" plastid signals, chloroplast metabolites may be involved in the regulation of nuclear gene expression [Kleine, Leister, 2016].

As a result of the research, a prediction was made that plastids, quite likely, send a signal to the nucleus, "notifying" it of its physiological and functional state. Currently, it is already known that a rather complex network of numerous

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retrograde signaling pathways operates in plastids, which interact quite closely with each other signaling systems of the cell [Singh et al., 2015, Sun, Guo, 2016, Page et al., 2017, de Souza et al., 2017]. At the same time, it is shown that one retrograde signal can regulate the expression of genes involved in different signaling pathways, and at the same time, different retrograde signals can regulate the expression of genes of the same signaling pathway. The most studied targets of retrograde signaling are nuclear genes related to photosynthesis. At the same time, retrograde signaling serves as the main pathway for the regulation and coordination of many processes in living organisms, including developmental processes, responses to biotic and abiotic stresses, protein transport, and remodeling of chromatin structure [Xiao et al., 2013].

Environmental stresses affect various physiological processes and cause changes in plant growth and development. To combat stress, plants have developed complex systems of signal cascades that perceive and transmit signals, activate gene expression and provide resistance against stressful stimuli. Various interactions of signaling systems ensure the uniqueness of the formation path of each process, despite the use of a small number of universal mediators for the transmission of information in the cell [Belyavskaya et al., 2018]. A stress-induced oxidative burst provokes the activation of various signaling pathways localized in the plasma membrane. At the same time, the formation of new signaling platforms that ensure the anchoring of signaling molecules can occur [Mynybaeva, 2010]. Plant nets are formed in plants, which initiate the expression of stress-regulated genes and the synthesis of stress proteins, which leads to an increase in resistance against a whole spectrum of stress factors of different nature [Kuznetsov, 2001; Knight, Knight, 2001; Deruyre, Kieber, 2002, Pastory, Foyer, 2002; Shinnusamy et al., 2004; Shinozaki, Yamaguchi-Shinozaki, 2007]. It was found that the redox state of mitochondria is a key point in the operation of the signal transduction system between mitochondria and other compartments of the

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plant cell. At the same time, phosphorylation or dephosphorylation of mitochondrial proteins probably plays a rather important role in redox signaling mechanisms [Subota et al., 2008]. As is known, their signaling systems take an active part in the regulation of growth and virulence of pathogenic microorganisms [Jimenez et al., 2012].

The processes of redox-dependent signal transduction are fast specific and reversible reactions, depend on the concentration of ROS and can occur in different areas of the cell or compartments at a specific time and regulate the activity of proteins key to the vital activity of the cell. The inclusion of ROS in the signal transduction system is undoubtedly related to their ability to migrate or their rapid inclusion in certain signaling systems. Hydrogen peroxide primarily possesses these properties and can activate kinases, MAP kinases [Kovtun et al., 2000]. It was also found that ROS can be involved in the transduction of hormonal signals in plant cells [Andreev et al., 2009, Andosch et al., 2015; Chmielowska-Bak et al., 2015; Hameed et al., 2016; Arena et al., 2017]. The level of ROS largely determines the state of conformation and performance of cellular proteins, having the ability to oxidize important amino acid residues, specifically, cysteine residues. Being in one or another degree of oxidative modification, such proteins act as redox sensors and affect a complexly coordinated network of signaling cascades, and the cell, in turn, gives an adequate response to the effect detected on it. The contribution of the glutathione-dependent system in response to the action of ROS is largely decisive in the fate of the cell [Menshchykova et al., 2006, Sies et al., 2017]. Under the influence of a number of exogenous stressors, nonspecific, i.e., independent of the nature of the stressor, secondary oxidative stress occurs, which is expressed in a significant increase in the level of ROS in the cell. The post-stress increase in ROS has at least two consequences for the vital activity of the cell: oxidative destructive and ( $O_2^{\cdot-}$  and  $H_2O_2$ )-signaling, mobilizing cellular systems to protect against the effects of stress. In addition,

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there are endogenous signaling systems that are not triggered by stress. One of these mechanisms is the light-dependent phytochrome system [Lyubimov et al., 2015].

