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INDICES OF ANTIOXIDANT AND OSMOPROTECTIVE SYSTEMS IN SEEDLINGS OF WINTER WHEAT CULTIVARS WITH DIFFERENT FROST RESISTANCE

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The functioning of the stress-protective systems of wheat under the action of cold at the early stages of plant development remains poorly studied. The aim of this work was a comparative study of antioxidant activity and the content of sugars and proline as indicators of osmoprotective activity during cold adaptation of seedlings of seven winter bread wheat (Triticum aestivum L.) cultivars that differ significantly in frost resistance. The 3-day-old etiolated seedlings were hardened at 2°C for 6 days and then frozen for 5 h at -6 or -9°C. Two days after freezing, the survival of seedlings was assessed by their ability to grow. A decrease in ROS content, an increase in the activity of antioxidant enzymes catalase and guaiacol peroxidase and accumulation of sugars in the shoots of high-frost-resistant cultivars during hardening were detected. The absolute values of catalase and guaiacol peroxidase activity correlated positively with the frost resistance of seedlings. The negative correlation between the frost tolerance of the cultivars and the accumulation of proline in the seedlings during hardening was recorded. The possibility of using the studied biochemical indices for frost resistance screening of winter wheat varieties at the seedling stage was stated.

K e y w o r d s: oxidative stress, antioxidant system, osmolytes, frost resistance, Triticum aestivum, cold hardening.

Despite the global trend towards higher temperatures, especially on the European continent [1], the problem of frost tolerance in winter cereal crops is not only not diminishing but, on the contrary, increasing. One of the manifestations of global climate warming is an increase in the frequency of winter thaws with a sharp change with low temperatures, which increases the probability of frost plant damage [2]. In Ukraine, in particular, the loss of winter cereal crops has been up to 35% in some years [3]. In this regard, the search for frost tolerance donors and the evaluation of this trait in new winter crop cultivars remains an important practical task.

Most plants have little or no constitutive capacity to survive exposure to sub-zero temperatures. However, after exposure to low positive temperatures (so-called cold hardening), many species, including winter cereals, develop induced frost resistance [4]. The nature of basic molecular genetic, physiological, and biochemical changes responsible for this property has now been elucidated. In particular, key families of transcription factors (CBF, WRKY, bZIP, MYB, bHLH, C2H2 NAC, and others) have been identified that regulate *COR* genes expression, leading to physiological responses to cold stress [5, 6]. Among these responses, the most important are an increase of unsaturated fatty acids in membrane lipids [7], accumulation of cryoprotectants [8], synthesis of various stress proteins [9], and activation of the antioxidant system [10]. The last mentioned is important for the frost resistance development due to the fact that a low temperature-induced increase in cell membrane rigidity increases the stochastic formation of reactive oxygen species (ROS) in electron-transport systems of mitochondria and chloroplasts [10]. In addition, low-temperature exposure causes increased ROS generation by NADPH oxidase at the cell surface [11].

Phenomena of increased gene expression and activity of key antioxidant enzymes – superoxide dismutase (SOD) [12, 13], catalase [12], non-specific peroxidase [14], and enzymes of the ascorbate-glutathione cycle [10] – in various objects during cold adaptation have now been recorded. The relationship between ascorbate peroxidase and guaiacol peroxidase activity and frost resistance of cereals of different genotypes subjected to cold hardening was shown [15, 16].

The main low-molecular-weight antioxidants, ascorbate and glutathione, are also involved in plant adaptation to cold, although the link between their content and resistance of individual genotypes is not always evident [17, 18]. The importance of the accumulation of polyfunctional low-molecular-weight compounds such as sugars and proline for the development of frost tolerance in plants is also well known [12, 19]. These compounds act as osmolytes, exhibit membrane-protective and anti-denaturation effects [20]. At the same time, they are powerful antioxidants [21, 22]. However, the relationship between the accumulation of sugars and proline and the low-temperature tolerance of cereals cannot be considered unambiguous. For example, the sugar content of the non-resistant triticale cultivar was higher than that of the frost-resistant one [23]. The cold-resistant rice genotype had lower proline content in leaves and shoots at normal and sub-zero temperatures than the non-resistant genotype [24].

In general, there has been a lot of investigation into the relationship between the individual components of the antioxidant system and the frost tolerance of plants [25-27]. However, the relationship between frost tolerance and the functioning of antioxidant and osmoprotective systems of wheat plants at the early stages of development using a wide range of cultivars developed in different geographical zones with contrasting climates has not been studied yet. However, obtaining such data can contribute to a better understanding of the adaptive strategies of resistant cultivars and the development of effective rapid screening methods for frost resistance of winter wheat cultivars.

The aim of this work was a comparative study of the ROS formation and the functioning of antioxidant and osmoprotective systems during cold adaptation of wheat seedlings of 7 cultivars that differ significantly in frost resistance.

