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Influence of Components of the Pro-Oxidant-Antioxidant System on the Immune Response Levels of Cereal Crop Varieties

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ABSTRACT

Objective of the research: To establish the relationship between the varietal immunoresistance of plants and quantitative changes in pro-oxidant-antioxidant balance parameters. The subject of the research is the effect of prooxidants and antioxidants on changing the resistance of a plant variety to the invasion of pathogens. Methodology: The following plants were used in the experiment: *Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf., Panicum miliaceum L.* The level of superoxide anion radical, TBA-active products, activity of superoxide dismutase, catalase, cytochrome oxidase, concentration of ascorbic acid, and glutathione were determined. The results of the research show that the grains of highly resistant varieties have significantly higher activity of superoxide dismutase, catalase, cytochrome oxidase, higher concentration of ascorbic acid, and glutathione, lower content of superoxide anion radical and TBA-active products. Practical consequences: expanding the sown areas of highly disease-resistant cereal varieties and replacing one variety with another in limited sown areas. Value/originality: The study of the biochemical bases of the stability of the variety provides a basis for obtaining new varieties of plants and improving existing varieties.

KEYWORDS: Plants, biological research, biochemical analysis, enzymes, vitamins.

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Influencia de los componentes del sistema Pro-oxidante-Antioxidante en los niveles de respuesta inmune en variedades de cultivos de cereales

RESUMEN

Objetivo de la investigación: Establecer la relación entre la inmunorresistencia varietal de las plantas y los cambios cuantitativos en los parámetros del balance pro-oxidante-antioxidante. El tema de la investigación es el efecto de los prooxidantes y antioxidantes en el cambio de la resistencia de una variedad vegetal a la invasión de patógenos. Metodología: en el experimento se utilizaron las siguientes plantas: Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf., Panicum miliaceum L. Se determinó el nivel de radical anión superóxido, productos activos de TBA, actividad de superóxido dismutasa, catalasa, citocromo oxidasa, concentración de ácido ascórbico y glutatión. Los resultados de la investigación muestran que los granos de variedades altamente resistentes tienen una actividad significativamente mayor de superóxido dismutasa, catalasa, citocromo oxidasa, mayor concentración de ácido ascórbico y glutatión, menor contenido de radical anión superóxido y productos activos de TBA. Consecuencias prácticas: ampliar las áreas de siembra de variedades de cereales altamente resistentes a enfermedades y reemplazar una variedad por otra en áreas limitadas de siembra. Valor/originalidad: El estudio de las bases bioquímicas de la estabilidad de la variedad proporciona una base para la obtención de nuevas variedades de plantas y mejoren las variedades existentes.

PALABRAS CLAVE: Plantas, investigación biológica, análisis bioquímico, enzimas, vitaminas.

Introduction

Ukraine is one of the world's most important grain producers. According to the European Commission, Ukraine accounts for 10 percent of the world wheat market, 15 percent of the corn market, and 13 percent of the barley market. According to statistics from the United States Department of Agriculture, in 2021-2022, Ukraine ranked seventh in the world for wheat production, sixth for corn production, and fourth for barley production. Even in the extremely difficult conditions of the war, according to the operational data of the State Customs Service, as of January 16, Ukraine had exported 24,472 million tons of grain and leguminous crops since the beginning of the 2022/23 marketing year, including 1,726 million tons in January. It is noted, however, that during the same period of the previous season, the specified indicator was 35,07 million tons, including 2,598 million tons in January 2022. The total export of Ukrainian flour on January 16 amounted to 73,8 thousand tons (4,8 thousand tons for the month), including 70,1 thousand tons of wheat (4,5 thousand tons).

The correct selection of varieties for sowing places great responsibility on farmers. They must implement the most effective planning of the use of sowing areas, taking into account not only the biological features of the cultivated plants, but also the influence of environmental factors, soil and climatic conditions, and pests. While certain factors can be regulated, protection against diseases and pests in planted plants is carried out through the use of chemical means of protection (insecticides, fungicides, antibiotics, etc.), which can introduce undesirable substances into finished plant products. As a result, allergic reactions of consumers, food poisoning, or accumulation of toxins with an unknown further path of their metabolism may occur.

That is why the competent introduction of highly resistant varieties of plants into the sown areas is of utmost practical importance. The problem of studying the molecular bases of the stability of the variety provides a basis for breeders to create highly productive and effective varieties of plants. Therefore, it is characterized by increased relevance.

