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RESPONSE OF THE ANTIOXIDANT SYSTEM OF WHEAT SEEDLINGS WITH DIFFERENT GENOTYPES TO EXOGENOUS PROOXIDANTS: THE RELATIONSHIP WITH RESISTANCE TO ABIOTIC STRESSORS

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Oxidative stress is an important component of heat- and drought-induced damage in plants. However, information on the relationship between the resistance of cultivated plants with different genotypes to environmental factors and their ability to maintain a pro-/antioxidant balance remains contradictory. This study aimed to compare the growth responses and adaptation ability of the antioxidant system in different wheat cultivars to oxidative stress agents hydrogen peroxide and iron(II) sulfate. Etiolated seedlings of common winter wheat (Triticum aestivum L.) Antonivka and Tobak (heat- and drought-tolerant), and Avgustina and Doskonala (not resistant to heat and drought) were used for the study. Three-day-old etiolated seedlings were exposed to 50 mM H_2O_2 or 5 mM $FeSO_4$ for one day. It was found that seedlings of Antonivka and Tobak cultivars treated with H_2O_2 or $FeSO_4$ were able to maintain relatively intensive growth, accumulated significantly lower amounts of endogenous hydrogen peroxide and lipid peroxidation products, significantly increased anthocyanin content and had a higher activity of SOD and catalase as compared with non-resistant cultivars. The non-tolerant cultivars' response to stress agents was only to increase proline content with a simultaneous decrease in SOD activity and anthocyanins content. The identified varietal markers of the antioxidant system adaptive strategy can be used to develop new approaches for screening wheat cultivars with cross-resistance to major abiotic stressors.

K e y w o r d s: oxidative stress, antioxidant system, H_2O_2, ferrous sulphate, heat tolerance, drought resistance, Triticum aestivum seedlings.

Exposure of plants to most (if not all) stressors leads to an imbalance between the generation and deactivation of reactive oxygen species (ROS). Even relatively small changes in this balance can lead to the disruption of cellular processes of redox regulation [1, 2], and significant accumulation of ROS in tissues leads to the well-known effect of oxidative stress, which causes lipid peroxidation (LPO) and oxidative damage to proteins and nucleic acids [3].

Oxidative stress is one of the main causes of plant damage during drought and high temperature. During heat stress, this effect is a consequence of the increased fluidity of the chloroplast and mitochondrial membranes, which leads to disruption of electron transport in these organelles [4]. The effects of activation of ROS-generating enzymes (primarily NADPH-oxidase) under heat stress are also known [5, 6]. Drought also has a similar effect on plants. Restriction of carbon dioxide influx into cells due to stomatal closure leads to an over-reduction of the electron transport chain in chloroplasts and increases the likelihood of ROS formation [7]. The significant contribution of mitochondria to the development of oxidative stress under drought conditions is also known. Thus, the carbonylated protein content in the mitochondria of wheat leaf cells under severe drought conditions was an order of magnitude

higher than that in chloroplasts [8]. In this regard, a relationship between plant tolerance to certain environmental stressors and resistance to oxidative stress is expected.

A network of genes ensures the maintenance of redox homeostasis in plants. To date, more than 150 genes have been identified as being involved in the regulation of ROS formation and detoxification [9]. In the last two decades, numerous studies have been conducted on the relationship between the functioning of the antioxidant system and the resistance of plants of different genotypes to various stress factors, including drought and high temperatures [10-15]. However, the multicomponent nature of the antioxidant system and the presence of functional relationships between different antioxidants make it difficult to establish such a relationship [16].

The effectiveness of modeling the relationship between the functioning of the antioxidant system and plant tolerance to abiotic environmental stressors can be improved if such studies are complemented with data on the relationship between plant tolerance to direct oxidative stress agents and tolerance to certain environmental factors. Iseki et al. [17] showed a close relationship between the ability of different rice genotypes to maintain membrane stability and photosynthetic activity under drought and oxidative stress agent methyl viologen. There is also evidence of an interrelationship between the resistance of different clover genotypes to the effects of ozone and drought [18]. When comparing two wheat cultivars differing in drought tolerance, it was shown that the more drought-tolerant cultivar retained more chlorophyll after hydrogen peroxide spraying than the less tolerant cultivar [19]. Sgherri et al. [20], evaluating the response of wheat cultivars to oxidative stress caused by excess copper in the medium, showed that the more drought-resistant cultivar maintained better redox homeostasis than the drought-sensitive cultivar. However, there are still very few studies of this kind, and they have only examined selected indicators of the pro-/antioxidant system. There is also an almost complete lack of comparative studies on the response of etiolated plants of different genotypes to oxidative stress agents. This makes it impossible to draw conclusions about the differences in adaptation strategies to oxidative stress between heat- and drought-tolerant and sensitive genotypes, especially in the early stages of plant development.

This study aimed to compare the growth response and antioxidant system adaptation strategies of etiolated wheat seedlings of four cultivars differing in heat and drought tolerance to the effects of the direct oxidative stress agents hydrogen peroxide and iron(II) sulfate.

Material and Methods

Plant materials and treatment. Ethiolated seedlings of four *Triticum aestivum* L. cultivars differing in heat and drought tolerance were used for the study: Avgustina and Doskonala (non-tolerant) and Antonivka and Tobak (resistant). The resistance of 4-day-old seedlings to high temperatures (heating at 45°C for 4 h) or osmotic stress (effect of 12% PEG 6000) was evaluated in our previous study [15] (Table 1).

Wheat seeds obtained from the National Center for Plant Genetic Resources of Ukraine (Kharkiv) were disinfected in 70% ethanol for 2 min, followed by 15 min in a 2% sodium hypochlorite solution, and then washed 10 times with sterile distilled water. They were germinated on water in Petri dishes in a thermostat at 24°C without light for three days. The seedlings were then incubated for 1 d in the presence of oxidative stress agents.

The oxidative stress agents used were 50 mM H_2O_2 and 5 mM $FeSO_4$. Exogenous iron(II) sulfate is used as an inducer of ROS formation in Fenton and Haber-Weiss reactions and is considered to be a typical activator of oxidative stress in plant cells [21]. The total exposure time of the roots of the intact seedlings to the solutions containing oxidative stress agents was 24 h. In the hydrogen peroxide exposure variant, after 12 h of exposure, the solution was carefully drained and replaced with freshly prepared solution for the next 12 h of incubation. The above concentrations of oxidative stress agent solutions that inhibited root and shoot growth, but not seedling death, were selected in preliminary experiments using the Doskonala and Tobak cultivars as examples (Tables 2 and 3).