Material and Methods

Plant material and treatments. Seeds of bread winter wheat (*Triticum aestivum* L.), obtained from the collections of the National Center for Plant Genetic Resources of Ukraine and Poltava State Agrarian University, were used in this work. The experiments were carried out on 7 cultivars of different ecological and geographical origins and, according to the scientific literature and resources of the Ukrainian Institute for Plant Variety Examination (Table 1), differing significantly in frost tolerance. The cultivar Albidum 114, in particular, is very frost-resistant, and the Doskonala is frost-resistant. The group with average or exceeding it frost resistance is made up of Zelenyi hai, Nordika, and Etana cultivars. The Malibu and Tobak have low frost resistance.

The seeds were disinfected for 15 min with a 1% NaClO solution, washed thoroughly with distilled water and germinated in Petri dishes on paper in a thermostat at 24°C. After germination for 3 days, etiolated seedlings were used for biochemical analyses and subjected to freezing for 5 h in a cham-

Table 1. Information about wheat cultivars used in the experiments

Cultivar	Country	Frost resistance	Source
Albidum 114	Russia	very high	https://doi.org/10.1078/0176-1617-00642
Doskonala	Ukraine	high (8.8 points*)	http://sort.sops.gov.ua/cultivar/view/12253
Etana	Germany	medium (5.0 points)	http://sort.sops.gov.ua/cultivar/view/4796
Malibu	Germany	low (2.0 points)	http://sort.sops.gov.ua/cultivar/view/3186
Nordika	Czech Republic	above average (7.2 points)	http://sort.sops.gov.ua/cultivar/view/4802
Tobak	Germany	low (2.5 points)	http://sort.sops.gov.ua/cultivar/view/3599
Zelenyi hai	Ukraine	above average (7.0 points)	http://sort.sops.gov.ua/cultivar/view/4798

Note. *On a 9-point scale, data from field trials of the Yuriev Plant Production Institute, National Academy of Agrarian Sciences of Ukraine

ber (Danfoss, Netherlands) in darkness at -6°C with a temperature decrease of 1°C/h. To thaw the seedlings, the chamber temperature was raised to 2°C at a rate of 1°C/h. The seedlings were then grown for an additional 2 days at 24°C and 150 W/m^2 and their survival rate was assessed. In the cold hardening variants, the 3-day-old etiolated seedlings were incubated for 6 days in refrigerated compartment (without light) at 2°C. The optimum hardening regime for the seedlings is based on the results of experiments described in a previous paper [28]. Biochemical analyzes of seedlings were carried out after 6 days of hardening at 2°C. As a control, 3-day-old etiolated seedlings that had not been hardened were used. As seedling growth was retarded at low temperatures, the 9-day-old hardened plants were the same as the 3-day-old control plants grown at 24°C.

After cold hardening, the seedlings were frozen for 5 h in the absence of light at -6 or -9°C with temperature reduction at a rate of 1°C/h. The samples were then thawed and grown further in the light to assess their survival rate as described above.

Generation of superoxide anion radicals (SAR – $O_2^{\bullet-}$) by the shoots was assessed by nitroblue tetrazolium (NBT) reduction. Ten shoots of equal size were placed in test tubes for 1 h with 5 ml of 0.1 M K,Na-phosphate buffer (pH 7.6) containing 0.05% NBT, 10 μM EDTA, and 0.1% Triton X-100 [29]. At the end of the exposure, the shoots were carefully removed from the incubation solution and the absorbance of the incubation solution at 530 nm was measured. The results were expressed in units of absorbance per shoot (×1000).

Evaluation of hydrogen peroxide content. To determine H_2O_2 content, shoots of seedlings 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 H_2O_2 concentration was determined in the supernatant using ferrothiocyanate method [30] with slight modifications. For this purpose, 0.5 ml of 2.5 M potassium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ammonium iron(II) sulfate were added to tubes. After mixing, the samples were poured into cuvettes and the absorbance at 480 nm was determined.

Evaluation of LPO product content. The lipid peroxidation (LPO) rate in seedling shoots was assessed by its products reacting with 2-thiobarbituric acid (TBA) (mainly malonic dialdehyde, MDA) [28]. Plant material was homogenized in 0.1 M Tris-HCl buffer (pH 7.6); then a 0.5% TBA solution in 20% TCA was added to the homogenate. After heating the mixture in a boiling water bath for 30 min, the samples were cooled and centrifuged at 8000 g for 10 min. Absorbance of the supernatant at 532 nm was then measured. Non-specific absorbance at 600 nm was also determined, the value of which was subtracted from the main result. Measurements were made relative to the reagent mixture that did not contain TBA.

Measurement of antioxidant enzyme activities. To evaluate the activity of antioxidant enzymes, shoots (200 mg) were homogenized in 10 ml of 0.15 M K,Na-phosphate buffer (pH 7.6) with EDTA (0.1 mM) and dithiothreitol (1 mM) on ice [28]. The homogenate was centrifuged for 15 min at 8000 g at a temperature not exceeding 4°C to obtain a supernatant, which was then analysed. SOD activity was determined at pH 7.6 by a method based on the enzyme's ability to compete with nitroblue tetrazolium for superoxide anions formed by the aerobic interaction of NADH with phenazine metosulphate. Catalase activity (CAT) was estimated by the amount of hydrogen peroxide decomposed per unit time [31]. Guaiacol peroxidase (GPX) was analysed at pH 6.2 (K,Na-phosphate buffer) using guaiacol as hydrogen donor and hydrogen peroxide as substrate [32].