The aim of the research is to investigate the relationship between the varietal immunoresistance of plants and the quantitative changes in the parameters of the pro-oxidant-antioxidant balance.

To achieve this aim, the following tasks have been identified:

1. To investigate the level of generation of reactive oxygen species (ROS) in plants, depending on the level of resistance of the variety to diseases.
2. To evaluate the degree of free-radical damage to the tissues of cereal grains of varieties with different levels of resistance.
3. To investigate the degree of antioxidant protection (AOP) in the tissues of experimental plants, depending on the level of resistance of the variety to diseases.
4. To conduct a comprehensive analysis of changes in the value of indicators of the state of the pro-oxidant-antioxidant system (PAS) depending on the level of resistance of the plant variety to diseases.
5. To outline the species- and variety-specific features of changes in the values of indicators of the state of the pro-oxidant-antioxidant balance of experimental plants.

1. Literature review

The concept of plant immunity is described in the works of M.I. Vavilov (Vavilov, 1986). A significant volume of material on the history of plant protection from pests and

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diseases in Ukraine is disclosed in the work of Vasiliev V.P. and Lisova M.P. (Vasilyev & Lisovoy, 1996). The classic basis for the study of phytoimmunity was laid by the work "General and molecular phytopathology" (Dyakov et al., 2001), which was significantly expanded and supplemented by the author in 2012 year (Dyakov, 2012). The question of the relationship between immunity and selection works to breed plants resistant to diseases and pests is thoroughly discussed in the work of L.Ya. Plotnikova (Plotnikova, 2007). Considerable attention was paid to the issue of the mechanism of the immune response of the plant organism to the invasion of pathogens in the works "Plant pathology" (Agrios, 2005) and "Phytopathology" (Semenkova & Sokolova, 2003; Churkov, 2007). In the work "Introduction to plant pathology" (Strange, 2003), it is possible to draw some parallels between the mechanisms of immune protection of plant and animal organisms. The specificity of the immune response of plants to the invasion of pathogens of a viral nature is disclosed in the work "Agricultural Phytovirusology" (Kartasheva, 2007). The molecular mechanisms of plant immunoprotection specific to bacterial lesions are described in the work "Bacterial plant pathology: Cell and molecular aspects" (Sigee, 2005). The general regularities of the plant immune response at the molecular level are covered in the book "Molecular plant immunity" (Sessa, 2012), while a detailed description of molecular changes in the plant organism during the invasion of pathogens can be found in the book "Molecular plant pathology" (Dickinson, 2003). Methods of laboratory research of plant immunity are described in the works of J. M. McDowell., R.N. Trigiano, M.T. Windham and A.S. Windham (McDowell., 2011; Windham, 2004).

The formation of ROS in response to the influence of physical, chemical and biological stress factors is described in the works of a number of scientists (Apel & Hirt, 2004; Foyer & Noctor, 2005; Gill & Tuteja, 2010; Pacheco et al., 2018; Kohen & Nyska, 2002). The role of ROS in the mechanisms of the protective immunological reaction of hypersensitivity is described in the works of I.V. Maksimova, I.A. Tarchevsky, A.A. Aver'yanov (Maksimov, 2006; Aver'yanov, 1991; Tarchevskiy, 2002). The connection between the generation of ROS and the activation of antioxidant protection during the development of acquired phytoimmunity is shown in the work "Active forms of oxygen and plant immunity" (Dmytriyev & Kravchuk, 2005). The double interdependence between ROS and AOP in the plant organism is systematically studied in the works of the school of biochemistry under the

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leadership of Dr. Nicholas Smirnoff (Smirnoff, 2005) and numerous works of Professor Kolupaev Y. E. (Kolupaev & Karpets, 2010, 2014, 2019). In general, the question of the importance of ROS and antioxidants (AO) in ensuring plant immunity is not unambiguous and exhaustive and requires careful further research and systematization.

