

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 109, No. 4 (2022), p. 313–322

DOI 10.13080/z-a.2022.109.040

## State of antioxidant and osmoprotective systems in etiolated winter wheat seedlings of different cultivars due to their drought tolerance

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### Abstract

Climatic changes bring the problem of drought tolerance of winter cereals to the fore at the earliest stages of development, immediately after autumn sowing. The reaction of antioxidant and osmoprotective systems of etiolated seedlings of winter wheat (*Triticum aestivum* L.) under osmotic stress was investigated. Seeds of seven cultivars were germinated for 4 days in Petri dishes on filter paper moistened with distilled water (control) or nonpenetrating osmotic agent PEG 6000 at a concentration of 12% (osmotic pressure 0.17 MPa) at 24°C. After that, the mass of shoots and roots of seedlings of experimental and control treatments were determined. Biochemical parameters were also determined in the shoots of 4-day-old seedlings. The highest ability to maintain shoot growth under osmotic stress was in the 'Tobak'; it was medium in the 'Antonivka', 'Lira Odeska, and 'Darynka Kyivska', and low in the 'Bogdana', 'Doskonala', and 'Avgustina'. In the least resistant 'Doskonala' and 'Avgustina', the content of lipid peroxidation product malondialdehyde was significantly increased in the shoots under osmotic stress. In the non-resistant cultivars and, also, in the medium-resistant 'Darynka Kyivska', an increase in hydrogen peroxide content was noted under osmotic stress. High- and medium-resistant cultivars were characterised by an increase in superoxide dismutase (SOD) activity in response to osmotic stress. Also, high- and medium-resistant cultivars showed a high catalase (CAT) activity. Osmotic stress caused an increase in guaiacol peroxidase activity in all cultivars, regardless of their drought tolerance. The proline content increased to some extent in all cultivars in response to stress exposure; however, no relationship was found between the amount of proline and drought resistance. The content of sugars under osmotic stress increased only in high and medium drought-resistant cultivars. Drought-resistant cultivars were also characterised by a high base content of flavonoid compounds absorbing UV-B and their retention under osmotic stress. Under osmotic stress, the strongest correlation ( $p \leq 0.05$ ) was found between the resistance to osmotic stress and the SOD activity ( $r = 0.93$ ) as well as the sugar content ( $r = 0.85$ ). In addition, a significant correlation was found between the drought tolerance and the flavonoid content and the CAT activity in seedlings. The complex of these indicators can be used to assess the drought tolerance of various genotypes of etiolated winter wheat seedlings.

Keywords: *Triticum aestivum*, drought tolerance, antioxidant enzymes, proline, sugars, reactive oxygen species.

### Introduction

In recent years, the average loss of grain crops due to drought in the world was about 10% (Lesk et al., 2016). At the same time, an increase in the frequency and intensity of drought is expected to be associated with both a decrease in precipitation and an increase in evaporation caused by global warming (Hasanuzzaman et al.,

2018). Climate change poses a serious threat to global food security, and the identification of drought-tolerant genotypes remains one of the most effective approaches to address these challenges (Chowdhury et al., 2021).

One of the early negative effects of drought is an excessive formation of reactive oxygen species (ROS)

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Kolupaev Y. E., Yastreb T. O., Saliy A. M., Kokorev A. I., Ryabchun N. I., Zmiievskaya O. A., Shkliarevskiy M. A. 2022. State of antioxidant and osmoprotective systems in etiolated winter wheat seedlings of different cultivars due to their drought tolerance. Zemdirbyste-Agriculture, 109 (4): 313–322. <https://doi.org/10.13080/z-a.2022.109.040>

due to the disruption of electron transport processes in chloroplasts and mitochondria. Restriction of carbon dioxide supply due to stomata closure leads to the over-reduction of chloroplast electron transport chain and ROS production (de Carvalho, 2008). It is also known that mitochondria contribute significantly to oxidative damage in plants under drought conditions. In wheat leaves, the content of carbonylated proteins increased by 2.5–2 times in chloroplasts and by 3–5 times in mitochondria under drought conditions (Bartoli et al., 2004).