It is assumed that the change in the fluidity of the plasma membrane is the primary act in the chain of events associated with the perception of high-temperature action, and the change in the conformation of membrane-bound proteins induced by rearrangements of the lipid bilayer activates a cascade of signaling pathways that ensure the transduction of the thermal action signal and further activation of protective mechanisms of cells. This is also confirmed by the fact that the state of the lipid bilayer of the plasma membrane and the synthesis of heat shock proteins are interconnected. But there is still no unequivocal answer to the question of whether there are fundamental differences in the response of cells and high-temperature action in different groups of plants, and how much tissue- and organ-specificity there is in the response of plants to thermal action [Nylova et al., 2017a].

Cellular signaling systems are of decisive importance in shaping the response of living organisms to the stressful impact of the external environment. These systems perceive signal impulses with the help of receptor proteins, transform, amplify and transmit them to the cell's genome, causing reprogramming of gene expression and changes in metabolism. A fairly important role in signal transmission in the cell is played by ROS and, first of all, hydrogen peroxide [Loshchynina, Nikitina, 2016]. It is believed that redox-dependent phosphorylation or dephosphorylation of mitochondrial proteins can play a rather important role in the transmission of regulatory signals in cells of higher plants [Subota et al., 2004, 2010]. Mitochondria are the intersection of almost all cell signaling systems. ROS and nitric oxide are formed in mitochondria [Blokchina, Fagerstedt, 2010]. Mitochondria also modulate intracellular calcium levels [Carraretto et al., 2016].

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It is shown that there is a two-way connection between signaling systems and the genome. On the one hand, enzymes and proteins of signaling systems are coded in the genome, and on the other hand, signaling systems control the genome, expressing some and suppressing other genes. Signaling molecules, as a rule, are distinguished by a fairly fast metabolic turnover and a fairly short life time. Research related to signaling systems is developing quite intensively, but the molecular mechanisms of signaling connections remain largely unexplained [Grechkin, Tarchevskii, 2000, Dmitriev et al., 2015]. To date, it has been established that with an increase in the concentration of ROS, there is a change in the activity of representatives of almost all known classes of effector proteins of signaling systems [Klamt, Kamp, 2011; Ballerstein et al., 2012, Dmitriev et al., 2015, Pradedova et al., 2017, Balasaraswathi et al., 2017; Kohli et al., 2017; Chmielowska-Bak et al., 2018; Georgiadou et al., 2018].

It is assumed, as in redox signaling, the presence of specialized protein redox sensors or redox sites in proteins that selectively contact certain ROS and are modified by them. For example, thiol groups of Cys and thioester groups of Met serve as such sensors. However, there are various types of ROS and the mechanisms of their selective action on these sensors, which do not have a narrow specificity for them, are quite often not understood [Chen, Schopfer, 1999, Wood et al., 2003, Sinkevich et al., 2011, Cherenkevich et al. , 2013]. Thus, as in the case of redox signaling, the mechanism of redox signal selectivity in redox regulation is still unknown [Cherenkevich et al., 2013]. However, the interaction of ROS with proteins is obvious, and this leads to structural modifications of proteins and changes their activity [Jones, 2008]. Therefore, oxidized ROS macromolecules can serve as secondary signaling mediators, which, by triggering certain signaling cascades, mediate the cell's response to stress [Moller et al., 2007].

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Glutathione, as a participant in redox signaling, is involved in the transduction of the salicylic acid signal into the genetic apparatus of plant cells. At the same time, it was established that in wild-type *Arabidopsis* plants, the expression of the PR1 gene increased under the influence of exogenous glutathione. Exogenous salicylic acid induced PR1 gene expression in wild-type *Arabidopsis* plants, while this effect was rather weak in *cad2* mutants deficient in glutathione [Han et al., 2013]. At the same time, it has been proven that one of the key elements in realizing the effects of salicylic acid as a signaling molecule is the NPR1 protein [Pieterse, Van Loon, 2004]. It is formed in plant tissues in small amounts, however, when infected with pathogens or treated with exogenous salicylic acid, its content increases [Mou et al., 2003, An, Mou, 2011].