After 24 h of exposure to H_2O_2 or $FeSO_4$, seedling shoot and root growth inhibition was estimated using the following formula:

$$I = \frac{(C_2 - C_1) - (E_2 - E_1)}{C_2 - C_1} \cdot 100\%,$$

where I is the growth inhibition (%); C_1 and C_2, E_1 and E_2 are the initial and final values of the fresh weight of the seedling organs in the control and experimental variants, respectively. After 24 h of exposure to the oxidative stress agents, biochemical

Table 1. Resistance of wheat seedlings to heat and osmotic stress (growth inhibition under stress calculated from [15])

Cultivar	Growth inhibition, %			
	Heat stress (45°C, 4 h)		Drought (12% PEG 6000, 4 d)	
	Shoots	Roots	Shoots	Roots
Avgustina	60.0 ± 3.2[a]*	43.7 ± 2.5[b]	52.9 ± 1.0[a]	38.9 ± 1.8[a]
Doskonala	60.9 ± 1.5[a]	62.6 ± 2.1[a]	54.1 ± 2.5[a]	32.0 ± 2.8[b]
Antonivka	15.1 ± 1.5[c]	17.8 ± 2.1[c]	44.7 ± 2.1[b]	35.6 ± 2.2[b]
Tobak	38.6 ± 2.9[b]	11.6 ± 0.4[d]	37.9 ± 0.5[c]	27.7 ± 1.0[c]

Note. *Here and in tables 2 and 3 different letters indicate values with significant differences ($P \leq 0.05$)

Table 2. Concentration dependence of the effect of hydrogen peroxide on the growth of wheat seedlings (roots and shoots growth inhibition, %)

Cultivars	Concentration, mM				
	10	25	50	100	250
			Roots		
Doskonala	42.5 ± 3.9[f]	51.2 ± 2.2[e]	63.4 ± 3.0[d]	71.3 ± 2.4[c]	100 ± 0.0[a]
Tobak	6.7 ± 0.3[h]	13.7 ± 1.4[h]	29.8 ± 2.3[g]	37.5 ± 3.3[f]	86.6 ± 4.7[b]
			Shoots		
Doskonala	7.4 ± 0.9[f]	9.8 ± 0.9[ef]	20.5 ± 2.5[d]	27.4 ± 1.9[c]	86.2 ± 3.9[a]
Tobak	0.0 ± 0.0[g]	3.9 ± 0.7[fg]	4.9 ± 0.7[fg]	15.3 ± 1.6[e]	59.8 ± 6.0[b]

Table 3. Concentration dependence of the effect of $FeSO_4$ on the growth of wheat seedlings (roots and shoots growth inhibition, %)

Cultivars	Concentration, mM		
	2	5	10
		Roots	
Doskonala	67.4 ± 2.9[c]	86.5 ± 3.9[b]	100.0 ± 0.0[a]
Tobak	44.6 ± 3.4[d]	61.4 ± 4.1[c]	80.1 ± 6.5[b]
		Shoots	
Doskonala	20.5 ± 1.2[c]	30.1 ± 2.3[b]	59.0 ± 5.1[a]
Tobak	4.9 ± 0.7[e]	12.8 ± 0.9[d]	27.3 ± 1.7[b]

analyses were performed. To avoid methodological artifacts, only shoots were used for analysis, which, unlike roots, were not exposed to direct contact with H_2O_2 or $FeSO_4$ solution.

Superoxide anion radical (SAR) generation by shoots was estimated using nitroblue tetrazolium (NBT) reduction. Ten identical shoots were placed for 1 h in a tube containing 5 ml of 0.1 M K, Na-phosphate buffer (pH 7.6) with 0.05% NBT, 10 μM EDTA, and 0.1% Triton X-100 [22]. Shoots were gently removed from the incubation solution at the end of exposure. The absorbance of the incubation solution was measured at 530 nm using. To test the specificity of the generation, some samples were supplemented with SOD (50 units/ml). SOD suppresses NBT reduction by at least 90%. The ratio (%) of the absorbance in the experimental and control variants was used to determine the change in SAR generation under stress.

Evaluation of H_2O_2 content. For determination of hydrogen peroxide content, seedling shoots were homogenized in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000 g for 10 min at 2-4°C on an MPW 350R centrifuge (MPW MedInstruments, Poland). The concentration of hydrogen peroxide was determined by the thiocyanate method as described previously [23].

Assessment of lipid peroxidation (LPO) intensity. The rate of LPO in the shoots of seedlings was assessed based on the content of its products reacting with 2-thiobarbituric acid (mainly malondialdehyde, MDA) [23].

Antioxidant enzyme activity measurement. The activities of the antioxidant enzymes superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6), and guaiacol peroxidase (GPX) (EC 1.11.1.7) were measured according to previously described protocols [23]. Seedling samples were homogenized in cold 0.15 M K, Na-phosphate buffer (pH 7.6) supplemented with 0.1 mM EDTA and 1 mM dithiothreitol. The homogenate was centrifuged for 15 min at 8000× g at 2-4°C. The supernatant was then assayed. SOD activity was determined at pH 7.6, using a method based on the ability of the enzyme to compete with NBT for SAR generated by the aerobic interaction between reduced nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate. CAT activity was evaluated based on the amount of H_2O_2 decomposed per unit of time. GPX activity was estimated using guaiacol as the hydrogen donor and H_2O_2 as the substrate, and the absorbance was measured at 470 nm.

Estimation of low-molecular-weight protectors. Proline content was determined using ninhydrin reagent [24]. Proline was extracted from the plant material using distilled water by boiling for 10 min. The extract was filtered, equal volumes of ninhydrin reagent and glacial acetic acid were added to the filtrate, and the samples were boiled in a water bath for 1 h. The absorbance of the colored reaction product was determined at 520 nm using L-proline as the standard.

Total sugar content in seedlings was determined by the Morris-Roe method using anthrone reagent with the modifications described earlier [23]. Sugars were extracted from the plant materials with distilled water by heating them in a boiling water bath for 10 min. The obtained extract was clarified by adding equal volumes (0.3-0.4 ml) of 30% zinc sulfate and 15% yellow blood salt to the reaction tubes and then filtering through a paper filter. The pre-measurement filtrate was diluted several times with distilled water if necessary. The reaction tubes contained 3 ml of anthrone reagent and 1 ml of filtrate. For the control sample, the filtrate was replaced with distilled water. The samples were boiled in a water bath for 7 min and then cooled to room temperature. Absorbance was measured at 610 nm relative to that of the control. D-glucose was used as the standard.

For the determination of total phenolic compounds and flavonoids, the seedlings were homogenized in 80% ethanol, extracted for 20 min and centrifuged at 8000× g (15 min). To analyze phenolic compounds, 0.5 ml supernatant, 8 ml distilled water, and 0.5 ml Folin reagent were added to reaction tubes, stirred, and after 3 min, 1 ml of 10% Na_2CO_3 was added. The absorbance of the reaction mixture was measured at 725 nm after 1 h [25]. The phenolic compound content was expressed as μmol of gallic acid per gram of raw material.

Before determining the content of anthocyanins and flavonoids absorbing in UV-B, the supernatant was acidified with HCl to a final concentration of 1%. The absorbance of the solutions was determined at 530 and 300 nm [26]. The results are expressed as absorbance per fresh weight of the plant material.

Experimental replication and statistical processing. The experiments were repeated three times. Data for each parameter were statistically analyzed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. Data are presented as mean ± SD values. Different letters indicate values with significant differences ($P \leq 0.05$).

Results

Inhibition of seedling growth by oxidative stress agents. Under the influence of 50 mM hydrogen peroxide, the shoot growth inhibition of Avgustina and Doskonala (heat- and drought-intolerant) was approximately 26 and 18%, respectively (Fig. 1). Meanwhile, the growth inhibition of roots that were in direct contact with the oxidative stress agent was stronger (46 and 67%, respectively). At the same time, the inhibition of organ growth in seedlings of the heat- and drought-tolerant cultivars Antonivka and Tobak was manifested to a much lesser extent (within 4-5% for shoots and about 30% for roots).