The drought tolerance in plants depends on the regulation of thousands of genes and multiple metabolic pathways (Fang, Xiong, 2015). The activation of antioxidant system and the accumulation of osmolytes belong to the key interrelated responses of plants to drought (An et al., 2013; Ajithkumar, Panneerselvam, 2014; Blum, 2017). These responses are measurable and can be used to characterise drought resistance properties (Laxa et al., 2019). The interaction between ROS and antioxidants plays a crucial role in the mechanisms that allow plants to conserve water, ensure biomass accumulation, and prevent crop losses during drought (Akinroluyo et al., 2020; Rane et al., 2022). It should be noted that a number of low-molecular-weight compounds that accumulate in response to drought are polyfunctional: they act as both osmolytes and antioxidants and some of them, such as proline, are also involved in the cell signalling processes (de Carvalho et al., 2013; Kolupaev et al., 2019).

Wheat is one of the most important cereals, the growth and development of which is severely limited by drought (Marček et al., 2019). Numerous studies have investigated the status of the antioxidant system of wheat in connection with the drought tolerance of different cultivars (Sheoran et al., 2015; Abid et al., 2018; Lou et al., 2018; Nasirzadeh et al., 2021). However, mostly only selected indicators of the antioxidant system were investigated using a minimal number of tolerant and sensitive cultivars (Laxa et al., 2019). Studies performed using green 20-day-old plants of four wheat cultivars showed that drought-resistant cultivars increased the activity of antioxidant enzymes in response to stress, while non-resistant ones accumulated non-enzymatic components of the antioxidant defence system (Kirova et al., 2021). Sufficiently detailed comparative studies of the possible relationship between the activity of antioxidant enzymes and the expression of their genes and drought resistance were carried out at the tillering stage of plants as well as at the generative phases (Sheoran et al., 2015). The protective systems at the earliest developmental stages of winter wheat have been less studied. However, seed germination and growth of etiolated seedlings are the first and very important stages of the plant life cycle (Hasanuzzaman et al., 2018). They determine the subsequent features of plant growth, development, and yield.

In recent years, the problem of autumn drought has become acute and has had an extremely negative impact on the further development of winter cereals for Ukraine and many other countries. In particular, over the past two decades, in the Steppe zone of Ukraine, the amount of precipitation in August decreased by 16.7 mm and in September by 3.5 mm. At the same time, the average temperature in August and September increased by 2.8°C and 1.9°C, respectively (Romanenko et al., 2018).

The functioning of antioxidant and osmoprotective systems in etiolated seedlings differs significantly compared to green vegetative plants (Altaf et al., 2021). In particular, young cereal seedlings are characterised by a high content of low-molecular-weight compounds (proline, sugars, flavonoids, etc.) that can perform

protective functions (Kolupaev et al., 2020). Previously, using three wheat cultivars of different ecotypes (steppe, forest-steppe, and forest), an association between the dehydration tolerance of seedlings, the activity of one of the key antioxidant enzymes superoxide dismutase (SOD) in them, and their field drought resistance (ability to maintain yield in dry years) has been shown (Обозний и др., 2013). In this regard, information on the functioning of stress-protective systems in seedlings can be partly extrapolated to characterise their resistance at later stages of development. However, a comprehensive study on the antioxidant and osmoprotective systems in etiolated wheat seedlings using a sufficient number of cultivars with different drought resistance has not yet been carried out. Obtaining such information can be useful both for a deeper understanding of differences in adaptive strategies of cultivars and for drought tolerance screening in winter wheat using germinating seeds.

In this regard, the aim of the experiment was to evaluate the state of antioxidant and osmoprotective systems in etiolated seedlings of seven winter wheat cultivars differing in drought resistance. Also, the tasks of the research included determining the correlations between the studied indicators.

## Material and methods

**Plant material.** In the experiment, winter wheat (*Triticum aestivum* L.) cultivars developed for cultivation in various climatic zones were used. Five cultivars created in Ukraine: the 'Doskonala', designed mainly for cultivation in the Forest-steppe and characterised by a low drought resistance (Karpets et al., 2016), the 'Darynka Kyivska' and 'Bogdana', characterised as moderately drought-resistant and intended for cultivation in different zones (Chernobai et al., 2019; Khomenko, 2020), and the 'Lira Odeska' and 'Antonivka', which have an ecological plasticity necessary for growing in the arid Steppe zone (Khakhula et al., 2013). Also, for the experiment, the cultivar 'Tobak' was used, designed for cultivation in Central Europe but capable of maintaining the normal functioning of the photosynthetic apparatus and productivity under drought and high temperatures (Hlaváčová et al., 2017; Urban et al., 2018). In addition, the cultivar 'Avgustina' was used, created for cultivation in the Belarusian Polesie with humid climate (Scientific and Practical Center of the National Academy of Sciences of Belarus).