In the signaling pathway of salicylic acid, glutathione can play a rather important role due to its influence on the redox state of the NPR1 protein [Foyer, Noctor, 2005; Mullineaux et al., 2008]. A shift in the balance of reduced to oxidized glutathione toward reduced glutathione leads to NPR1 activation [Janda, Ruelland, 2015]. Restoration of NPR1 can also occur as a result of salicylic acid-induced "oxal outbreak" and subsequent increase in the level of reducing agents, specifically thioredoxin [Kolupaev, Yastreb, 2013].

It is likely that glutathione is also involved in jasmonic acid signal transduction. In *Arabidopsis cad2* mutants, a low level of expression of VSP2 and PDF1.2a genes, which are controlled by jasmonic acid, was noted. In wild-type *Arabidopsis* plants, the expression of these genes decreased when they were treated with buthionine sulfoximine, an inhibitor of glutathione synthesis. It was also established that exogenous glutathione stimulated the expression of jasmonate-dependent genes LOX3, JAZ10, VSP2 [Han et al., 2013a]. Identifying new components of signaling pathways and studying their interaction with previously identified genes allows finding optimal ways to control growth processes and create new forms of plants resistant to stress [Sklyarova, 2006].

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Thiol groups of proteins are considered as "nanoswitches" or "gray switches" in signal transduction processes [Go, Jones, 2013, Foyer et al., 1997]. In this capacity, they regulate signaling pathways, but do not themselves participate in signal transmission. This function of thiols is defined as "orthogonal control". This concept was presented and analyzed in detail [Go, Jones, 2013]. The signal transmission pathway is modulated by changes in sulfhydryl homeostasis (RSH, RS-SR, RSOH) and in the glutathione mode of specific proteins [Conte, Carroll, 2013]. For example, S-glutathionylation of some kinases, on the contrary, serves as an activating factor [Bylan et al., 2015, Kalinina et al., 2014]. Thus, through the thiol groups of proteins, control over signaling and other intracellular processes is carried out, and at the same time, regulation is usually mediated by a change in the activity of proteins without changing the molecular mechanisms as a whole [Go, Jones, 2013].

Lipoxygenases are common plant enzymes that catalyze the peroxidation of polyunsaturated fatty acids. This reaction is key in the enzymatic cascade of formation of a wide range of regulators of plant metabolism - oxylipins. The action of these biologically active compounds is associated with the formation of protective mechanisms under conditions of biotic and abiotic stresses [Pokotylo et al., 2015]. The signaling role of oxylipins as products of the lipoxygenase cascade has been sufficiently studied. It is known that oxylipins as electrophilic compounds can form conjugates with cellular nucleophiles, including thiols and amino groups of nucleic acids, proteins, and peptides, which was also shown for glutathione [Davoine et al., 2006]. All this can play a rather important role in the protective reactions of plants - reactions of hypersensitivity.

At the same time, the significant contribution of glutathione to the redox regulation of catalytic and signaling proteins is quite obvious. It serves as a substrate in the reactions of protein Cys reduction and glutathione [Go, Jones, 2013, Conte, Carroll, 2013, Kalinina et al., 2014]. Researchers are trying to

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integrate all the reactions of glutathione and thiol groups of proteins into effective models, which can be used in the future for testing the plant organism, which is quite important for the diagnosis of physiological abnormalities and pathological changes. However, such integration, according to some scientists, is hindered by a significant number of various processes involving glutathione. Numerous information about the role of glutathione in detoxification and antioxidant protection shield the main function of glutathione, which is seen in redox regulation, in particular, in redox signaling and redox control [Jones, 2008, Go, Jones, 2013, Pradedova et al., 2017].

The role of glutathione-S-transferase in the regulation of cell signaling through protein-protein interactions with kinases activated by oxidative stress is quite significant. Under physiological conditions, part of the GSTP1-1 isoform is in a bound state with the JNK1 (c-Jun N-terminal kinase) kinase, making it inactive and thus regulating the basal level JNK1 [Adler et al., 1999, Board, Menon, 2013].