Similar effects were observed with another oxidative stress agent, 5 mM $FeSO_4$, although the growth-inhibiting effect was generally more severe (Fig. 1). In the non-resistant cultivars Avgustina and

Doskonala, shoot growth was inhibited by 21 and 29%, and root growth by 73 and 81%, respectively. At the same time, in the resistant cultivars Antonivka and Tobak, under the action of ferrous sulfate, shoot growth was inhibited by 11-15%, and root growth by 52-59%.

ROS generation by wheat seedlings under the action of oxidative stress agents. Hydrogen peroxide treatment caused an increase in SAR generation only in seedlings of the Avgustina cultivar; in seedlings of all other cultivars, this index did not change significantly (Fig. 2, *A*). Under the influence of another oxidative stress agent, $FeSO_4$, $O_2^{\bullet-}$ generation was reduced by 23% in the Avgustina cultivar and by approximately 40% in all other cultivars.

The pattern of changes in the hydrogen peroxide content of seedlings after their treatment with oxidative stress agents differed from the changes in $O_2^{\bullet-}$ generation. Thus, in both cultivars that are not resistant to abiotic stresses (Avgustina and Doskonala), the content of endogenous hydrogen peroxide in shoots increased approximately to the same extent (by 25-32%) under the influence of both H_2O_2 and $FeSO_4$ (Fig. 2, *B*). No significant changes in the content of endogenous hydrogen peroxide were observed in resistant cultivars Antonivka and Tobak after the treatment with oxidative stress agents.

The levels of one of the LPO end products, MDA, in the non-tolerant Avgustina and Doskonala cultivars increased by approximately 25% upon treatment with hydrogen peroxide; this effect was even more significant upon exposure to $FeSO_4$ (Fig. 2, *C*). At the same time, the resistant cultivars Antonivka and Tobak did not show a significant increase in LPO in shoots.

Activity of antioxidant enzymes in wheat seedlings. The basal values of SOD activity in the seedlings of different cultivars differed. The highest enzyme activity was recorded in the Antonivka and Tobak cultivars; slightly lower values were observed in the Avgustina cultivar, and the lowest in the Doskonala cultivar (Fig. 3, *A*). Hydrogen peroxide treatment caused a significant decrease in SOD activity in the non-tolerant cultivars Avgustina and Doskonala. Under the influence of $FeSO_4$, these cultivars also showed a decrease in enzyme activity; it

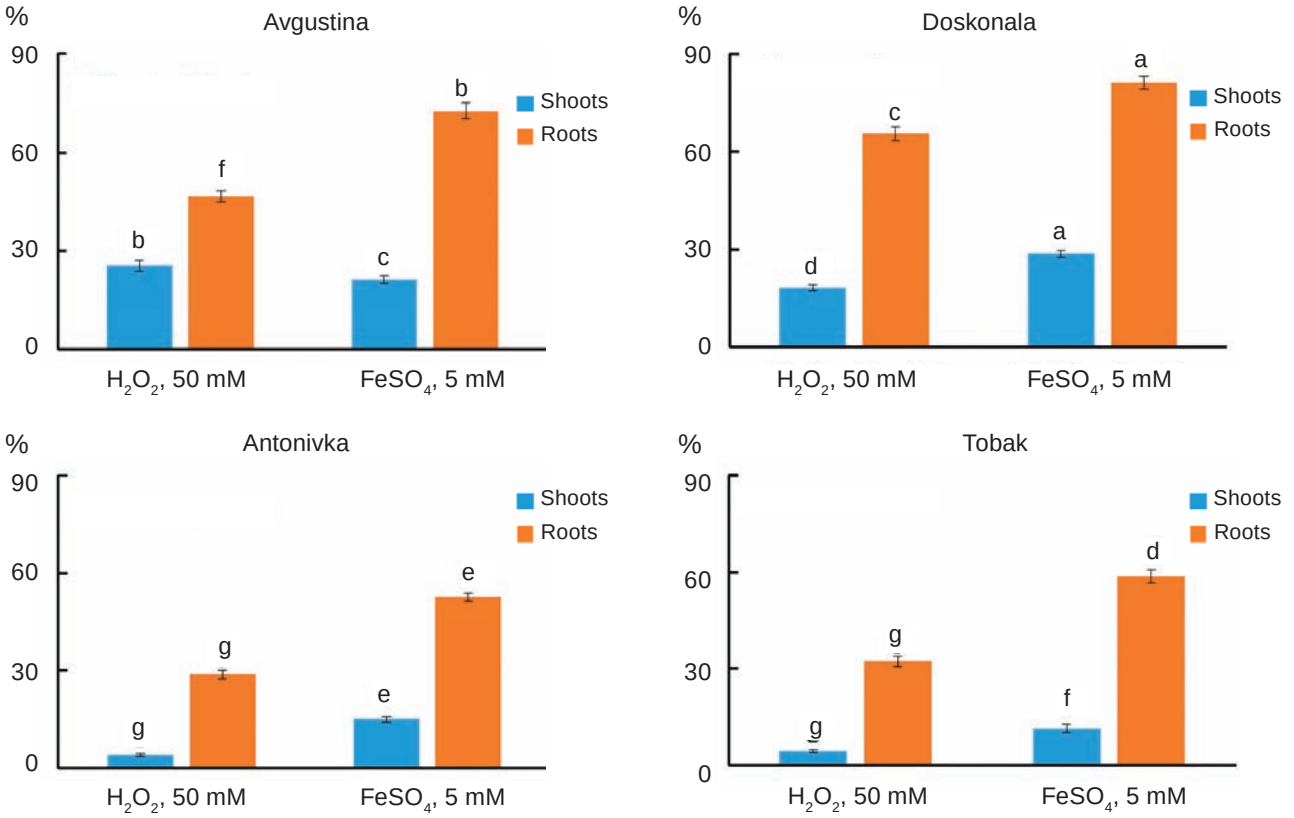

Fig. 1. Growth inhibition (%) of shoots and roots of wheat seedlings under H_2O_2 and $FeSO_4$ action. The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$