The grains were disinfected for 30 min with a 6% H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) solution and washed with distilled water. Then, they were germinated for 4 days in Petri dishes on filter paper moistened with distilled water (control treatment) or non-penetrating osmotic agent PEG 6000 at a concentration of 12% (osmotic pressure 0.17 MPa) (Money, 1989) at 24°C temperature. The level of the stress effect, which causes the inhibition of the growth of seedlings by approximately 35–50%, was chosen based on the preliminary experiment using PEG 6000 solutions with an osmotic pressure in a range of 0.12–0.24 MPa. In 4-day-old control and experimental seedlings, the mass of roots and shoots, their water content and biochemical parameters were measured. The stress level at which the biomass accumulation of seedling organs was approximately 50–65% of control values was selected based on the preliminary experiment using the PEG 6000 solutions with an osmotic pressure in a range of 0.12–0.24 MPa.

**Determination of water content** in organs of seedlings was carried out gravimetrically by drying at 103°C to a constant weight of a sample of shoots or roots (500 mg of fresh material).

**Evaluation of  $H_2O_2$  content.** To determine  $H_2O_2$  content, the shoots of seedlings were homogenised in the cold with 5% TCA (trichloroacetic acid). The samples were centrifuged at  $8000\times g$  for 10 min in a centrifuge MPW 350R (MedInstruments, Poland) at  $2-4^\circ C$ . The concentration of  $H_2O_2$  was determined in a supernatant using the ferrothiocyanate method (Sagisaka, 1976) with minor modifications. To do this, 0.5 ml of 2.5 M KSCN (potassium thiocyanate), 0.5 ml of 50% TCA, 1.5 ml of the supernatant, and 0.5 ml of 10 mM iron (II)  $(NH_4)_2SO_4$  (ammonium sulphate) were added to the tubes. After mixing, the samples were poured into cuvettes and the absorbance at 480 nm was determined.

**Evaluation of the content of lipid peroxidation (LPO) products.** The rate of LPO in the shoots of seedlings was assessed by the content of its products reacting with 2-thiobarbituric acid (TBA), mainly malondialdehyde (MDA) (Kolupaev et al., 2015). The plant material was homogenised in a 0.1 M Tris-HCl buffer (pH 7.6); the homogenate was then supplemented with 0.5% TBA solution in 20% TCA. After heating the mixture in a boiling water bath for 30 min, the samples were cooled and centrifuged at  $8000\times g$  for 10 min. The absorbance of the supernatant was then measured at 532 nm. The 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 the activity of antioxidant enzymes.** Activity of 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), was measured according to the protocols described earlier (Kolupaev et al., 2015). Seedling samples were homogenised in cold 0.15 M K, Na-phosphate buffer (pH 7.6) with the addition of EDTA (0.1 mM) and dithiothreitol (1 mM). The homogenate was centrifuged at  $8000\times g$  for 15 min in a centrifuge MPW 350R (MedInstruments) at temperature no more than  $4^\circ C$  to prepare the supernatant, which was then assayed. SOD activity was determined at pH 7.6 using a method based on the enzyme ability to compete with nitroblue tetrazolium for superoxide anions produced by the aerobic interaction between NADH (nicotinamide adenine dinucleotide reduced) and PMS (phenazine methosulfate). CAT was evaluated through the amount of  $H_2O_2$  decomposed in a time unit (Aebi, 1984). For this purpose, 1 ml of 30 mM  $H_2O_2$  was added to 2 ml of the supernatant in the cuvette, and the decrease in absorbance of the solution at 240 nm was determined for 3 min. The reference solution contained 0.15 M K, Na-phosphate buffer instead of the supernatant. GPX activity was estimated using guaiacol as a donor of hydrogen and  $H_2O_2$  as a substrate. The absorbance was measured at 470 nm. The protein content in the samples was measured according to Bradford (1976) using bovine serum albumin as a standard.