2. Research methodology

The following types of cereal plants were used in the experiment to quantitatively assess the state of PAS: *Panicum miliaceum L., Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf.* For each species, three varieties were selected, differing in the level of resistance to diseases and pests. In this way, we obtained the following list of research objects (*Table 1*):

Table 1: Characteristics of research plant varieties

№	Species of plants	Varieties of plants		
		The level of resistance to diseases and pests		
		High	Medium	Low
1	*Panicum miliaceum L.*	«Kozatske»	«Yuvileyne»	«Yardush»
2	*Oryza sativa L.*	«Vikont»	«Ukraine 96»	«Yantarnyy»
3	*Avena sativa L.*	«Spurt»	«Iren»	«Synelnykivskyi 1321»
4	*Zea mays L.*	«DK Burshtyn»	«DK Veles»	«DN Ajamka»
5	*Hordeum vulgare L.*	«Sozonivskyi»	«Vakula»	«SN-28»
6	*Triticum durum Desf.*	«Podolyanka»	«Kuyalnik»	«Khersonska bezosta»

Biochemical analysis was performed on grain tissues that were in a resting state. Each experimental group included 10 samples, resulting in the analysis of 1260 samples in the experiment.

Since the superoxide anion radical ($•O_2^-$) is the most potent and key APO in cells, which is formed as a result of the singlet oxygen transformation (Halliwell, 2006), monitoring the content of $•O_2^-$ is advisable for determining the pro-oxidant link's power precisely. Quantitative determination of $•O_2^-$ was carried out using the spectrophotometric NBT test, following a standardized method. Photometry was carried out at 540 nm on a microphotoelectrocolorimeter in a 1 cm^3 cuvette that was 0.5 cm thick. Super oxide

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production in nmol per sample (n nmol $\bullet O_2^-$) was found according to the calibration graph and was converted into nmol per g of tissue per second of incubation.

When ROS acts on cell membranes, it forms TBA active products (TBA_{ap}), and the key product is malondialdehyde (MDA). Determination of the change in MDA level was carried out by reacting with thiobarbituric acid in a pro-oxidant iron-ascorbate buffer. The formed trimethine complex was determined photometrically at 540 nm.

The detailed step-by-step method of determining the value of all the above-described PAS indicators is given in the work "The effect of hypothermia on the prooxidant-antioxidant system's state of plants" (Bobrova et al., 2021).

Superoxide dismutase (SOD) and catalase are the key enzymatic antioxidants (Xu, D.-P., et al., 2017), while the main low molecular weight compounds with AO activity are ascorbic acid (AA) and glutathione (GSH) (Hasanuzzaman et al., 2019; Pacheco et al., 2018). Therefore, the AOP's state assessment was carried out by monitoring changes in the indicators' values of these AOs. SOD activity was determined by noting the time of change in the sample's extinction when the oxidation of $\bullet O_2^-$ adrenaline is inhibited into adrenochrome. The results were expressed in conventional units OD (1 OD corresponds to inhibition of the reaction rate by 50%). Catalase activity was determined by direct titrimetry with a solution of potassium permanganate in an acidic environment.

The content of GSH was analyzed using the reaction with Ellman's reagent, followed by photometry of the formed complex at 412 nm in a 1 cm cuvette. The results were calculated by constructing a calibration graph. AA concentration was measured by direct titrimetry according to Tillmans. A detailed step-by-step method for determining the value changes of all the above-mentioned AOs is given in our previous works (Bobrova et al., 2020).

Cytochrome oxidase is a marker of the degree of free radical peroxidation (FRPO) of membrane biopolymers. Therefore, a change in its activity is an essential indicator of the balance between pro-oxidants and the level of AOP in the body. Cytochrome oxidase activity was determined by the reaction with cytochrome c in the presence of α-naphthol and N,N-dimethyl-para-phenylenediamine hydrochloride. Photometry was performed at 540 nm (Kaznachieiva & Tsebrzhynskyi, 2011).

All the results obtained by us underwent mathematical and statistical processing according to generally accepted methods.

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3. Results and discussion

Results of the research of changes in the value of PAS indicators of *Oryza sativa L.*, grain tissues are shown in *Table 2*.

According to the results obtained, the level of $\bullet O_2^-$ generation in the grains of the high-resistant variety "Vikont" is 1,97 times lower than that of the medium-resistant variety "Ukraine 96" and 2,39 times lower than that of the low-resistant variety "Yantarnyy". The difference in the values of the indicators between the "Ukraine 96" and "Vikont" varieties is 1,23 times. The value of ΔTBA_{ap}, which is inversely proportional to the stock of antioxidants in tissues, changes in proportion to the level of resistance of the variety. Thus, ΔTBA_{ap} is highest in the grains of the "Yantarnyy" variety, which is 2,04 times greater than that of the "Ukraine 96" variety and 2,97 times greater than that of the "Vikont" variety.