Content of low-molecular protective compounds. The total sugar content of the plant material was determined by the Morris-Roe method based on anthrone reagent [33] in our modification. Sugars from the plant material were extracted with distilled water and heated for 10 min in a boiling water bath. The resulting extract was clarified by adding equal volumes (0.3 ml) of 30% zinc sulfate and 15% blood yellow salt to the reaction tubes. Samples were filtered through a paper filter and, if necessary, diluted several times with distilled water before measurement. The reaction tubes were filled with 3 ml of anthrone reagent and 1 ml of filtrate, and distilled water was added to the control sample instead of the filtrate. After boiling for 7 min in a water bath, samples were cooled and absorbance at 610 nm was determined relative to the control solution. D-glucose was used as the standard.

Proline content in the shoots was determined according to Bates et al. [34] modified method. Proline was extracted from the plant material with distilled water by boiling for 10 min. The extract was then filtered, mixed in equal volumes with ninhydrin

reagent and glacial acetic acid and the samples were boiled in a water bath for 1 h. The absorbance of the coloured reaction product was determined at 520 nm using L-proline as standard.

Replication of experiments and statistical processing of results. The experiments had 3 biological replicates. When evaluating seedling survival after freezing, each biological replicate consisted of at least 80 seedlings. In the biochemical analysis, there were 12-15 shoots in the sample. Data were statistically processed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and Table 2 show the mean values and their standard errors. Different letters denote values, which differences are significant at $P \leq 0.05$. Correlation coefficients were estimated using programming language R, version 4.1.1 (R Core Team).

Results

Wheat seedling survival after freezing. The unhardened seedlings of most cultivars died almost completely after exposure to -6°C. In the most resistant cultivar, Albidum 114, a survival rate of 23.3% was recorded (Table 2). A small number of seedlings of Nordika and Zelenyi hai also retained viability. In general, however, it was not possible to differentiate between unhardened seedlings of cultivars in terms of frost tolerance (Table 2).

At the same time, hardening caused differential development of the frost resistance in seedlings of all the cultivars. The seedlings of Albidum 114 showed the highest resistance. The survival of seedlings of Doskonala after freezing at -6 and -9°C was slightly lower (Table 2). The seedlings of Zelenyi hai and Nordika were almost equally frost-resistant, but their survival after sub-zero temperatures was lower than that of the Albidum 114 and Doskonala. These values were even lower in the Etana cultivar. Finally, the Malibu and Tobak seedlings showed the least resistance (Table 2). Thus, studied cultivars represented the following series with significant differentiation in terms of seedling frost tolerance: Albidum 114 > Doskonala > Zelenyi hai ≥ Nordika > Etana > Malibu ≥ Tobak.

ROS generation and MDA content in unhardened and hardened seedlings. The level of SAR generation in unhardened seedlings of the different cultivars varied slightly, regardless of their frost tolerance (Fig. 1, *A*). Hardening caused a reduction in this indicator in high- and medium-resistant cultivars Albidum 114, Doskonala, Zelenyi hai, and Etana, while in the other ones, such effect was only a tendency.

The hydrogen peroxide content in the shoots of unhardened seedlings also varied, with no significant association with frost tolerance (Fig. 1, *B*). After hardening, there was a significant reduction in H_2O_2 content of shoots of frost-resistant cultivars Albidum 114, Doskonala, and Zelenyi hai. In other cultivars, this effect was small or not manifested at all.

The amount of LPO products (mainly MDA) in unhardened seedlings, as well as other indicators, varied slightly regardless of cultivar resistance. After hardening temperatures, MDA content decreased in the high and medium frost-resistant cultivars, while it did not change in the two least resistant ones, Malibu and Tobak (Fig. 1, *C*).

Antioxidant enzyme activity in unhardened and hardened wheat seedlings. The SOD activity in the shoots of unhardened seedlings of different cultivars varied irrespective of their resistance (Fig. 2, *A*). For example, high values were recorded for one of the least frost-resistant cultivars (Malibu) and for the

Table 2. Survival of wheat seedlings (%) after 5-h freezing at -6 and -9°C

Cultivar	Freezing of unhardened seedlings (5 h at -6°C)	Freezing of cold-hardened seedlings (5 h at -6°C)	Freezing of cold-hardened seedlings (5 h at -9°C)
Albidum 114	23.3 ± 1.4	93.6 ± 2.4	88.2 ± 1.9
Doskonala	0.5 ± 0.4	84.3 ± 1.1	73.5 ± 0.7
Etana	0.0	63.3 ± 2.1	53.3 ± 2.0
Malibu	0.0	44.5 ± 3.1	21.9 ± 1.3
Nordika	15.5 ± 2.6	69.5 ± 2.4	60.0 ± 1.9
Tobak	0.0	37.4 ± 1.6	20.0 ± 1.5
Zelenyi hai	10.4 ± 1.0	76.5 ± 1.6	60.3 ± 3.0
$LSD_{0.05}$	2.9	5.2	4.7

most frost-resistant, Albidum 114. After hardening, the enzyme activity increased markedly only in Albidum 114 and Zelenyi hai. In cultivars characterized by low and medium tolerance (Malibu, Tobak, and Etana), SOD activity decreased after hardening temperatures (Fig. 2, *A*).