**Estimation of low-molecular-weight protectors.** Total sugar content in the seedlings was assayed by the Morris-Roe method using an anthrone reagent with the modifications described earlier (Kolupaev et al., 2015). Sugars were extracted from plant materials with distilled water by 10-min heating in a boiling water bath. Clarification of the obtained extract was carried out by adding equal volumes (0.3–0.4 ml) of 30%  $ZnSO_4$  (zinc sulphate) and 15% yellow blood salt to the reaction tubes. The samples were filtered through paper filter and, if necessary, the pre-measurement filtrate was diluted several times with distilled water. The reaction

tubes contained 3 ml of an anthrone reagent and 1 ml of a filtrate, and distilled water was added to the control samples instead of the filtrate. The samples were then boiled for 7 min in a water bath, followed by cooling to room temperature. The absorbance of the samples was determined relative to the control solution at 610 nm using D-glucose as a standard.

Proline content was evaluated using a ninhydrin reagent (Bates et al., 1973). Proline was extracted from the plant material with distilled water by boiling for 10 min. Afterwards the extract was filtered, equal volumes of the 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 coloured reaction product was determined at 520 nm using L-proline as a standard.

To assay the content of anthocyanins and UV-B absorbing flavonoids, samples of the shoots were homogenised in 1% HCl (hydrochloric acid) in methanol. After the homogenate centrifugation at  $8000\times g$  for 15 min, the absorbance of the supernatant was estimated at 530 and 300 nm (Nogués, Baker, 2000). The results were expressed in absorbance per dry matter weight of the plant material.

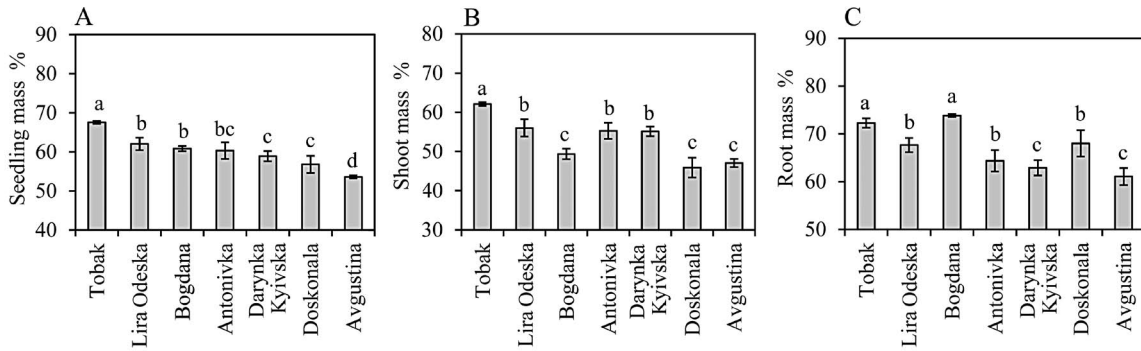
**Replicate of experiment.** The experiment had 3–4 biological replicates. When assessing the weight of seedling organs and their water content, each biological replicate consisted of 30 seedlings. When measuring biochemical parameters, each sample of plant material was taken from 12–15 seedlings.

**Statistical analysis** of the results was carried out using the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and the table show the mean values from three biological replicates and their standard errors. Different letters indicate values with differences that are significant at  $P \leq 0.05$ . Correlation coefficients were estimated using the programming language R, version 4.1.1 (R Core Team).

## Results

**Biomass accumulation of winter wheat seedlings.** Cultivar 'Tobak' showed the greatest ability to accumulate the total biomass of seedlings under osmotic stress (Figure 1A). Slightly lower biomass accumulation under osmotic stress was observed in the 'Lira Odeska' and 'Antonivka', designed for cultivation in the southern Steppe zone as well as in the 'Bogdana', designed for cultivation in various climatic zones of Ukraine. There were no significant differences in this trait between these cultivars. A significant inhibition of the whole seedling biomass accumulation under the PEG 6000 action was noted in the 'Darynka Kyivska' and 'Doskonala'. Finally, an almost twofold decrease in the accumulation of seedling biomass was found in the 'Avgustina', created in the humid conditions of the Belarusian Polesie.