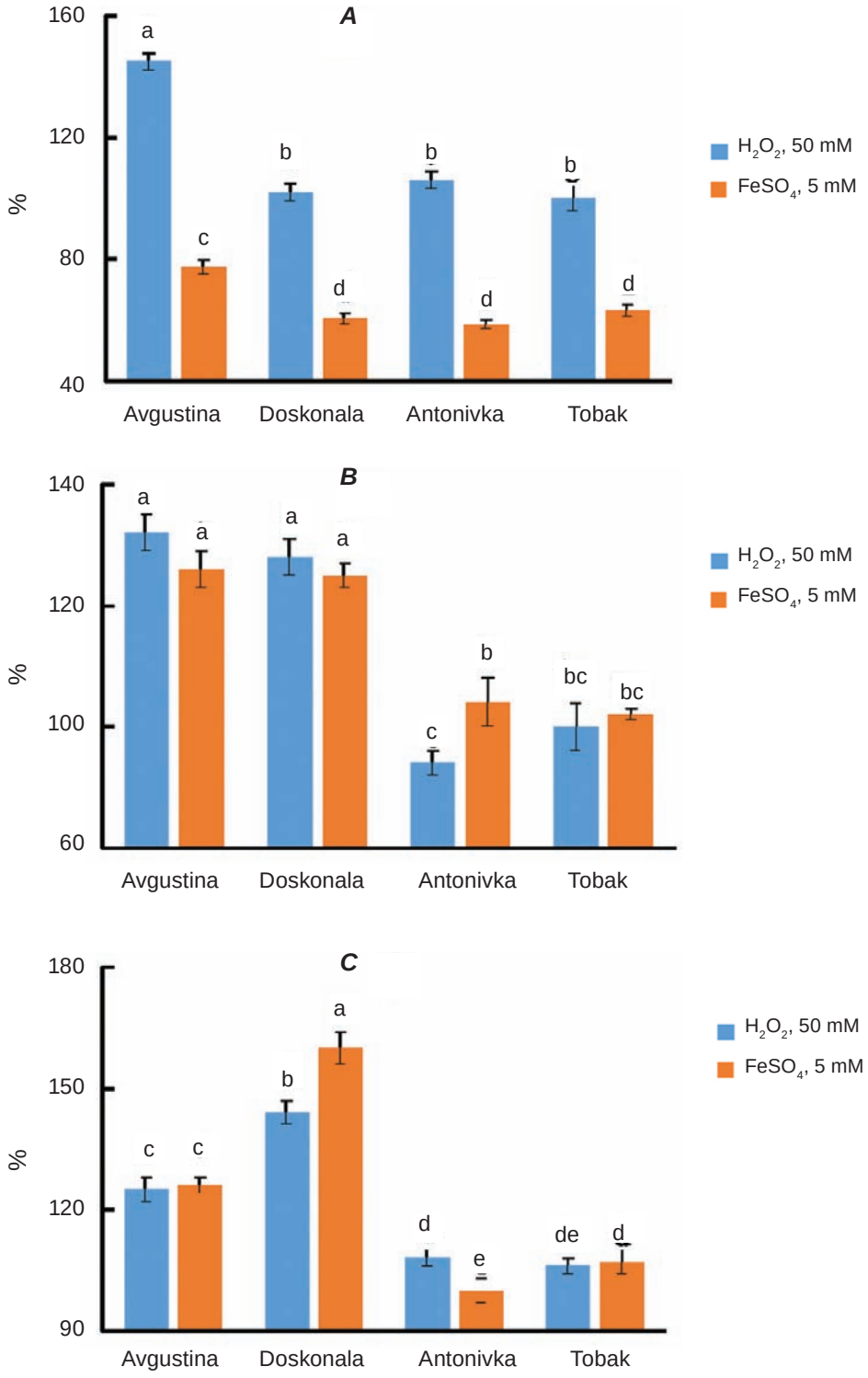

*Fig. 2. Generation (% to control) of superoxide anion radical (**A**), content of hydrogen peroxide (**B**) and malondialdehyde (**C**) in shoots of wheat seedlings under the action of oxidative stress agents. The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

was significant at $P \leq 0.05$ in the Doskonala cultivar, while it was at the trend level in the Avgustina cultivar. A different pattern was observed in cultivars resistant to abiotic stress. Thus, only a slight decrease in SOD activity was observed in the Antonivka cultivar after H_2O_2 treatment, whereas it remained stable in the Tobak cultivar (Fig. 3, *A*). In the $FeSO_4$ variant, there was an increase in enzyme activity in the Antonivka cultivar, whereas there was no change in the Tobak cultivar.

Catalase activity in the control variants differed slightly among cultivars, regardless of their tolerance to stress factors (Fig. 3, *B*). Enzyme activity did not change in the presence of either oxidative stress agent in the non-resistant cultivars Avgustina and Doskonala. In the resistant cultivars, Antonivka and Tobak, it was increased by both hydrogen peroxide and ferrous sulfate treatments (Fig. 3, *B*).

The activity of guaiacol peroxidase in the non-tolerant cultivars Avgustina and Doskonala was not

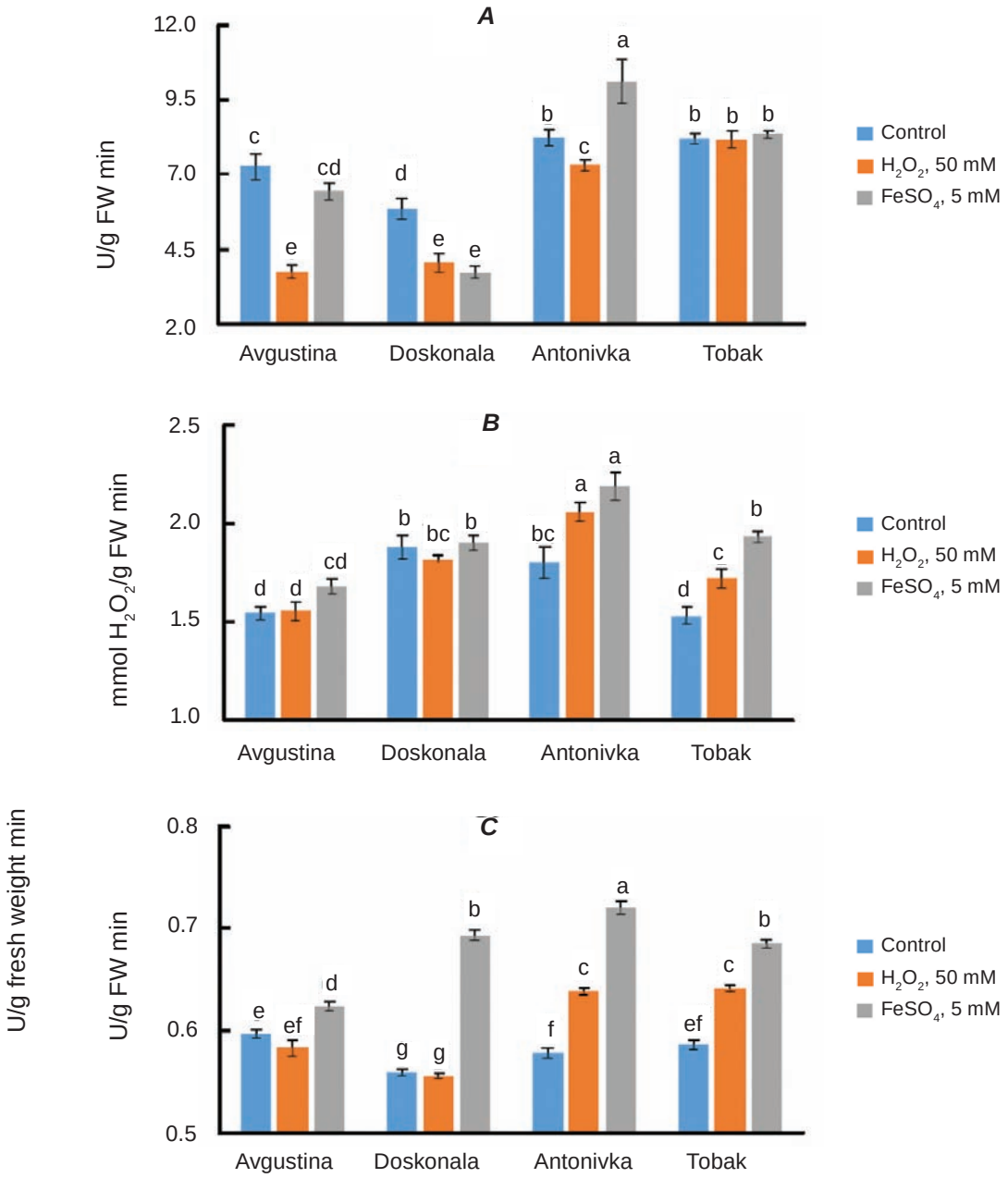

*Fig. 3. Activity of SOD (**A**), catalase (**B**), and guaiacol peroxidase (**C**) in shoots of wheat seedlings under the action of oxidative stress agents. The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