The CAT activity in unhardened seedlings, as well as the other studied parameters, varied independently of cultivar frost tolerance (Fig. 2, *B*). Hardening caused an increase in the enzyme activity only in the most resistant cultivar Albidum 114. At the same time, the non-tolerant cultivars Malibu and Tobak showed a decrease in CAT activity, significant at $P \leq 0.05$. In the other cultivars, this indicator did not change significantly after the hardening temperature (Fig. 2, *B*).

The GPX activity in the shoots of unhardened seedlings, like the other antioxidant enzymes studied, varied regardless of cultivar resistance (Fig. 2, *C*). After hardening, there was more than a twofold increase in enzyme activity in the most frost-resistant Albidum 114. A significant increase in GPX activity was also observed in the relatively frost-resistant cultivars Doskonala and Nordika. The other cultivars showed little change in the activity of this enzyme, while the non-frost-resistant cultivar Malibu showed a marked decrease in GPX after hardening temperature (Fig. 2, *C*).

Sugars and proline content in unhardened and hardened seedlings. In non-hardened seedlings, the sugars content varied considerably regardless of the resistance of cultivars (Fig. 3, *A*). Thus, two medium-resistant varieties, Etana and Nordika, had the highest and lowest values for this indicator, respectively. In response to hardening temperature, the sugars content increased significantly in all high and medium frost-resistant cultivars. In contrast, in the non-frost-resistant cultivars, it decreased or remained unchanged (Fig. 3, *A*).

The constitutive proline content of the seedling shoots of different cultivars, as well as other indicators, varied irrespective of their resistance (Fig. 3, *B*). Cold hardening caused an increase in proline content in all cultivars. However, this effect was most pronounced in the non-frost-resistant Malibu and Tobak (4.5 and 3.7 times, respectively). At the same time, the proline content in response to hardening temperature increased least (by only 13%) in the most frost-resistant cultivar, Albidum 114 (Fig. 3, *B*).

Correlation between indicators of pro-/antioxidant balance, osmolyte content, and frost resistance of cultivars. The $O_2^{\bullet-}$ generation, H_2O_2 and MDA levels in hardened seedlings were negatively correlated with their survival after freezing (Fig. 4). However, at $P \leq 0.05$, this was significant only for the hydrogen peroxide content. At the same time, when expressing these indicators in the cold hardening variant as a percentage of unhardened control, they all had a strong negative correlation with seedlings frost resistance (-0.93, -0.75, and -0.94 for SAR generation, H_2O_2 and MDA content, respectively).

A significant positive correlation with seedling survival after freezing was observed for CAT and GPX activity as well as sugar content (Fig. 4). At the same time, there was a significant negative correlation between the proline content of hardened seedlings and their survival. Also noteworthy is the high negative correlation between hydrogen peroxide content and such indicators of protective systems as catalase activity and sugars content (Fig. 4).

Discussion

Cold hardening caused the seedlings of all the cultivars used in the experiment to develop frost resistance (Table 2). The winter wheat cultivars selected for the study differed significantly in their frost tolerance, which was clearly manifested at the seedling stage. For example, after freezing at -9°C, the survival rate of the most resistant cultivars Albidum 114 and Doskonala was between 74-88%, while that of the least resistant Malibu and Tobak was only 20-22%. Other cultivars were intermediate. These results indicated that such a set of varieties could be used as a model to investigate the relationship between frost resistance itself and the complex of indicators characterizing resistance to oxidative stress and functioning of the osmoprotective system.

It is also important that clear differences in frost resistance between the cultivars were evident after cold hardening. It was the biochemical changes caused by the action of moderately low temperatures that contributed to the frost-resistance property. Among these processes, regulation of pro-/antioxidant balance seems to play a particularly important role. Thus, despite the different values of $O_2^{\bullet-}$ generation, H_2O_2 and MDA content in seedlings of different cultivars, after hardening they decreased in most of them except for the least resistant Malibu and Tobak. When expressing these indicators in hardened seedlings as a percentage of those in unhardened ones, their significant negative correlation with frost resistance (survival after freezing)