In the presence of PEG 6000, the reduction in shoot biomass accumulation was more significant than that of roots in all cultivars tested. Thus, in the 'Doskonala', 'Avgustina', and 'Bogdana', the biomass of the aerial parts under osmotic stress was less than 50% of the control values (Figure 1B). 'Lira Odeska' and 'Antonivka' showed a more pronounced ability to maintain shoot growth under osmotic stress. The highest accumulation of shoot biomass as well as seedlings in general (mass of shoots and roots) under osmotic stress was noted in the 'Tobak'. The distribution of cultivars according to the root growth under osmotic stress was somewhat different (Figure 1C). It was the highest in



Note. Mean values and their standard errors for three replicates are given; different letters indicate the values that are significant at  $p \leq 0.05$ .

Figure 1. Weight of winter wheat seedlings (A), shoots (B), and roots (C) mass under osmotic stress (12% PEG 6000)

the ‘Bogdana’ and ‘Tobak’ and slightly lower in the ‘Doskonala’, ‘Lira Odeska’, and ‘Antonivka’. Finally, the strongest inhibition of root growth was observed in the ‘Avgustina’ and ‘Darynka Kyivska’.

The studied cultivars differed in the constitutive shoot-to-root ratio (Table). In the control conditions, it was the highest in the ‘Avgustina’ and ‘Bogdana’. This ratio also was more than one in the ‘Darynka Kyivska’ and ‘Doskonala’. Smaller base values were typical of the ‘Antonivka’, ‘Tobak’, and ‘Lira Odeska’. Under stress exposure, the shoot-to-root mass ratio in the ‘Bogdana’, ‘Doskonala’, and ‘Avgustina’ significantly decreased. A less noticeable shift in this ratio was in the ‘Darynka

Kyivska’, ‘Tobak’, ‘Antonivka’, and ‘Lira Odeska’, which indicates a greater resistance of these cultivars in comparison to the ‘Avgustina’, ‘Bogdana’, and ‘Doskonala’.

**Water content in organs of winter wheat seedlings.** The basic water content in shoots of winter wheat seedlings did not differ significantly (Figure 2A). A slightly lower water content than in the other cultivars was found in the ‘Tobak’, ‘Lira Odeska’, and ‘Bogdana’. When seedlings were grown in the presence of PEG 6000, the amount of water in the shoots was significantly reduced in all cultivars, except for the ‘Tobak’.

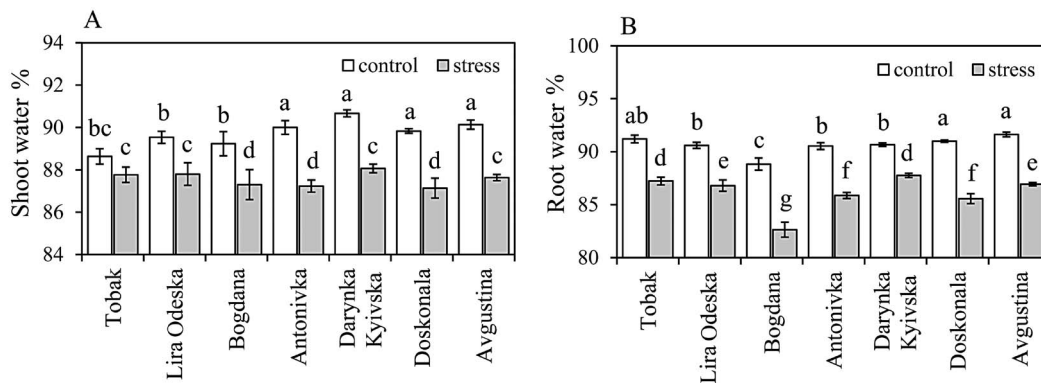
Table. Shoot-to-root ratio of winter wheat seedlings under normal moisture (control) and osmotic stress (12% PEG 6000)