The transmembrane circulation of reduced glutathione can be of great importance not only in bacteria that contain glutathione, but also in eukaryotic cells, where the release of reduced glutathione and disruption of ion currents are early events that determine the transition to apoptosis [Ghibelli et al., 1998], and numerous signaling pathways have redox-sensitive sites [Thannickal, Fanburg, 2000; Filomeni et al., 2002]. The specificity of the activation of individual small GTPases, rather, indicates the individual role of these gases as signal indicators of various stresses [Novikova, Moshkov, 2015].

It has been established that the vacuoles of plant cells can perform signaling functions by initiating intracellular signals. The activity of the signaling system of vacuoles is closely related to the physiological state of the root crop. All this makes a certain contribution to the development of the theory of intracellular signal transduction in plants and the participation of vacuoles in this process



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[Rykun et al., 2013]. In cultured cells of *Arabidopsis thaliana* (L.) Heynh. insensitivity to ethylene affects ABA signaling. In normal conditions, exogenous ABA reduces DNA synthesis and subsequent cell proliferation, and in mutant strains it "rescues" from negative consequences associated with the absence of ethylene signaling [Stepanchenko, 2011]. It is rather difficult to attribute the components of the ethylene signal transmission identified up to this point to the same pathway [Moshkov et al., 2009].

It was established that the key components of the signaling cascade triggered by oxidized glutathione, which leads to the increase of divalent calcium ions in macrophages, are tyrosine kinases, tyrosine phosphatases, phosphatidylinositol kinases, small G-proteins of the ras family, the most important enzymes of the phosphoinositide signaling system, phospholipase C and protein kinase C, and as well as elements of the actin cytoskeleton. The obtained scientific results testify to the participation of cyclooxygenases or products of the cyclooxygenase pathway of arachidonic acid oxidation in the signaling cascade, which is triggered by oxidized glutathione or glutoxime and leads to an increase in divalent calcium ions in macrophages [Naumova et al., 2010].

The influence of low-molecular antioxidants and their derivatives on the in planta expression of the auxin-dependent genetic construct Dr5::GUS, which was created on the basis of the promoter of the primary auxin response gene, was studied. Experiments were carried out on 3-4-day-old seedlings of transgenic *Arabidopsis*, with a short exposure of 4 hours. Inducers and inhibitors were added to the solution in which the seedlings were incubated. The effect on the expression of these genetic constructs was determined quantitatively by the activity of GUS in vitro by the fluorometric method. It was shown that in concentrations of 0.1-1 mM thiols (L-cysteine, glutathione) mimicked the effect of auxin on the expression of this genetic construct, increasing its expression 2-4 times. At the

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same time, with the combined use of thiols and auxin, they inhibited its effect on expression by 30-47%. Dithiothreitol alone did not enhance expression, but at a concentration of 1 mM it enhanced the effect of auxin by 44%. L-cysteine and the oxidized form of glutathione at a concentration of 1 mM enhanced the effect of auxin by 17% and 143-154%, respectively. The effect of ascorbate at a concentration of 1 mM was also studied, in which it did not show a noticeable effect on the expression of Dr5::GUS. It was shown that chlormercurybenzoate, which binds thiol groups at a concentration of 0.01 to 1 mM, as well as sodium sulfite, a compound that breaks lysulfide bridges at a concentration of 0.01 to 1 mM, inhibited the effect of auxin by 28-90% and 22-57%, respectively. All these results show that a change in the level of the thiol pool has a noticeable effect on the intracellular signaling of auxins [Vershinkin, Romanov, 2007].