The activity of catalase in the grains of the "Vikont" variety is 1,39 times higher than that of the "Ukraine 96" variety and 2,26 times higher than that of the "Yantarnyy" variety. The change in SOD activity indicators follows a similar pattern: there is a difference of 1,41 times between the "Vikont" and "Ukraine 96" varieties, and a difference of 1,97 times between the "Vikont" and "Yantarnyy" varieties.

Analyzing the content of low-molecular antioxidants, it was established that the amount of AA in the grains of the "Yantarnyy" variety is 1,48 times less than that of the "Vikont" variety and 1,19 times less than that of the "Ukraine 96" variety. The "Yantarnyy" variety also has a 1,14 times lower GSH content compared to the "Vikont" variety. The difference in values between the "Ukraine 96" variety and the other two varieties is unreliable.The activity of cytochrome oxidase changes in proportion to the level of resistance of the variety. Therefore, the grains of the "Vikont" variety have the highest activity, with a value 1,27 times greater than that of the "Ukraine 96" variety and 1,51 times greater than that of the "Yantarnyy" variety.

The results of the research on changes in the value of PAS indicators of *Avena sativa L.***,** grain tissues are shown in *Table* 3.

After analyzing the pro-oxidant link using the table of obtained results, the ratios of the levels of $\bullet O_2^-$ generation were found to be 1:1,71:4,43 and the ratios of Δ TBAap were 1:1,34:4,15, in the order of the varieties mentioned: "Spurt", "Iren", "Synelnykivskyi 1321". In analyzing the antioxidant link using the same table, the ratios of catalase activity levels were

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1,91:1,22:1, and the ratios of SOD activity levels were 2,45:2,3:1, also in the order of the mentioned varieties. The ratios of AA content values were 1,74:1,42:1 and the ratios of GSH concentration were 1,10:1,06:1, while the ratios of cytochrome oxidase activity were 1,33:1,11:1, following the order of "Spurt", "Iren", "Synelnykivskyi 1321".

The results of the research of changes in the value of PAS indicators of the tissues of *Zea mays L.*, grain sare shown in *Table 4*.

Table 2: Comparative characteristics of the PAS status indicators in the tissues of *Oryza sativa L.*, grains

№	Indicators of the PAS state	Varieties of plants		
		«Vikont»	«Ukraine 96»	«Yantarnyy»
Indicators of prooxidant activity				
1.	NBT test (base level), nmol $\bullet O_2^-/(g*s)$	0,222±0,008	0,437±0,010	0,531±0,014
2.	Δ TBA_{ap}, %	14,88 ± 0,99	21,63 ± 1,10	44,12 ± 2,83
Enzymatic antioxidants				
3.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,43 ± 0,02	0,31 ± 0,01	0,19 ± 0,01
4.	SOD activity, OD	0,59 ± 0,01	0,42 ± 0,01	0,30 ± 0,01
Low molecular weight antioxidants				
5.	Concentration of AA, $\frac{mmol}{kg}$	0,114 ± 0,01	0,092 ± 0,01	0,077 ± 0,01
6.	Concentration of GSH, $\frac{mmol}{kg}$	46,81 ± 0,12	45,18 ± 0,78	41,22 ± 0,31
Indicators of the effects of PAS changes				
7.	Cytochrome oxidase activity, OD	0,505 ±0,004	0,398 ±0,006	0,334 ±0,011

Upon analyzing the obtained results, we have observed patterns similar to those described above. The level of $\bullet O_2^-$ production increases inversely proportional to the resistance of the variety, with the highest level found in the tissues of "DN Ajamka" at 1,10 times higher than "DK Veles" and 1,40 times higher than "DK Burshtyn". The ratio of ΔTBAap content is 1:1,72:2,17 for the high-, medium-, and low-resistant varieties, respectively. SOD activity is highest in the tissues of "DK Burshtyn", with a predominance of 1,79 times in relation to "DK Veles" and 3,10 times in relation to "DN Ajamka". The intervarietal difference in the value of catalase activity indicators is unreliable. The ratio of cytochrome oxidase

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activity is 1,60:1,20:1 for the varieties "DK Burshtyn", "DK Veles", and "DN Ajamka", respectively.

The results of the research of the PAS indicators of *Hordeum vulgare L.*, grain tissues are shown in *Table 5*.