altered by H_2O_2 treatment, but was increased by $FeSO_4$ (Fig. 3, *C*). In resistant cultivars Antonivka and Tobak, increased enzymatic activity was observed when exposed to both oxidative stress agents.

Low-molecular-weight multifunctional compounds in wheat seedlings. The constitutive proline content of the Avgustina and Doskonala cultivars was higher than that of the Antonivka and Tobak cultivars (Fig. 4, *A*). Under the influence of both oxidative stress agents, a significant increase in proline content occurred only in the non-resistant cultivars Avgustina and Doskonala, while in the resistant cultivars Antonivka and Tobak it did not change significantly.

Sugar content did not change significantly in response to hydrogen peroxide in the non-tolerant Avgustina and Doskonala cultivars, but increased when treated with $FeSO_4$ (Fig. 4, *B*). In resistant cultivars Antonivka and Tobak, the sugar content increased significantly after exposure to hydrogen peroxide. In response to ferrous sulfate treatment, the sugars increased significantly in seedlings of the Tobak cultivar and less markedly in the Antonivka cultivar.

The total phenolic compounds content was higher in Avgustina cultivar compared to other cultivars studied (Fig. 5, *A*). No clear relationship between the pattern of change in this index and the resistance of cultivars to oxidative stress agents could be found. Thus, in response to hydrogen peroxide treatment, a significant increase in the content of phenolic compounds was found in the non-tolerant cultivar Doskonala and in the resistant cultivar Antonivka, while no noticeable changes were found in the other two cultivars. Upon $FeSO_4$ treatment, the content of phenolic compounds decreased in the Avgustina cultivar and did not change in the other cultivars.

The constitutive content of anthocyanins in wheat seedlings varied independently of varietal resistance to oxidative stress (Fig. 5, *B*). Under the influence of H_2O_2 and $FeSO_4$, the anthocyanin con-

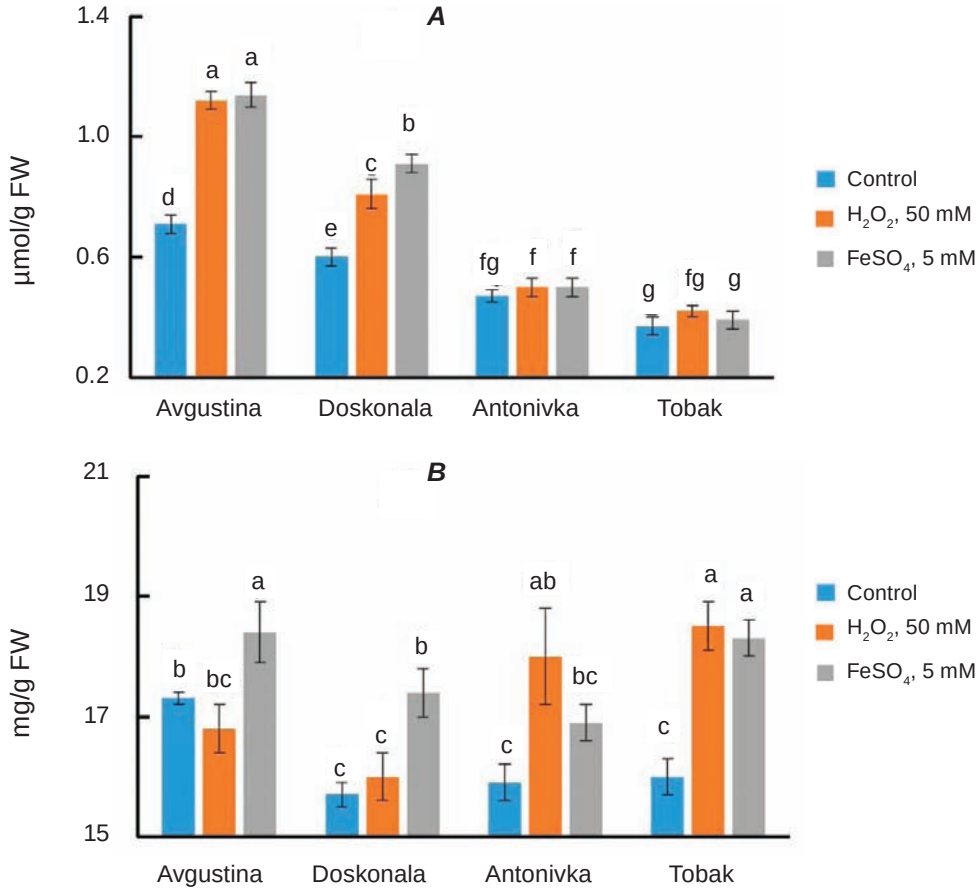

*Fig. 4. The content of proline (**A**) and sugars (**B**) in shoots of wheat seedlings under the action of oxidative stress agents. The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