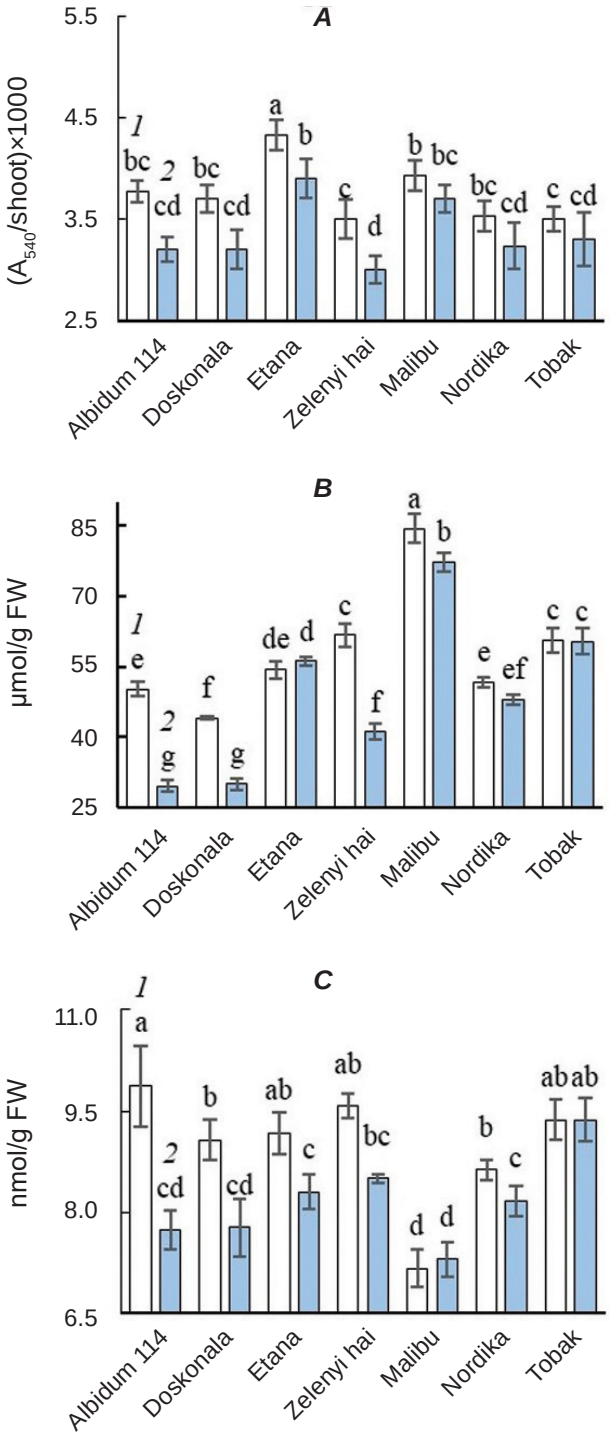

*Fig. 1. Superoxide anion radical (SAR) generation (**A**), hydrogen peroxide (**B**) and MDA contents (**C**) in wheat seedlings. 1 (white bars) – control; 2 (blue bars) – cold hardening (2°C, 6 days). The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