It was shown that oxidized glutathione and its synthetic analogue, the drug glutoxime (the disodium salt of oxidized glutathione with platinum in a nanoconcentration), cause a biphasic increase in the intracellular concentration of  $Ca^{2+}$ ,  $[Ca^{2+}]_i$ , which reflects the mobilization of  $Ca^{2+}$  from thapsigargin-sensitive  $Ca^{2+}$  depots and the subsequent entry of calcium ions from the external environment. In addition, using agents that cause depolymerization of actin filaments, latrunculin B and cytochalasin D, it was shown that short-term 5-minute incubation of cells with these agents causes an increase in both phases of the  $Ca^{2+}$  response induced by glutoxime or oxidized glutathione, while a longer preincubation, for 20 minutes, leads to almost complete suppression of  $Ca^{2+}$ -responses, caused by glutoxime or oxidized glutathione. Using the fluorescent  $Ca^{2+}$  probe Fura-2AM, the effect of the actin filament stabilizer - calyculin A on  $Ca^{2+}$  responses induced by oxidized glutathione or glutoxime in macrophages was investigated. At the same time, it was shown that pre-incubation of cells with 100 nM calyculin A for 10 minutes before the introduction of 200  $\mu$ g/ml of oxidized glutathione or glutoxime causes a significant (by 50%) decrease in the

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phase of mobilization of  $\text{Ca}^{2+}$  from the depot and the subsequent entry of  $\text{Ca}^{2+}$  from the external environment caused by oxidized glutathione or glutoxime. The obtained information indicates the participation of actin filaments in a complex signaling cascade caused by oxidized glutathione or glutoxime, which leads to an increase in  $[\text{Ca}^{2+}]_i$  in macrophages [Kurilova et al., 2010].

The NADPH oxidase signaling system is closely related to the calcium and MAP kinase signaling systems. Direct regulation of various enzymes and transcription factors by hydrogen peroxide is also possible. Oxidation of the SH group of the cysteine residue with the help of  $\text{O}_2$  or hydrogen peroxide can lead to a change in conformation, the formation of disulfide bridges, and the crosslinking of proteins. Usually, such changes transfer enzymes to an inactive state, but there are proteins whose activity increases upon oxidation [Thannickal, Fanburg, 2000]. It has been shown that S-nitrosoglutathione can be extremely important in nitric oxide signaling [Wilson et al., 2008]. It has been established that the activity of NADPH oxidase in plant cells can increase under the influence of various signaling mediators, in particular calcium ions, nitric oxide, salicylic acid and a number of stress phytohormones. All this information is consistent with the idea of ROS signaling not as an autonomous network, but as an integrated signaling pathway that functions in close conjunction with other signaling networks [Mittler et al., 2011]. In addition, the effect of NADPH oxidase activation by the hydrogen peroxide molecules themselves has been established [Bailey-Serres, Chang, 2005]. Experimental confirmation was also obtained, which indicates that the NADPH oxidase homolog RbohD is necessary for the rapid propagation of systemic signals throughout the plant at a speed of approximately 8 cm/min. [Suzuki et al., 2012].

During temperature stress, mitochondrial signaling functions in plant cells, which includes the interaction of the information and energy systems of the cell. It is shown that temperature fluctuations cause changes in the energy activity of

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plant mitochondria. These changes are associated with the rearrangement of lipid composition of mitochondrial membranes, which is most likely a signal of the onset of temperature stress. The redox state of mitochondrial membranes changes and a stress signal is formed. After signal transduction in the nucleus, the expression of stress genes changes and the synthesis of stress proteins occurs, which enter different compartments of the cell, changing its metabolism and resistance to stress. Thus, under stress, a program of selective gene expression is implemented in plant cells, in the regulation of which mitochondria take a direct part. The change in their energy state determines the redox state of the mitochondrial membrane and regulates the expression of nuclear genes. Therefore, nuclear-mitochondrial integration is a very important component in the perception of the signal about the action of the stress factor on the cell and in the implementation of the mechanisms of genetic determination of resistance of plants against stress loads [Voinikov, 2010]. However, the analysis of the available information about the possible nature of the signal under temperature action indicates that, most likely, this function in the plant is performed by the action potential or hydraulic impulse [Tytov, Talanova, 2011].

Currently, the mechanisms of the interdependence of the redox status of cells and intracellular signaling events are being studied quite actively, including the redox regulation of enzymes that perform protein phosphorylation-dephosphorylation on tyrosine residues - protein tyrosine kinases and protein tyrosine phosphatases [Karimova, Petrova, 2016].