Table 3: Comparative characteristics of the PAS status indicators in the tissues of *Avena sativa L.,* grains

№	Indicators of the PAS state	Varieties of plants		
		«Spurt»	«Iren»	«Synelnykivskyi 1321»
Indicators of prooxidant activity				
1.	NBT test (base level), nmol $\bullet O_2^-/(g^*s)$	0,021±0,009	0,036±0,004	0,093±0,004
2.	Δ TBA_{ap}, %	8,43 ± 1,04	11,27 ± 2,01	34,98 ± 1,33
Enzymatic antioxidants				
3.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,61 ± 0,04	0,39 ± 0,03	0,32 ± 0,01
4.	SOD activity, OD	0,49 ± 0,01	0,46 ± 0,02	0,20 ± 0,02
Low molecular weight antioxidants				
5.	Concentration of AA, $\frac{mmol}{kg}$	0,136 ± 0,09	0,111 ± 0,03	0,78 ± 0,04
6.	Concentration of GSH, $\frac{mmol}{kg}$	56,12 ± 0,88	54,19 ± 0,34	51,02 ± 0,19
Indicators of the effects of PAS changes				
7.	Cytochrome oxidase activity, OD	0,501 ±0,004	0,418 ±0,009	0,376 ±0,011

The spectrophotometrically determined basic level of $\bullet O_2^-$ was highest in the tissues of barley grains of the "SN-28" variety, which exceeded the "Vakula" indicator by 1,40 times and the "Sozonivskyi" indicator by 2,12 times. Inter-varietal comparison of the Δ MDA indicator can be presented in the following ratio - 1:1,94:2,84.

The results of the determination of non-enzymatic AOs indicate that the content of AA in barley grains varies according to the level of disease resistance of the plant variety. Thus, the AA content of the highly resistant grade "Sozonivskyi" is 1,09 times higher compared to "Vakula" and 2,02 times higher compared to "SN-28". The difference between the varietal comparison of "Vakula" and "SN-28" indicators was 1,85 times. The comparison

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of GSH concentration can be reduced to the following ratio: 1,05:1:0,87, respectively, for the varieties "Sozonivskyi", "Vakula" and "SN-28".

Table 4: Comparative characteristics of the PAS status indicators in the tissues of *Zea mays L.*, grains

№	Indicators of the PAS state	Varieties of plants		
		«DK Burshtyn»	« DK Veles»	« DN Ajamka»
	Indicators of prooxidant activity			
1.	NBT test (base level), nmol $\bullet O_2^-/(g{*}s)$	0,998±0,009	1,273±0,015	1,394±0,011
2.	Δ TBA_{ap}, %	65,12 ± 3,22	111,83 ± 5,19	141,32 ± 4,68
	Enzymatic antioxidants			
3.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,11 ± 0,01	0,09 ± 0,01	0,09 ± 0,01
4.	SOD activity, OD	0,34 ± 0,02	0,19 ± 0,01	0,11 ± 0,01
	Low molecular weight antioxidants			
5.	Concentration of AA, $\frac{mmol}{kg}$	0,101 ± 0,03	0,085 ± 0,02	0,078 ± 0,01
6.	Concentration of GSH, $\frac{mmol}{kg}$	41,16 ± 0,64	37,16 ± 0,99	34,54 ± 0,27
	Indicators of the effects of PAS changes			
7.	Cytochrome oxidase activity, OD	0,213±0,004	0,159±0,008	0,133±0,006

As a result of the research, a connection between the level of resistance of the barley variety to diseases and the activity of AOP enzymes was revealed. Thus, catalase activity in barley grains of the Sozonivskyi variety is 1,22 times higher than that of "Vakula". Catalase activity in the grains of the "SN-28" variety is reduced by 1,47 times compared to the "Sozonivskyi" variety and by 1,21 times compared to the "Vakula" variety. Biochemical analysis showed the superiority of SOD activity of barley grains of the variety "Sozonivskyi" over the indicators of "SN-28" and "Vakula" by 1,65 and 1,34 times, respectively. The activity of SOD decreases by 1,24 when switching from the variety "Vakula" to "CH-28". The activity of cytochrome oxidase in the tissues of the variety "Sozonivskyi" is 1,29 times higher compared to the variety "SN-28" and 1,25 times compared to "Vakula".

The results of the research on the PAS indicators of *Triticum durum Desf.*, grain tissues are shown in *Table* 6.