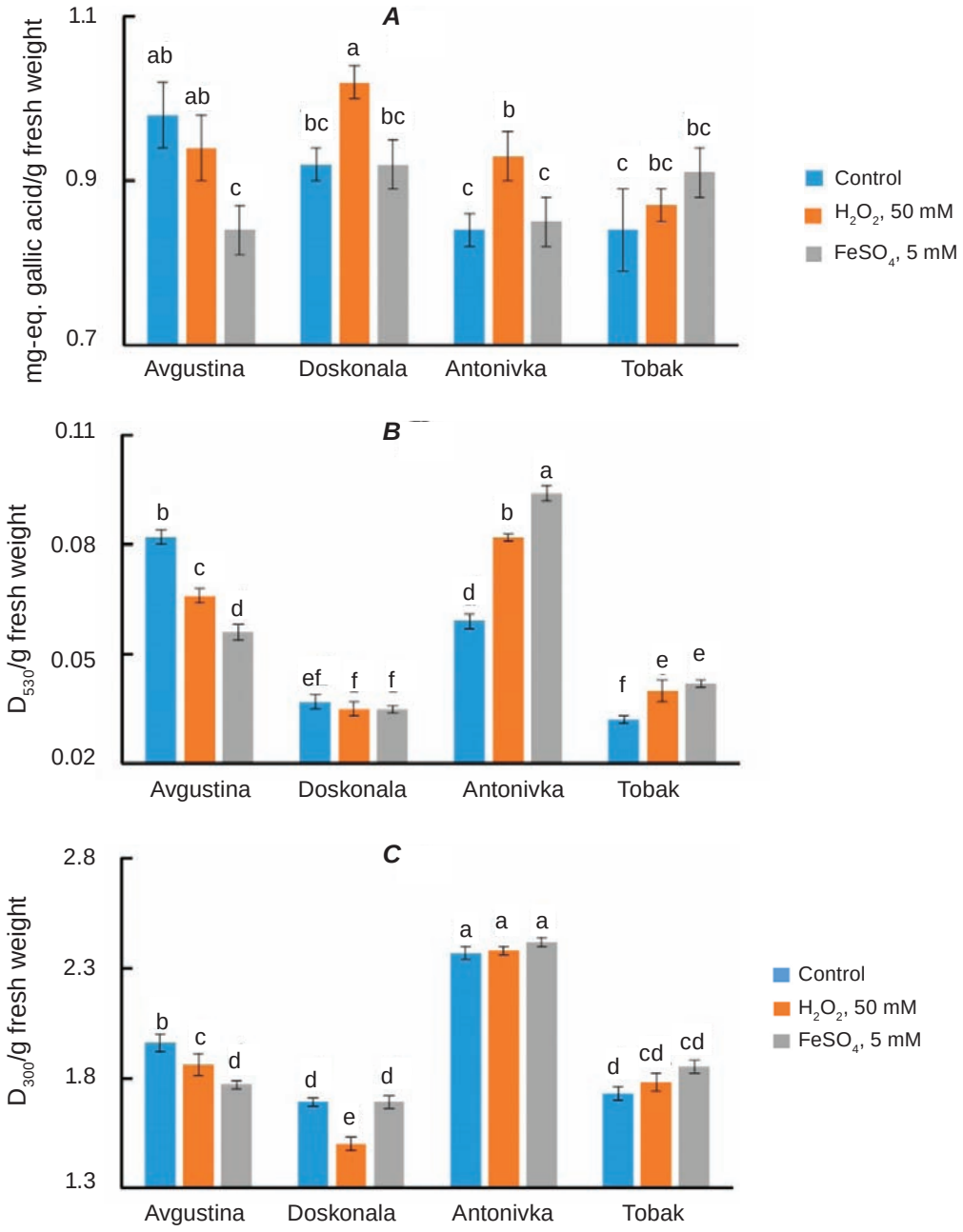

*Fig. 5. The total content of phenolic compounds (**A**), anthocyanins (**B**), and UV-B absorbing flavonoids (**C**) in the shoots of wheat seedlings under the action of oxidative stress agents. The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

tent of the Avgustina cultivar decreased significantly, while it did not change in another non-tolerant cultivar (Doskonala). In the resistant cultivars Antonivka and Tobak, the anthocyanin content increased in response to treatment with both oxidative stress agents, with high absolute values of this index being characteristic of the Antonivka cultivar.

The constitutive content of flavonoids absorbing in the UV-B region was highest in the resistant cultivar Antonivka (Fig. 5, *C*). Treatment of seedlings with hydrogen peroxide resulted in a decrease in the susceptible Avgustina and Doskonala cultivars and no significant change in the resistant Antonivka and Tobak cultivars. In the presence of ferrous sulfate, the content of flavonoid compounds decreased in the non-tolerant cultivar Avgustina and did not change in the other cultivars studied.

Discussion

The results obtained in the present study indicate a relationship between the resistance of wheat plants of different genotypes to abiotic stresses and direct agents of oxidative stress. Thus, the growth-inhibitory effects of two different oxidative stress agents (H_2O_2 and $FeSO_4$) on wheat cultivars Antonivka and Tobak, which are characterized by high heat and osmotolerance at the seedling stage [15] (Table 1), were much weaker than those of the non-tolerant cultivars Avgustina and Doskonala (Fig. 1).

The effect of exogenous oxidative stress agents on the generation of superoxide anion radicals by the shoots of wheat seedlings has been equivocal. Thus, under the influence of hydrogen peroxide, $O_2^{\bullet-}$ generation was enhanced only in the non-tolerant cultivar, Avgustina (Fig. 2, *A*, 6), which showed the strongest inhibition of shoot growth after a 24-h incubation on medium supplemented with H_2O_2 (Fig. 1). One of the reasons for changes in SAR generation by plant tissues under the influence of hydrogen peroxide may be modification of SOD activity. Thus, it is well known that one of the major forms of this enzyme, Cu,Zn-SOD, is strongly inhibited *in vitro* by hydrogen peroxide [27], which may lead to increased levels of SAR. At the same time, in response to the action of exogenous hydrogen peroxide *in vivo*, both inhibition of SOD activity and its increase have been reported on different objects. For example, an increase in SOD activity was observed in isolated wheat coleoptiles when treated with 1 mM hydrogen peroxide; this effect was accompanied by a decrease in $O_2^{\bullet-}$ generation [28]. On the other hand, a decrease in SOD activity under the influence of hydrogen peroxide was recorded in the leaves of adult wheat, and it was more significant in the less drought-resistant cultivar than in the drought-resistant cultivar [19]. It is possible that, depending on the changes in the endogenous content of hydrogen peroxide under the influence of exogenous treatments as well as the time of exposure, there may be both suppression of SOD (e.g., as a result of the direct action of hydrogen peroxide on the enzyme molecules) and induction of expression of genes encoding different molecular forms of this enzyme due to the action of hydrogen peroxide as a signaling molecule [29]. Under the conditions of our experiments, SOD inhibition seems to be a probable cause of the enhanced SAR generation under the influence of hydrogen peroxide in the Avgustina cultivar. Thus, the most pronounced decrease in SOD activity after 24 h of treatment of seedlings with exogenous H_2O_2 was observed in the Avgustina cultivar, whereas in the other cultivars, SOD activity decreased insignificantly during such treatment, and in the resistant cultivar Tobak, it did not change at all (Fig. 3, *A*). Other reasons for changes in SAR generation under the influence of hydrogen peroxide may be modulation of the activity of ROS-generating enzymes, NADPH oxidase and extracellular peroxidase. Notably, NADPH oxidase can be activated by direct agents of oxidative stress, such as paraquat [30]. On the other hand, hydrogen peroxide has been shown to inhibit extracellular peroxidase activity [28]. The degree of manifestation of these or those processes probably depends on many factors, including the species and varietal characteristics of the plants.

The change in $O_2^{\bullet-}$ generation by wheat seedlings depended on the nature of the agent causing oxidative stress. Thus, while treatment with hydrogen peroxide caused an increase in SAR in the Avgustina cultivar and did not affect this index in the other three cultivars, treatment with ferrous sulfate led to a decrease in $O_2^{\bullet-}$ formation in all four cultivars studied (Fig. 2, *A*). One of the reasons for this effect may be an increase or maintenance of SOD activity at a sufficiently high level. Thus, in Antonivka cultivar, there was an increase in SOD activity during iron sulfate treatment, in Tobak and Avgustina, cultivars the enzyme activity remained stable, and only in Doskonala cultivar it decreased (Fig. 3, *A*). It should be noted that in the work of Yang et al., the long-term action of moderately toxic doses of iron and copper in the roots of plants also resulted in a decrease in the generation of superoxide radicals, despite the increase in hydrogen peroxide and MDA content. This study also showed an increase in the activity of extracellular forms of SOD in the presence of excess iron. Another possible reason for the decrease in SAR generation in the presence of iron is the inhibition of NADPH oxidase [31].