Cultivar	Mass of shoots/roots		Stress/control
	control	stress	
Tobak	0.87 ± 0.005 c	0.75 ± 0.020 d	0.86 ± 0.018 a
Lira Odeska	0.92 ± 0.036 c	0.76 ± 0.002 d	0.83 ± 0.031 ab
Bogdana	1.13 ± 0.022 a	0.76 ± 0.024 d	0.67 ± 0.021 c
Antonivka	0.80 ± 0.011 d	0.69 ± 0.013 e	0.86 ± 0.005 a
Darynka Kyivska	1.06 ± 0.012 b	0.93 ± 0.010 c	0.88 ± 0.015 a
Doskonala	1.03 ± 0.004 b	0.69 ± 0.038 e	0.68 ± 0.038 c
Avgustina	1.15 ± 0.012 a	0.89 ± 0.054 c	0.77 ± 0.039 b

Explanation under Figure 1

The water content in the roots of cultivars grown under normal moisture conditions (control treatment) also differed insignificantly – it was the highest in the ‘Avgustina’ and the lowest in the ‘Bogdana’ (Figure 2B).

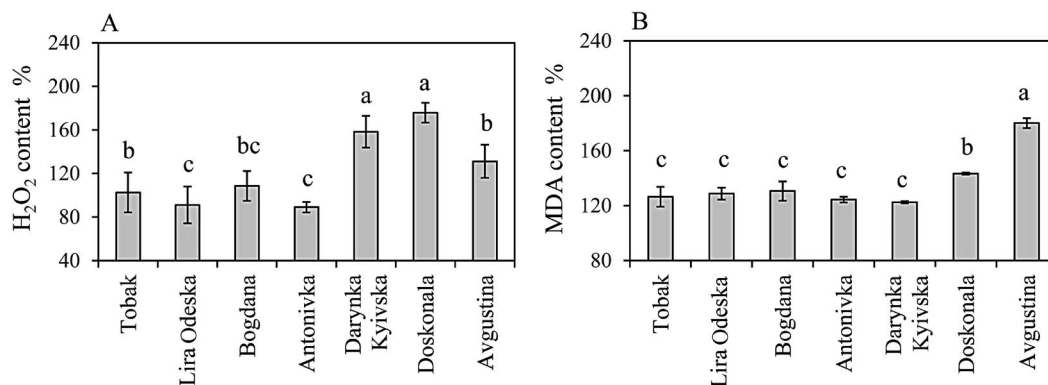
Under osmotic stress, the water content decreased in the roots of all studied cultivars. The lowest water content during the germination under osmotic stress was recorded in the roots of ‘Bogdana’ seedlings.



Explanation under Figure 1

Figure 2. Water content in the shoots (A) and roots (B) of winter wheat seedlings under osmotic stress (12% PEG 6000)

**Content of  $H_2O_2$  and MDA.** In the presence of PEG 6000, the shoots of ‘Doskonala’ and ‘Darynka Kyivska’ seedlings showed a significant (by 75% and 55%, respectively) increase in the content of  $H_2O_2$  (Figure 3A). In the ‘Avgustina’, this increase was more than 20%. At the same time, no significant changes in the  $H_2O_2$  content in shoots were observed in other tested cultivars. The content of LPO products (mainly MDA) under moderate osmotic stress conditions increased significantly only in the ‘Avgustina’ and ‘Doskonala’ (Figure 3B).



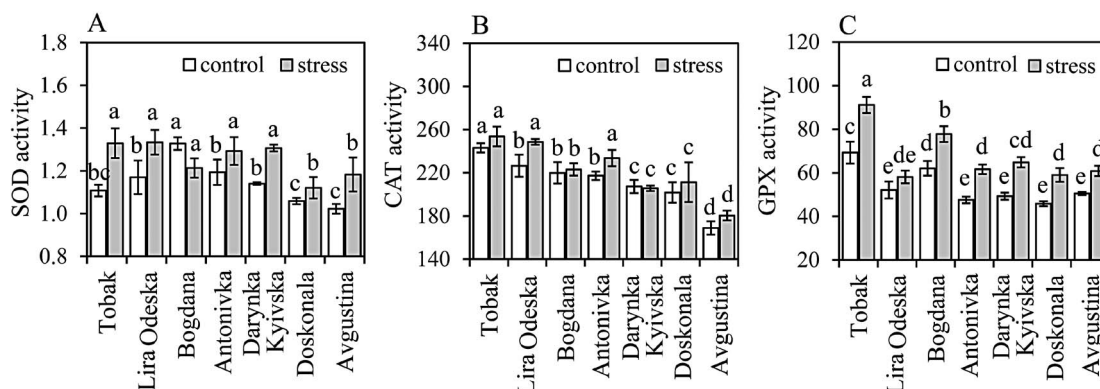
Explanation under Figure 1

**Figure 3.** Content of hydrogen peroxide ( $H_2O_2$ ) (A) and malondialdehyde (MDA) (B) in the shoots of winter wheat seedlings under osmotic stress (12% PEG 6000)