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Table 5: Comparative characteristics of the PAS status indicators in the tissues of *Hordeum vulgare L.*, grains

№	Indicators of the PAS state	Varieties of plants		
		«Sozonivskyi»	«Vakula»	«SN-28»
	Indicators of prooxidant activity			
1.	NBT test (base level), nmol $\bullet O_2^-/(g*s)$	0,065 ± 0,021	0,091±0,009	0,138±0,012
2.	TBA_{ap0}, мкмоль/кг	10,03 ± 0,65	17,67±0,68	25,39±1,11
3.	Δ TBA_{ap}, %	66,10 ± 6,89	128,45 ± 18,35	187,61 ± 24,50
	Enzymatic antioxidants			
4.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,28 ± 0,02	0,23 ± 0,02	0,19 ±0,01
5.	SOD activity, OD	0,39 ± 0,02	0,29 ± 0,01	0,23 ±0,01
	Low molecular weight antioxidants			
6.	Concentration of AA, $\frac{mmol}{kg}$	0,083 ± 0,01	0,076±0,01	0,041±0,004
7.	Concentration of GSH, $\frac{mmol}{kg}$	50,65 ± 0,68	48,05 ± 0,1	42,00±0,53
	Indicators of the effects of PAS changes			
8.	Cytochrome oxidase activity, OD	0,346 ± 0,006	0,276±0,005	0,268±0,009

The spectrophotometric NBT test revealed the highest background level of $\bullet O_2^-$ generation in the grains of the "Khersonska bezosta" variety, which is 1.26 times higher than the $\bullet O_2^-$ level of the "Kuyalnik" variety and 1,66 times higher than the "Podolyanka" variety. The concentration ratio of $\bullet O_2^-$ in the tissues of wheat varieties "Podolyanka" and "Kuyalnik" is reliably 1,32.

The concentration of low molecular weight AO is the highest in the grains of the highly disease-resistant variety "Podolyanka" and the lowest for the variety "Khersonska bezosta". Thus, the content of AA in the grains of wheat "Podolyanka" is higher than that in the variety "Kuyalnik" and "Khersonska bezosta" by 1,28 and 1,87 times, respectively, and the concentration of GSH is higher by 1,11 and 1,12 times. When comparing the indicators determined for the "Kuyalnik" and "Khersonska bezosta" varieties, a 1,46-fold superiority over the medium-resistant variety was found in the analysis of AA.

Biochemical analysis of the activity of enzymatic AOs shows that the wheat grains of the "Podolyanka" variety have the highest activity of catalase, which is 2,67 times higher than

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the Kuyalnik variety and 4,00 times higher than the "Khersonska bezosta" variety. The ratio of catalase activity in wheat grains of the "Kuyalnik" variety to that of "Khersonska bezosta" is 1,5 times. The intervarietal comparison of SOD activity can be reduced to the following ratio – 1,60:1:1,01, according to the order of the varieties "Podolyanka", "Kuyalnik" and "Khersonska bezosta". As a result of research on the activity of cytochrome oxidase, a pattern was revealed, according to which, when moving from the highly disease-resistant variety "Podolyanka" to the medium-resistant "Kuyalnik", the activity of the enzyme decreases by 1,57 times, and when moving to the low-resistant variety "Khersonska bezosta", it decreases by 1,61 times.

Table 6: Comparative characteristics of the PAS status indicators in the tissues of *Triticum durum Desf.,* grains

№	Indicators of the PAS state	Varieties of plants		
		«Podolyanka»	«Kuyalnik»	«Khersonska bezosta»
	Indicators of prooxidant activity			
1.	NBT test (base level), nmol $\bullet O_2^-/(g^*s)$	0,068 ± 0,002	0,090 ±0,009	0,113 ± 0,009
2.	Δ TBA_{ap}, %	46,00 ± 6,26	27,86 ± 4,11	25,64 ± 2,77
	Enzymatic antioxidants			
3.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,24 ± 0,04	0,09 ± 0,02	0,06 ± 0,02
4.	SOD activity, OD	0,35 ± 0,01	0,22 ± 0,01	0,22±0,01
	Low molecular weight antioxidants			
5.	Concentration of AA, $\frac{mmol}{kg}$	0,073 ± 0,01	0,057 ± 0,01	0,039±0,005
6.	Concentration of GSH, $\frac{mmol}{kg}$	45,22 ± 0,81	40,79 ± 0,25	40,23±0,56
	Indicators of the effects of PAS changes			
7.	Cytochrome oxidase activity, OD	0,542 ± 0,031	0,346 ±0,001	0,336±0,007

The results of the research of the PAS indicators of *Panicum miliaceum L.*, grain tissues are shown in *Table 7*.