As a result of differences in the parameters of the redox state in certain types of cells, redox-active molecules can activate different types of proteins. Thus, a set of molecules that determine the intracellular redox state can be considered as a kind of transducer that regulates the transmission of signals to intracellular effectors. The sensor systems inside the cells are quite sensitive to changes in the parameters of the intracellular redox state and, with the help of

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mediator proteins, form a functional cellular response. At the same time, it is claimed that the functioning of redox messenger systems is a new way of transducing, recording and reading information in cells [Cherenkevich, Martynovych, 2012].

The signaling function of nitric oxide, carried out through direct and mediated interactions, can be realized in individual cells or even in microcompartments, which fully corresponds to the known concept of calcium, hydrogen peroxide and cyclic nucleotides. At the same time, nitric oxide is able to form complexes with metal-containing proteins, cytosolic and mitochondrial aconitase, ascorbate peroxidase, catalase, cytochrome C oxidase [Besson-Bard et al., 2008]. In addition, special attention is paid to covalent post-translational modifications of proteins caused by nitric oxide synergistically with other active forms of nitrogen and oxygen. Protein nitrosylation at specific cysteine (S-nitrosylation) or glutathione (S-glutathione) residues were the first discovered mechanisms of direct action of nitric oxide on the cell [Wilson et al., 2008]. Non-enzymatic molecules, which can act as direct antioxidants, regulate the redox potential of the cell, affecting signaling cascades, the cell cycle, and the synthesis of various metabolites [Wong et al., 2007; Zhang et al., 2010, Foyer, Noctor, 2012, Noctor et al., 2012, Gest et al., 2013].

The analysis of induced ABA in the closing cells of the stomata leads to the conclusion that we are not talking about a signaling chain, but about a signaling network that allows for alternative signal passage, and that, in addition, it is quite important, the possibility of interaction of the ABA signal with the signals of other phytohormones, which could lead to to well-known phenomena in plant physiology: 1) antagonism in the action of various hormones, 2) mutual amplification of their signals, 3) to partial "replacement" of the action of one hormone by another, and 4) to the necessity of the simultaneous action of several hormones to achieve a certain physiological effect. All this is extremely important

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to know for successful manipulation of the hormonal system of plants in genetic engineering to obtain economically valuable genotypes [Hetherington, 2001]. The transient nature of changes in the content of ROS in seedlings and the apoplast of cultivated cells and the superoxide-producing activity of the plasmolemma when the growing temperature changes indicate the possible involvement of NADPH oxidase in the system of cellular signaling reactions [Piotrovsky, 2012].

A model of the effect of brassinosteroids on lipid signaling and metabolism of plant cells under the effects of stress was constructed. According to this model, exogenous brassinosteroids in the extracellular environment bind to specific receptors (BRI1/BAK1 complex) at the level of cell membranes, which leads to the activation of FH-FLS and diacylglycerol kinases. Further, according to our results, diacylglycerol is formed, which is phosphorylated with the formation of phosphatidic acid - a secondary mediator of signaling cascades. Phosphatidic acid, in turn, regulates the activity of BS-dependent genes. The brassinosteroid-initiated increase in calcium levels in the cytosol of cells, which is regulated by the CAX1 antiporter, promotes the activation of antioxidant enzymes of the ascorbate-glutathione cycle: ascorbate peroxidase and glutathione reductase. As a result, an excess of active forms of oxygen, in particular, hydrogen peroxide, which were formed as a result of salt stress, is utilized [Kretinin, 2015].

At present, the role of glutathione in the redox-dependent control pathways of apoptosis induction associated with mitogen-activated protein kinases has not yet been fully elucidated. Due to the fact that glutathione has a significant role in maintaining redox homeostasis in the middle of the cell and has antioxidant properties, it is quite likely that it is also a rather important link in the modulation of MAPK-dependent signaling pathways. Thus, studies on cell models showed that the imbalance of the ratio of reduced to oxidized glutathione with increased ROS generation or impaired recycling of reduced glutathione acted as an activator

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of signaling due to the functioning of MAPK and provoked apoptosis [Cuadrado et al., 2003, Lu et al., 2007].