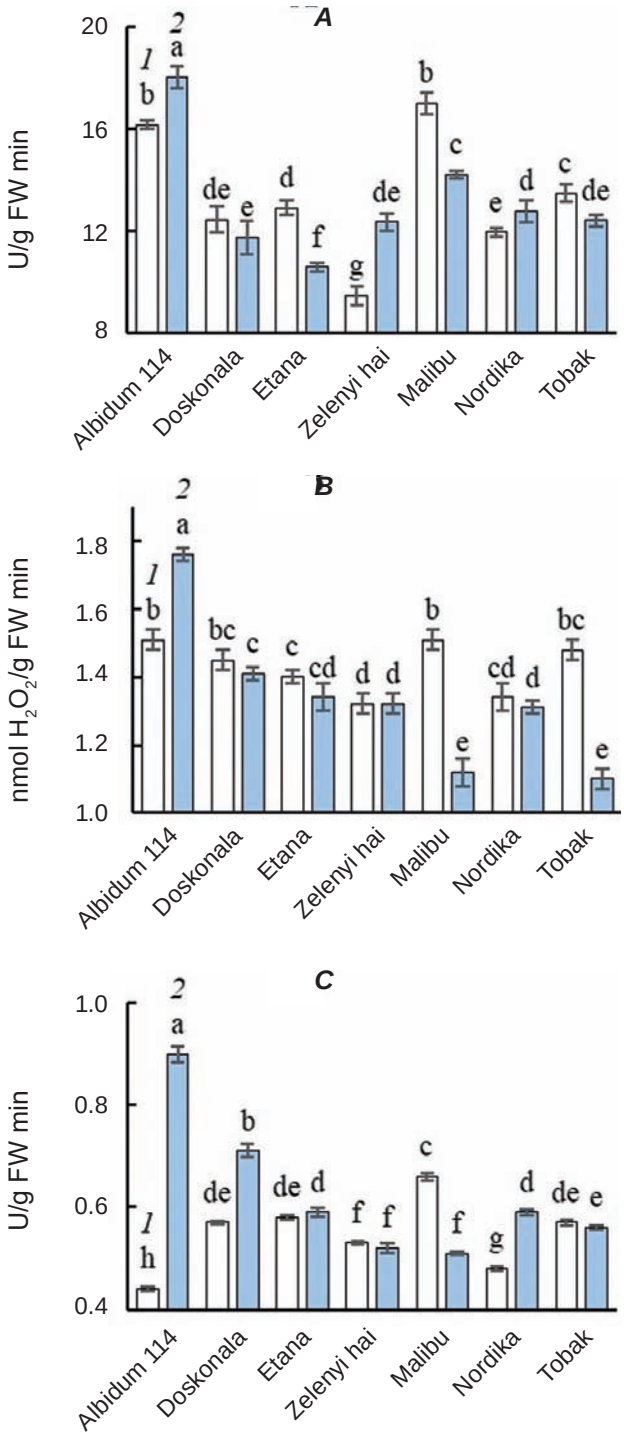

*Fig. 2. SOD (**A**), CAT (**B**) and GPX (**C**) activity in wheat seedlings. 1 – control; 2 – cold hardening (2°C, 6 days). The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

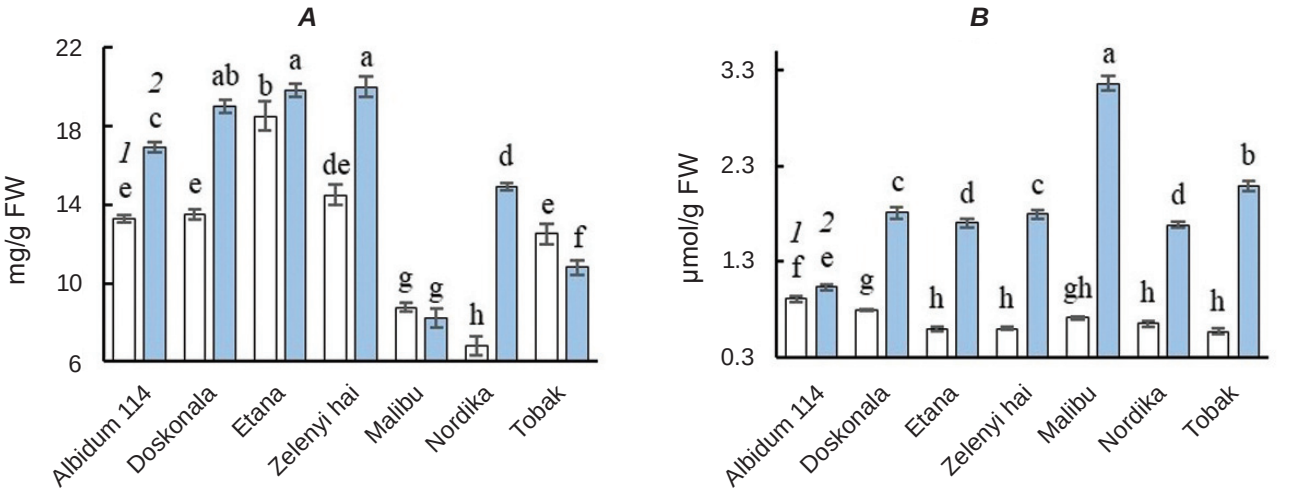

*Fig. 3. Sugars (**A**) and proline (**B**) contents in wheat seedlings. 1 – control (white bars); 2 – cold hardening (2°C, 6 days – blue bars). The same Latin letters denote quantities between which differences are not reliable for $P \leq 0.05$*

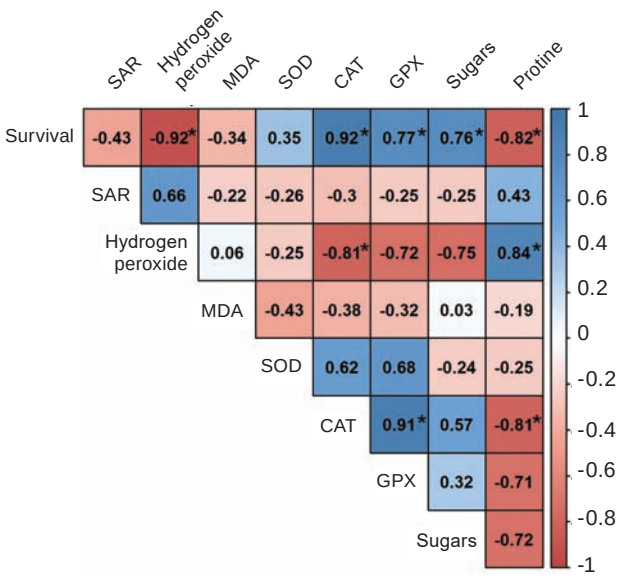

*Fig. 4. Correlation coefficients between survival rate and biochemical indices of wheat seedlings of cultivars studied under cold hardening (6 days at 2°C). *Significant at $P \leq 0.05$*

was evident. Therefore, the effect of hardening temperatures on the seedlings shifted the pro-/antioxidant balance towards the antioxidants and this effect was more pronounced in the frost-resistant cultivars.