CAT was the highest in the shoots of ‘Tobak’ seedlings; it was also quite high in the ‘Lira Odeska’ (Figure 4B).

‘Avgustina’ had a very low activity of the enzyme. Moderate osmotic stress did not cause significant changes in the CAT in most cultivars; a significant increase ( $p \leq 0.05$ ) in the CAT was recorded only in the ‘Lira Odeska’. The highest absolute values

of enzyme activity under osmotic stress were observed in the ‘Tobak’, ‘Lira Odeska’, and ‘Antonivka’. In the absence of stress, GPX activity was higher in the ‘Tobak’ and ‘Bogdana’ compared to other cultivars (Figure 4C). Osmotic stress caused a noticeable increase in the enzyme activity in most cultivars. Absolute values were the highest in the ‘Tobak’ and ‘Bogdana’.



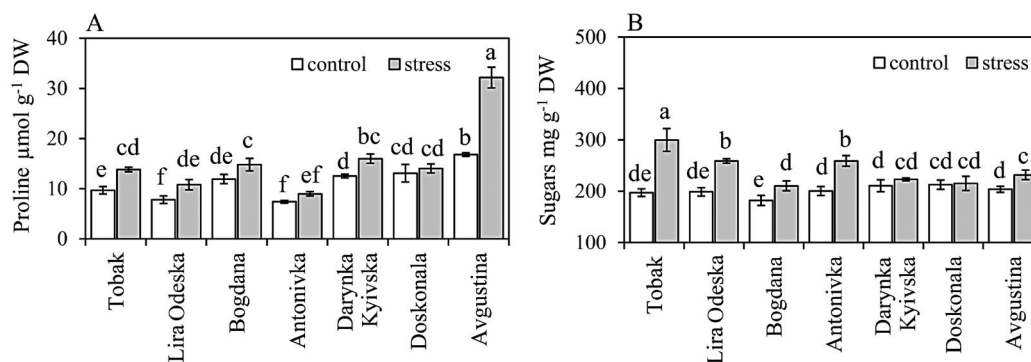
Explanation under Figure 1

**Figure 4.** Activity ( $U \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) of superoxide dismutase (SOD) (A), catalase (CAT) (B), and guaiacol peroxidase (GPX) (C) in the shoots of winter wheat seedlings under osmotic stress (12% PEG 6000)

**Content of proline and sugars.** The proline content in shoots in the absence of stress varied: the highest values were typical for the ‘Avgustina’ and the lowest for the ‘Antonivka’ and ‘Lira Odeska’ (Figure 5A). Under osmotic stress, the content of proline increased in most cultivars, no significant changes were noted only in the ‘Doskonala’ and ‘Antonivka’. The constitutive total content of sugars in the shoots of seedlings differed only slightly between the cultivars. In response to osmotic stress, the sugars content increased most significantly

in the resistant ‘Tobak’, ‘Lira Odeska’, and ‘Antonivka’ (Figure 5B).

**Content of flavonoid compounds.** During the seed germination under optimal moisture conditions, the content of anthocyanins in seedling shoots varied quite significantly. The highest base content of anthocyanins was in the ‘Avgustina’, and it was also quite high in the ‘Lira Odeska’ and ‘Antonivka’. Under osmotic stress, the content of anthocyanins slightly increased only in the ‘Tobak’. At the same time, in the ‘Avgustina’ and