Analyzing the results of the research of the pro-oxidant link, the ratio of the level of generation $\bullet O_2^-$ 1:1,19:1,29, and the ratio Δ TBA_{ap} 1:1,44:2,16 according to the order of counting varieties: "Kozatske", "Uuvileyne", "Yardush".

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Table 7: Comparative characteristics of the PAS status indicators in the tissues of *Panicum miliaceum L.,* grains

№	Indicators of the PAS state	Varieties of plants		
		«Kozatske»	«Yuvileyne»	«Yardush»
Indicators of prooxidant activity				
1.	NBT test (base level), nmol $\bullet O_2^-/(g^*s)$	0,912±0,012	1,086±0,011	1,177±0,009
2.	Δ TBA_{ap}, %	99,95 ± 4,39	144,09 ± 9,51	215,62 ± 12,84
Enzymatic antioxidants				
3.	Catalase activity, $\frac{\mu mol}{kg*min}$	0,18 ± 0,01	0,07 ± 0,01	0,05 ± 0,01
4.	SOD activity, OD	0,23 ± 0,02	0,16 ± 0,01	0,11 ± 0,01
Low molecular weight antioxidants				
5.	Concentration of AA, $\frac{mmol}{kg}$	0,071 ± 0,01	0,037 ± 0,01	0,024 ± 0,01
6.	Concentration of GSH, $\frac{mmol}{kg}$	49,21 ± 0,18	43,14 ± 0,67	40,97 ± 0,12
Indicators of the effects of PAS changes				
7.	Cytochrome oxidase activity, OD	0,152±0,008	0,118±0,006	0,076±0,004

Analyzing the antioxidant link from the table of obtained results, the ratio of the level of catalase activity was 3,60:1,40:1, and the ratio of the level of SOD activity was 2,09:1,46:1, in accordance with the order of mention of the varieties: "Kozatske", "Uuvileyne", "Yardush".

The values of AA content indicators have the following ratio: 2,96:1,54:1, GSH concentration – 1,20:1,05:1, cytochrome oxidase activity – 2,00:1,55:1 according to the order of varieties: "Kozatske", "Uuvileyne", "Yardush".

Summarizing all the above digital calculations of the results of the biochemical analysis of the components of the PAS state of the grains of experimental cereal plants and intervarietal comparison of the values of the obtained indicators, the following patterns were revealed:

1. There was a significant increase in the baseline level of $\bullet O_2^-$ generation when transitioning from highly disease-resistant varieties to low-resistant varieties.
2. The tissues of highly resistant varieties of all experimental cereals had the lowest level of ΔTBA_{ap}, which could be explained by the experimentally confirmed increased activity of enzymatic and non-enzymatic antioxidants.

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3. It was found that the grains of highly resistant varieties of all experimental cereals had significantly higher activity of enzymatic antioxidants: SOD and catalase. This can be explained by the leading role of SOD in the elimination of $•O_2^-$. At the same time, $•O_2^-$ decomposes to H_2O_2, the excess of which is decomposed by catalase. Therefore, the series of decrease in activity of SOD and catalase showed a significant similarity.
4. The total content of AA in the grains of experimental cereals is quite low, which can be explained by the seeds being at rest. However, even taking into account this feature, it was found that the content of low molecular weight antioxidants in the grains of all experimental cereals changed in proportion to the change in the variety's resistance to diseases. Thus, GSH is a hydrogen donor for GSH-peroxidase, and, together with AA, ensures the recovery of oxidized forms of AO, breaks the FRPO chain, and oxidized GSH in seeds inhibits membrane ATPases, hexokinase, glucose-6-phosphate dehydrogenase, phosphorylation, and nuclear synthesis of RNA.
5. As a result of the assessment of the consequences of changes in PAS, the highest activity of cytochrome oxidase was found in grains of highly disease-resistant varieties, and its natural weakening when transitioning to low-resistant varieties, which may be explained by a decrease in the intensity of peroxide destruction of mitochondrial membranes as a result of increased AOP.