It has been established that oxidative modifications of macromolecules are a rather important component of redox-mediated intracellular signals that regulate the physiological processes of growth, development, and stress reactions of the plant organism [Ryabovol, 2014]. Biochemical control of signaling systems that regulate phytoimmunity can become one of the promising areas of integrated plant protection [Tyuterev, 2008].

It should also be noted that dozens of different protein kinases and phosphoprotein phosphatases function on the path of signal propagation, which regulate the degree of protein phosphorylation and their activity. In plant organisms, selective phosphorylation of free side OH groups of serine, threonine, or tyrosine residues in proteins is generally quite widespread. It is carried out by protein kinases that use ATP as phosphate donors. Depending on the type of protein, its functional activity either increases or decreases as a result of phosphorylation. The activity of protein kinases, in turn, is regulated with the help of universal intracellular messengers (carriers). Phosphorylation of proteins is reversible, because there are numerous phosphoprotein phosphatases in the cytosol, which separate the phosphate from the phosphorylated enzyme, that is, the protein, as a result of which its activity returns to the initial level. A special role in signaling systems is played by regulation by phosphorylation or dephosphorylation of nuclear factors of transcription regulation, which interact with the promoter regions of genes, allowing or prohibiting their expression. It is assumed that transcription regulatory factors have several phosphorylation sites in amino acid residues capable of attaching orthophosphate under the action of "their" types of protein kinases, which determines the specificity of activation of transcription factors [Dyakov et al., 2001]. It is likely that two-component signaling systems play a key role in the transmission of hormonal signals in plants,

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while signaling systems combined with G-proteins cover a relatively narrow range of hormones, which makes the signal transduction systems of plants close to those of fungi [Shpakov, 2009] . It is believed that G-protein signal perception is ensured with the help of a system of GPCRs (G-proteins coupled receptors) - special protein receptors containing seven transmembrane helical regions and adapted to transmit various signals [Tuteja, Sopory, 2008]. Features of GPCRs of plant cells have been studied so far mainly by methods of biological informatics [Johnston et al., 2007]. The cytosolic domains of GPCRs contact the G-protein. It is known that extracellular loops of GPCRs can be glycosylated [Tuteja, Sopory, 2008].



## CONCLUSIONS

A fairly effective link of metabolic protection is the glutathione system, the activity of which is determined by the content of reduced glutathione in plant cells and is induced by the influence of various factors [Pukacka, Ratajczak, 2006]. It is shown that reduced glutathione and glutathione-dependent enzymes play a rather significant role in the system of antioxidant protection of plants and redox-dependent regulation. In recent years of research, fundamentally new features of the participation of glutathione-dependent enzymes in many life processes of the plant organism have been revealed. Reduced glutathione is a rather important intracellular antioxidant and plays a special role in maintaining the cellular redox status due to participation in thiol-disulfide exchange, which ensures the regulation of a number of plant cell functions, including the regulation of the expression of necessary genes, the activity of certain enzymes and enzymatic systems [Kalinina et al., 2014].

The functional role of glutathione is diverse, because it is contained in almost all plant tissues and participates in many physiological and biochemical processes: it protects the body from active oxygen compounds, restores and isomerizes disulfide bonds, affects the activity of enzymes, protein biosynthesis and the proliferation of nucleic acids, supports the functions of membranes, is a reserve of cysteine, increases the resistance of plant cells to the action of chemical and physical environmental factors, causes resistance to heavy metal cations [Dolgova, SamoiloVA, 2009].

A change in the amount of ROS changes the amount of antioxidants, which is reflected in the balance of reduced to oxidized glutathione, which affects the state of transcription regulatory factors and causes changes in gene expression, including those that provide protective reactions and activation of antioxidant

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systems [Noctor et al., 2002] . Over the past decades, several hundred gene families have been isolated and characterized, the activation of whose expression contributes to the increase of plant resistance to stress. Previously unknown xenobiotic ion transporter proteins were also discovered. Information has appeared on the activation of genes that encode components of the stress signal transmission chain and signaling molecules.