The antioxidant system is involved in plant adaptation to stressors of various nature [35, 36], including exposure to low temperatures. Our results indicate the involvement of all the antioxidant enzymes studied in the regulation of ROS content in wheat seedling cells. Thus, in the most frost-resistant cultivar Albidum 114 and the moderately resistant Zelenyi hai, the decrease in SAR generation after cold hardening was accompanied by a simultaneous increase in SOD activity (Fig. 1, *A*, 2, *A*). On the other hand, the least resistant cultivars Malibu and Tobak did not show a significant reduction in superoxide radical content after hardening temperature, while they showed a decrease in SOD activity under low-temperature hardening conditions. In other cultivars, no relationship was observed between $O_2^{\bullet-}$ generation and SOD activity. SOD is known to be the only enzyme that neutralizes SAR [36, 37]. However, the $O_2^{\bullet-}$ content in tissues depends not only on the SOD activity, but also on the activity of enzymes that generate it, especially NADPH oxidase [11], as well as on the content of low-molecular-weight antioxidants, in particular ascorbic acid [37]. Apparently, combination of these factors is reflected in changes in the SAR amount in wheat seedlings of different cultivars during cold hardening.

Another ROS, hydrogen peroxide, decreased most markedly in high- and medium-resistant wheat cultivars under the influence of cold hardening (Fig. 2, *B*). Wherein, a high negative correlation (r = -0.92, Fig. 4) was observed between the H_2O_2 amount in tissues and frost resistance of hardened seedlings.

Excessive amounts of hydrogen peroxide in the cells are eliminated by a complex of antioxidant enzymes. Among these, CAT plays an important role. This enzyme requires no additional substrates

for activity and is very efficient at neutralizing high concentrations of H_2O_2 [37]. The results obtained indicate a relationship between hydrogen peroxide content in seedling shoots and CAT activity. For example, the most frost-resistant cultivar Albidum 114 showed a decrease in hydrogen peroxide content and an increase in CAT activity after hardening. At the same time, in the least resistant cultivar Tobak, exposure to hardening temperature did not cause a change in hydrogen peroxide content, while CAT activity decreased (Fig. 1, *B*, 2, *B*). The contribution of CAT to the regulation of hydrogen peroxide content in hardened seedlings is also indicated by the high negative correlation coefficient between these indices (r = -0.81, Fig. 4).

The results also point to the GPX contribution to the maintenance of redox balance. The enzyme activity after cold hardening increased significantly only in the frost-resistant cultivars Albidum 114 and Doskonala and, conversely, in one of the least resistant cultivars (Tobak), it decreased (Fig. 2, *C*). A fairly high correlation (r = 0.77, Fig. 4) was observed between GPX activity values and the survival of hardened seedlings. A fairly high (r = 0.72), although not significant at $P \leq 0.05$, correlation coefficient (Fig. 4) was also found between hydrogen peroxide content and the activity of this enzyme.

Other studies have found the effects of increased CAT activity in the cold adaptation in both etiolated [38] and green [10] seedlings of various cereal species. Thus, increased CAT activity and the induction of several peroxidase molecular forms were observed during cold adaptation of etiolated maize seedlings [38]. In wheat plants under low-temperature adaptation, an expansion of the electrophoretic spectrum of peroxidase was also found [39].

Thus, we can speak of an important role of antioxidant enzyme complex in the regulation of ROS content in cereal seedlings during adaptation to cold and of a close relationship between catalase and peroxidase activity and frost tolerance of wheat seedlings.

Thus, the important role of antioxidant enzymes complex in the regulation of ROS content in cereal seedlings during adaptation to cold and the close relationship between the activity of catalase and peroxidase and the frost resistance of wheat seedlings should be noted.

The group of low-molecular-weight organic compounds whose contribution to the frost resistance property has been studied for many decades are soluble carbohydrates. A moderate action of cold, which usually causes a hardening effect, leads to the accumulation of sugars in plants of different species [19]. In our experiments, the total content of sugars during hardening increased in most of the cultivars studied (Fig. 3, *A*). The exceptions were the most non-tolerant cultivars, Malibu and Tobak, which had no increase or even a decrease in sugars. Wherein a positive correlation significant at $P \leq 0.05$ was found between the absolute content of sugars in hardened seedlings and the frost resistance of cultivars (Fig. 4). It should be noted, however, that the contribution of sugars to the frost resistance property of cereals does not seem to be absolutely unambiguous and may differ according to species and cultivar characteristics. For example, the sugars content of the non-tolerant triticale cultivar was higher than that of the frost-resistant ones [23]. In general, sugars are considered to be polyfunctional protective compounds of plant cells. By interacting with membrane lipids and proteins, they can stabilize the structure and fluidity of cell membranes under hypothermic conditions [21]. Sugars are also seen as components of the plant cell's non-enzymatic antioxidant system [22]. Another function of sugars, important for adaptation to stress factors, may be related to their involvement in cell signaling processes [40].