In order to identify species-specific signs, we also calculated the average grade values of each indicator of the state of PAS and created a consolidated *Table 8*:

Table 8: Average class values of PAS state indicators

№	Species of plants	The average value of PAS state indicators:						
		NBT test	ΔTBA_{ap}	Catalase activity	SOD activity	Concentration of AA	Concentration of GSH	Cytochrome oxidase activity
1	*Panicum miliaceum L.*	1,058	153,22	0,10	0,17	0,044	44,44	0,115
2	*Oryza sativa L.*	0,397	26,88	0,31	0,44	0,094	44,40	0,412
3	*Avena sativa L.*	0,050	18,23	0,44	0,38	0,108	57,78	0,432
4	*Zea mays L.*	1,222	106,09	0,10	0,21	0,088	37,62	0,168
5	*Hordeum vulgare L.*	0,098	127,39	0,23	0,30	0,067	46,90	0,297
6	*Triticum durum Desf.*	0,090	33,17	0,13	0,26	0,056	42,08	0,408

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Analyzing *table* 8, the following species-specific patterns can be identified:

1. Due to the increase in the level of $\bullet O_2^-$ generation in the tissues of grains, the experimental plant species can be placed in the following sequence: *Avena sativa L., Triticum durum Desf., Hordeum vulgare L., Oryza sativa L., Panicum miliaceum L., Zea mays L.*
2. As the value of ΔTBA_{ap} increases in the tissues, the experimental plant species form the following series: *Avena sativa L., Oryza sativa L., Triticum durum Desf., Zea mays L., Hordeum vulgare L., Panicum miliaceum L.*
3. Due to the increase in the activity of catalase in the tissues of grains, experimental cereals form the following sequence: *Panicum miliaceum L., Zea mays L., Triticum durum Desf., Hordeum vulgare L., Oryza sativa L., Avena sativa L.*
4. For increasing SOD activity, experimental plants can be placed in the following sequence: *Panicum miliaceum L., Zea mays L., Triticum durum Desf., Hordeum vulgare L., Avena sativa L., Oryza sativa L.*
5. In the row *Panicum miliaceum L., Triticum durum Desf., Hordeum vulgare L., Oryza sativa L., Zea mays L., Avena sativa L,.* AA concentration increases.
6. In the row *Zea mays L., Triticum durum Desf., Oryza sativa L., Panicum miliaceum L., Hordeum vulgare L., Avena sativa L.,* GSH concentration increases.
7. Cytochrome oxidase is characterized by increasing indicators in the following sequence of experimental plants: *Panicum miliaceum L., Zea mays L., Hordeum vulgare L., Triticum durum Desf., Oryza sativa L., Avena sativa L.*

The general laws of PAS, inherent in all types of experimental plants, became:

1. The content of AA in grain tissues is low, which may be explained by the low metabolism of grain tissues.
2. The inter-varietal and inter-species indicator of GSH content is relatively stable, which is possibly explained by the narrow norm of the reaction of this characteristic and the value of GSH in the performance of basic biochemical functions in the tissues of the grains that are in a state of rest.
3. The tissues of grains in a state of rest have a predominance of AO links, which is necessary to ensure homeostasis and protection from stressors in tissues with a low level of metabolism.

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4. The low-molecular-weight link among AOs plays a leading role in ensuring the stability of the variety in the tissues of grains that are at rest. This may be explained by the inducibility of enzyme AOs, where the battery capacity is inherent only to low molecular weight AOs.

Conclusions

1) A significant increase in the basal level of $\bullet O_2^-$ generation was observed with decreasing resistance of experimental plant varieties to diseases.
2) The tissues of highly resistant varieties of all experimental cereals have the lowest levels of ΔTBA_{ap}.
3) It was found that the grains of highly resistant varieties of all experimental cereals have significantly higher activity of enzymatic antioxidants: SOD and catalase.
4) The total content of AA in cereal grains is quite low, which is explained by the seeds being in a state of rest. However, even taking into account this feature, it was found that the content of low molecular weight antioxidants in the grains of all experimental cereals changes proportionally to the change in the variety's resistance to diseases.
5) As a result of the assessment of the consequences of the change in PAS, the highest activity of cytochrome oxidase was found in the grains of highly disease-resistant varieties, and its natural weakening during the transition to low-resistant varieties was observed.
6) The general patterns of PAS found in the tissues of grains of all types of experimental plants were: low content of AA, relatively constant inter-varietal and inter-species indicator of GSH content, and predominance of AO links (low molecular weight AOs play a leading role).

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