A key role in the detoxification of hydrogen peroxide in the plant cell is played by the ascorbate-glutathione cycle, the mechanism of which is the reduction of hydrogen peroxide to water with the participation of ascorbate and ascorbate peroxidase. In this reaction, ascorbate is oxidized to dehydroascorbate, which is again transformed into a reduced form due to reduced glutathione.

In recent years, new participants in the redox exchange of cell walls have been discovered - glutathione and redox-sensitive proteins. They are thought to be required for redox signaling, which affects the rate of cell growth. In this connection, it is interesting that not only glutathione was found in the cell walls, but also glutaredoxin and thioredoxin, which regulate the restoration of SH-groups of proteins. The ratio of ascorbic acid to dehydroascorbic acid in the apoplast probably plays a rather important role in redox signaling. Thus, numerous scientific studies prove that redox reactions in the apoplast have a rather strong influence on the growth rate of cells, but the specific mechanisms by which this influence is achieved remain unexplored. It is not yet clear how the ratio of pro- and antioxidant activities of the main participants of redox metabolism is regulated, and how redox signals are formed and prescribed in cell walls [Sharova, Medvedev, 2017]. It has been proven that the change in glutathione concentration under stress is a consequence of a sharp disruption of transmembrane membrane flows.

Glutathione is compatible with glutathione-S-transferases - a rather important component of the antioxidant defense system of plants. And that is why

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it is quite important to carry out experimental works on genetic engineering and genome editing, where the genes of enzymes of biological synthesis of glutathione and glutathione-dependent enzymes act as targets. A number of scientific studies have shown that overexpression of glutathione synthetases, glutamate cysteine ligase, and glutathione-S-transferase in transgenic plants increases their stress resistance, which leads to increased productivity [Baymukhametova et al., 2016]. It should also be noted that the genomes of cultivated plants contain a significant number of genes for glutathione biological synthesis enzymes and glutathione-S-transferases, and many of them still remain unstudied. At the same time, some of these genes may turn out to be quite effective targets for increasing plant stress resistance and productivity.

The importance of glutathione in the adaptive reactions of plants can be multifaceted: related to its antioxidant effect, restoration of thiol groups in protein molecules, maintenance of the pool of ascorbic acid; due to participation in the transduction of cellular signals, as well as in the binding of heavy metals and detoxification of xenobiotics [Szalai et al., 2009; Miteva et al., 2010; Han et al., 2013; 2013a].

The interchangeability of antioxidants, their functional interaction with each other, certainly provides stability and reliability to the antioxidant system as a whole. However, these features also create significant problems in studying the contribution of individual components to the functioning of the antioxidant system and its role as a whole in the adaptation of plants against the action of stressors. Another problem in studying the role of antioxidants, especially low-molecular antioxidants (glutathione, etc.) is due to their polyfunctionality, and especially the participation of many of them in cellular signaling [Kolupaev, Yastreb, 2015].

Numerous scientific publications show that reduced glutathione and glutathione-dependent enzymes play a significant role in the system of antioxidant

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protection and redox-dependent regulation. Reduced glutathione is a rather important intracellular antioxidant and plays a special role in maintaining the cellular redox status due to participation in thiol-disulfide exchange, which ensures the regulation of a number of cell functions, including the regulation of gene expression and the activity of certain enzymes and enzymatic systems [Kalinina and etc., 2014].

Currently, the glutathione system is considered as a component of antioxidant protection. It is believed that glutathione binds into a single complex the functioning of the combined redox system, the oxidation of acetate in the Krebs cycle and the oxidation of glucose in the pentose phosphate pathway [Tereshina et al., 2015]. There is a close functional interaction between the components of the signaling, hormonal and stress-protective systems. Its presence greatly complicates the study of each of these systems separately [Kolupaev et al., 2017].

Despite the fact that the number of scientific publications dedicated to the study of the role of glutathione in the vital activity of cells is in the thousands, but still, quite a few molecular mechanisms of glutathione's participation in various metabolic pathways remain largely unknown. And that's why researchers have an increased interest in discovering new properties of this peptide. At the same time, more detailed studies of factors regulating its metabolism, mechanisms of its transport and participation in detoxification of xenobiotics in plants are needed.

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