Along with sugars, proline is a very important stress metabolite involved in low-temperature adaptation in plants. This imino acid acts as an osmoprotectant, membrane-protecting compound, and antioxidant [41]. An increase in proline content during cold hardening was recorded in Bermuda grass [42]. A report by Tantau et al. [43] showed a fairly close relationship between proline accumulation and the frost tolerance of barley lines grown *in vitro*. However, under the conditions of our experiments, the most significant increase in proline content in response to hardening temperatures was found in the least frost-resistant cultivars (Fig. 3, *B*), and a strong inverse correlation was observed between the absolute amount of proline in hardened seedlings and their frost tolerance (Fig. 4). This phenomenon may be a feature of etiolated seedlings. Although an inverse correlation between proline content and cold tolerance has been reported in several other studies, including those performed on green plants. As already noted, the organs of the cold-tolerant rice genotype exhibited lower proline content than those of the non-tolerant genotype [24]. No difference has been shown in the basal proline content in the leaves of winter and spring bread wheat [44]. After

Table 3. Pattern of changes in ROS generation, antioxidant enzyme activity, and osmolytes content under the action of hardening temperature (2°C, 6 days) on etiolated wheat seedlings of different cultivars

Indicators		High-resistant cultivars (Albidum 114, Doskonala)	Medium-resistant cultivars (Zelenyi hai, Nordika, Etana)	Low-resistant cultivars (Malibu, Tobak)
ROS generation	$O_2^{\bullet-}$	↓	↓	→
	H_2O_2	↓	↓ →	→
	MDA	↓	↓ →	→
Antioxidant enzyme activity	SOD	↑ →	↑ ↓	↓
	Catalase	↑ →	→	↓
	Guaiacol peroxidase	↑↑	↑ →	↓ →
Osmolytes content	Sugars	↑	↑	↓ →
	Proline	↑	↑	↑↑

Note. ↑ – increase; ↑↑ – strong increase; ↓ – decrease; → – no effect.

cold hardening, proline content increased, but there were no differences between the varieties. Proline content was found to increase during cold hardening of Arabidopsis plants, but no relation was found between the dynamics of proline accumulation and the development of frost tolerance [45]. The authors consider proline accumulation to be a consequence of the effects of low temperatures on plants, rather than a cause of resistance. In general, an increase in proline content is a non-specific response to thermal stress [46]. Apparently, when exposed to low positive temperatures, proline accumulation as an adaptive response is strongly activated precisely in non-tolerant genotypes.

Conclusions. The studied wheat varieties can be roughly divided into high- (Albidum 114, Doskonala), medium- (Zelenyi hai, Nordika, Etana), and low-resistant (Malibu, Tobak) to frost (Table 2). Adaptation of soft winter wheat seedlings of different cultivars includes marked changes in the pro-/antioxidant balance, antioxidant and osmoprotective system status indicators. The high-frost-resistant cultivars were characterized by a decrease in ROS content during hardening, an increase in the activity of antioxidant enzymes (especially catalase and guaiacol peroxidase), and an accumulation of sugars (Table 3).

In contrast, the non-frost-resistant cultivars were characterized by a lack of ROS reduction effect in response to hardening temperatures. Also in seedlings of non-resistant cultivars under the influence of low positive temperatures, CAT activity was reduced, sugars content was reduced or not changed, but proline content was sharply increased. In the group of medium-resistant cultivars, these values were mostly intermediate between the contrasting groups of cultivars (Table 3). Thus, there are indications of differences in the low-temperature adaptation strategies of wheat varieties that are evident at the germination stage. A complex of indicators of ROS content and the functioning of antioxidant and osmoprotective systems can be used to characterize frost tolerance when screening this property at the seedling stage.

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ПОКАЗНИКИ АНТИОКСИДАНТНОЇ ТА ОСМОПРОТЕКТОРНОЇ СИСТЕМ ПРОРОСТКІВ СОРТІВ ОЗИМОЇ ПШЕНИЦІ З РІЗНОЮ МОРОЗОСТІЙКІСТЮ

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Функціонування стрес-протекторних систем пшениці за дії холоду на ранніх етапах розвитку рослин залишається малодослідженим. Метою роботи було порівняльне дослідження антиоксидантної активності та вмісту цукрів і проліну як індикаторів осмопротекторної активності за холодової адаптації проростків семи сортів пшениці м'якої озимої (*Triticum aestivum* L.), які суттєво відрізняються за морозостійкістю. 3-денні етіольовані проростки загартовували при 2°С протягом 6 днів, а потім проморожували протягом 5 годин при -6 або -9°С. Через дві доби після проморожування оцінювали виживаність проростків за їх здатністю до росту. Виявлено зниження вмісту супероксидного аніон-радикала, підвищення активності антиоксидантних ензимів каталази та гваяколпероксидази та накопичення цукрів у пагонах високоморозостійких сортів під час загартування. Абсолютні значення активності каталази та гваяколпероксидази позитивно корелювали з морозостійкістю проростків. Зафіксовано негативний кореляційний зв'язок між морозостійкістю сортів і накопиченням проліну в проростках під час загартування. Зроблено висновок про можливість використання досліджених біохімічних показників для скринінгу морозостійкості сортів озимої пшениці у фазі проростків.

Ключові слова: окислювальний стрес, антиоксидантна система, осмоліти, морозостійкість, *Triticum aestivum*, холодове загартування.

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