Thus, the modulation of $O_2^{\bullet-}$ generation by exogenous oxidative stress agents and the activity of enzymes on which this process depends can be highly complex and vary depending on the type of oxidative stress agent (hydrogen peroxide or Fe^{2+} ions) and cultivar characteristics.

The effect of oxidative stress agents on the levels of a relatively stable ROS hydrogen peroxide and MDA, the end product of LPO, in shoots of seedlings of different wheat cultivars was more

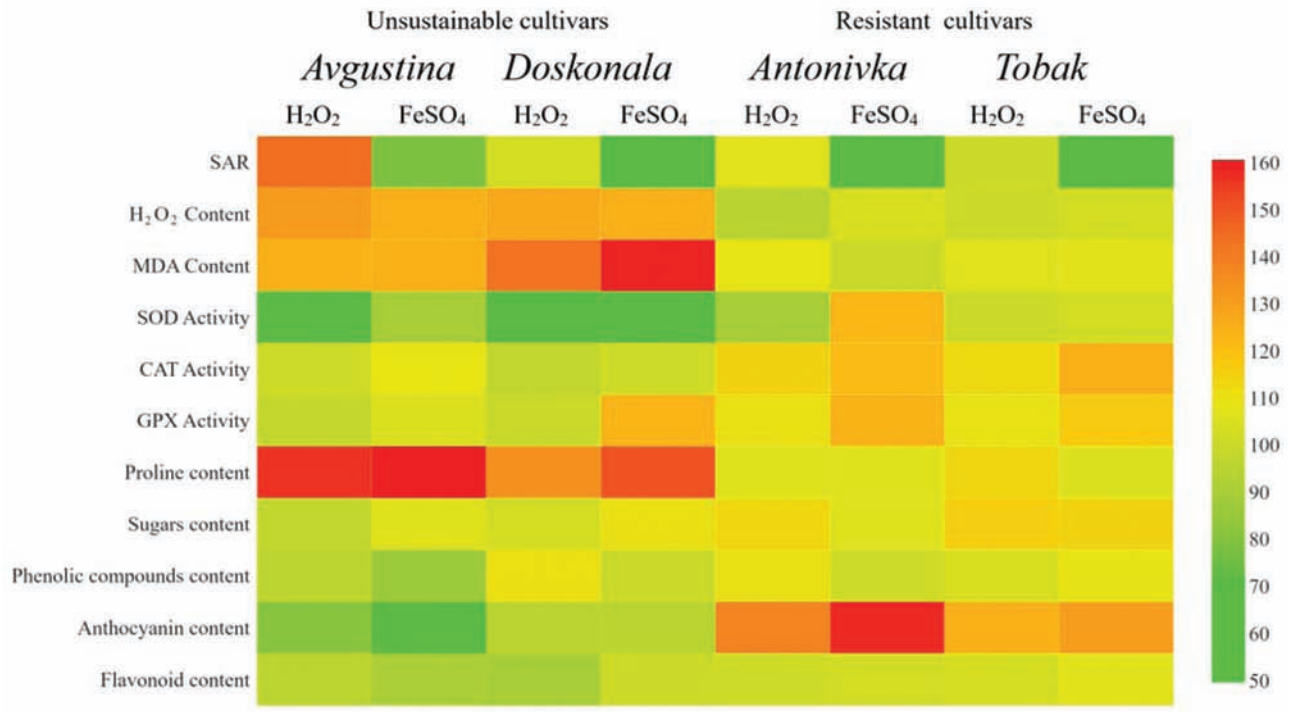

Fig. 6. Heat map of changes in biochemical parameters under the action of oxidative stress agents (50 mM H_2O_2 and 5 mM $FeSO_4$) in wheat seedlings of different cultivars. During building, all indicators were expressed as percentages of the control

unambiguous. Heat- and drought-tolerant cultivars Antonivka and Tobak were distinguished by the absence of a significant increase in the content in shoots of the main markers of oxidative stress hydrogen peroxide and MDA when seedlings were treated with exogenous H_2O_2 and $FeSO_4$, while in heat- and dehydration-intolerant cultivars Avgustina and Doskonala the content of these compounds increased approximately 1.3-1.6 times (Fig. 2, 6). Increased hydrogen peroxide accumulation and LPO process under the influence of high doses of iron, acting as an agent of oxidative stress, has been recorded in plants of different taxonomic groups [31-33]. Both inhibition and enhancement of the activity of key antioxidant enzymes (catalase, non-specific peroxidase, ascorbate peroxidase, and other enzymes of the ascorbate-glutathione cycle) have been reported when plants are exposed to excess iron [31, 33].

Our experiments showed that the effect of oxidative stress agents on the activity of hydrogen peroxide-neutralizing antioxidant enzymes depends on cultivar resistance. Thus, catalase activity increased in response to H_2O_2 and $FeSO_4$ in the resistant to oxidative stress agents and abiotic factors cultivars Antonivka and Tobak, while it did not change in the non-resistant cultivars (Fig. 3, *B*). The important role of catalase in plant resistance to environmental stresses, particularly thermal and osmotic stresses, has been reported in a number of works [11, 15, 34]. Its importance in heat tolerance is clearly demonstrated by the increased heat tolerance of Arabidopsis plants overexpressing the *BoCAT2* gene, transformed from broccoli plants [35].

Guaiacol peroxidase activity in resistant cultivars Antonivka and Tobak was also increased by oxidative stress agents (Fig. 3, *C*). However, when iron ions were used as an oxidative stress agent, an increase in the activity of this enzyme was also observed in the non-tolerant cultivars Avgustina and Doskonala. Other studies have also shown an increase in the activity of various peroxidases in plants under the influence of iron [33]. Apparently, the action of Fe^{2+} as an oxidative stress agent is more complex compared to the effects of hydrogen peroxide due to its influence as a micronutrient (at low tissue accumulation) and its toxic effect as a heavy metal (at high concentrations).

In general, cultivars that were resistant to both environmental stresses and exogenous agents of oxidative stress showed a greater increase in the activity

of antioxidant enzymes (catalase and guaiacol peroxidase) and retention of SOD activity when exposed to exogenous hydrogen peroxide or Fe^{2+} (Fig. 3). It is noteworthy that under the action of the osmotic stress agent PEG 6000, the activity of these enzymes was higher in resistant wheat cultivars than in non-tolerant ones [23]. The relationship between drought tolerance and resistance to oxidative stress agents is also indicated by the data on the increased activity of antioxidant enzymes (catalase and guaiacol peroxidase) in oat plants that were exposed to artificial drought before exposure to herbicide-induced oxidative stress (fenoxaprop-P-ethyl treatment) [36].

The character of changes in the content of the main multifunctional protective compounds in response to the action of H_2O_2 and $FeSO_4$ differed significantly in cultivars with different heat and drought resistance. Thus, only the non-tolerant cultivars Avgustina and Doskonala showed increased proline levels when treated with these oxidative stress agents (Fig. 4, *A*, 6). The same cultivars were characterized by a significant increase in oxidative stress markers upon treatment with hydrogen peroxide and ferrous sulfate, as mentioned above. Notably, a very high correlation between the accumulation of proline and LPO product MDA under heat stress (r = 0.91) was previously found in etiolated seedlings of seven wheat cultivars (including those studied in the present work) [15]. Approximately the same high positive correlations between proline and MDA levels were also found in leaves of adult wheat plants of different cultivars under heat stress and drought conditions [37]. In this regard, it can be assumed that proline accumulation may correlate not with the resistance of seedlings to the stress factor, but, on the contrary, with the manifestation of oxidative damage. For a clear interpretation of such phenomena, however, there are still insufficient grounds. It has been reported that high proline content in plant cells can lead to the development of pro-oxidative processes in mitochondria and even induce programmed cell death [38, 39]. On the other hand, numerous data on the antioxidant, membrane-protective, and chaperone effects of proline [40] do not allow us to consider its accumulation as a clear cause of the development of oxidative stress. The question remains under which conditions the sign of the proline action can be reversed.

Sugar content was not significantly altered by hydrogen peroxide treatment in cultivars with low resistance. At the same time, this index increased significantly in resistant cultivars (Fig. 4, *B*, 6). Treatment with ferrous sulfate did not show similar clear differences between cultivars: an increase in sugars occurred in all cultivars, regardless of their resistance to other factors. It can be hypothesized that accumulating sugars is a specific response to excess iron stress. It is known that the accumulation of sugars in cells reduces ionic activity, which prevents the development of toxic effects [41, 42]. In general, sugars are now considered to be compounds that exhibit direct and indirect antioxidant effects [43]. In particular, disaccharides and fructans have a high free radical scavenging capacity, and monosaccharides may induce an increase in antioxidant enzyme activity through an as yet unknown mechanism [44].

Total phenolic compounds did not change significantly under hydrogen peroxide or ferrous sulfate stress. At the same time, some cultivars had an increase in their content upon exposure to hydrogen peroxide (Fig. 5, *A*, 6). More clear patterns can be seen in the character of changes in anthocyanin content in the studied cultivars under oxidative stress: in the non-tolerant cultivar Avgustina it was the highest under normal conditions, but decreased in response to both agents of oxidative stress (Fig. 5, *B*, 6). In the non-tolerant cultivar Doskonala, this index was low in the control and did not change under stress. At the same time, the resistant cultivars were characterized by a significant increase in the amount of anthocyanins when exposed to hydrogen peroxide or ferrous sulfate. The levels of another group of secondary metabolites, UV-B-absorbing flavonoids, varied among cultivars, but decreased in non-resistant cultivars when exposed to oxidative stress agents and remained stable in resistant cultivars (Fig. 5, *C*, 6). It should be noted that the ability to increase or maintain at a high level the content of various polyphenolic compounds under stress conditions has been recorded for plants of different taxonomic groups. For example, a comparison of sweet potato (*Ipomea batatas* L.) cultivars differing in drought tolerance revealed the ability of a resistant cultivar to maintain high levels of total secondary metabolites under dehydration conditions [45]. Similar trends have been observed in woody species, for example, a more drought-tolerant olive (*Olea europaea* L.) cultivar accumulated higher levels of phenolic compounds under stress conditions compared to an intolerant cultivar [46].

Conclusions. Thus, significant differences in response to oxidative stress agents were found in wheat

cultivars with different heat and drought tolerance. Seedlings of highly resistant cultivars Antonivka and Tobak were characterized by the ability to maintain growth under the action of oxidative stress agents (Fig. 1) and did not show obvious signs of oxidative damage development as determined by endogenous hydrogen peroxide and MDA content (Fig. 6). At the same time, cultivars with low heat/drought tolerance, Avgustina and Doskonala, showed strong growth inhibition and significant accumulation of oxidative stress markers under the action of H_2O_2 and $FeSO_4$ (Figs. 1, 6). Significant differences in the response of components of the antioxidant system in resistant and non-resistant cultivars to the action of exogenous agents of oxidative stress were also revealed. The resistant cultivars in response to H_2O_2 or $FeSO_4$ treatment were characterized by a significant increase in anthocyanin content, catalase activity and maintenance of SOD activity at a high level (Fig. 6). At the same time, in the non-tolerant cultivars, the expressive response to stress was only an increase in proline content with a simultaneous decrease in SOD activity and the content of anthocyanins and other secondary metabolites (Fig. 6).

In the present study, the response of different wheat genotypes to oxidative stress agents was for the first time extensively investigated in etiolated seedlings instead of green plants. The detected differences in response to the action of such agents in wheat cultivars differing in resistance to adverse environmental effects can be used for express screening of genotypes with high cross-resistance to abiotic stress factors.

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РЕАКЦІЯ АНТИОКСИДАНТНОЇ СИСТЕМИ ПРОРОСТКІВ ПШЕНИЦІ РІЗНИХ ГЕНОТИПІВ НА ДІЮ ЕКЗОГЕННИХ ПРООКСИДАНТІВ: ЗВ'ЯЗОК ЗІ СТІЙКІСТЮ ДО АБІОТИЧНИХ СТРЕСОРІВ

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Оксидативний стрес є важливим компонентом у розвитку пошкодженнь рослин, спричиненого спекою та посухою. Однак інформація про взаємозв'язок між стійкістю культурних рослин з різними генотипами до чинників навколишнього середовища та їхньою здатністю підтримувати про/антиоксидантний баланс залишається суперечливою. Метою цього дослідження було порівняти ростові реакції та адаптаційні можливості антиоксидантної системи у різних сортів пшениці до дії пероксиду водню та сульфату заліза(II). Для дослідження використовували етіольовані проростки пшениці м'якої озимої (*Triticum aestivum* L.) сортів Антонівка і Тобак (жаро- та посухостійкі), а також Августина і Досконала (нестійкі до спеки та посухи). Триденні етіольовані проростки піддавали дії агентів окислювального стресу 50 мМ H_2O_2 або 5 мМ $FeSO_4$ протягом однієї доби. Встановлено, що проростки сортів Антонівка і Тобак, оброблені H_2O_2 або $FeSO_4$, здатні підтримувати відносно інтенсивний ріст, накопичують значно меншу кількість ендогенного пероксиду водню та продукту пероксидного окислення ліпідів малонового діальдегіду, значно підвищують вміст

антоціанів та мають вищу активність антиоксидантних ензимів (супероксиддисмутази і каталази) порівняно з нестійкими сортами. Реакція нестійких сортів на дію стресових агентів полягала лише у збільшенні вмісту проліну з одночасним зниженням активності СОД та вмісту антоціанів. Виявлені сортові маркери адаптивної стратегії антиоксидантної системи можуть бути використані для розробки нових підходів до скринінгу сортів пшениці з перехресною стійкістю до основних абіотичних стресорів.

Ключові слова: оксидативний стрес, антиоксидантна система, активні форми кисню, сульфат заліза(II), теплостійкість, посухостійкість, *Triticum aestivum*.

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