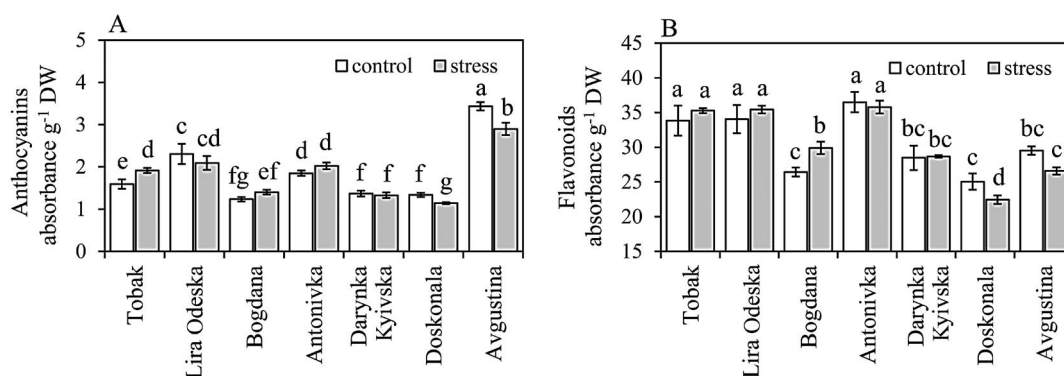
Explanation under Figure 1

**Figure 5.** Content of proline (A) and sugars (B) in the shoots of winter wheat seedlings under osmotic stress (12% PEG 6000)

‘Doskonala’ the amount of anthocyanins decreased under osmotic stress (Figure 6A). Under normal moisture conditions (control treatment), the content of “colourless” flavonoids capable of absorbing UV-B was highest in the ‘Antonivka’, ‘Lira Odeska’, and ‘Tobak’ (Figure 6B).

The impact of osmotic stress caused minor and multidirectional changes in the content of these

compounds. In response to stress, a slight increase in the content of flavonoids was noted in the ‘Bogdana’. At the same time, the ‘Avgustina’ and ‘Doskonala’ showed a decrease in their content. In other studied cultivars, this parameter practically did not change. The highest values against the stress background were characteristic of the ‘Antonivka’, ‘Lira Odeska’, and ‘Tobak’ (Figure 6B).



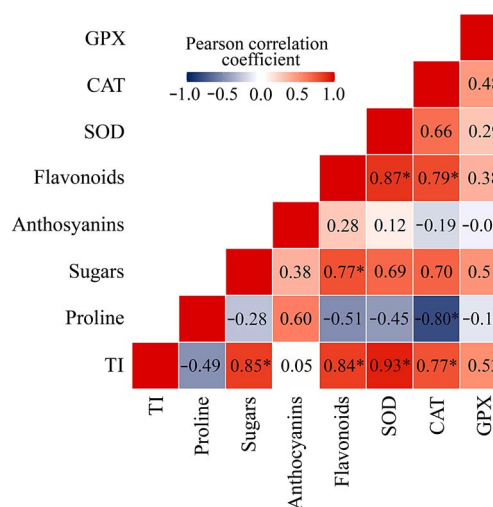
Explanation under Figure 1

**Figure 6.** Content of anthocyanins (A) and flavonoids absorbing UV-B (B) in the shoots of winter wheat seedlings under osmotic stress (12% PEG 6000)

#### Correlation between the tolerance index (TI) and indicators of stress protection system functioning.

Based on the obtained results, the correlation between indicators of the antioxidant system status and the growth rate of shoots under osmotic stress (tolerance index) was also calculated (Figure 7).

Under osmotic stress, the SOD activity reached high values in the cultivars with a high and medium drought tolerance: ‘Tobak’, ‘Lira Odeska’, ‘Antonivka’, and ‘Darynka Kyivska’. The correlation coefficient between the accumulation of shoot biomass and the SOD activity turned out to be very high, 0.93. Under drought stress, the CAT activity increased only in certain cultivars (‘Lira Odeska’ and ‘Antonivka’). However, at the same time, the correlation between the enzyme activity and the index of plant tolerance to osmotic stress was significant at  $p \leq 0.05$ . Nevertheless, the correlation between the GPX activity indices and seedling drought tolerance was not significant. A negative correlation was found between the drought resistance index and the proline content, although it was not significant. Under the PEG 6000 action, in three high- and medium-resistant cultivars (‘Tobak’, ‘Lira Odeska’, and ‘Antonivka’), a significant increase in sugar content was observed as well as a strong



\* – significant at  $P \leq 0.05$

**Figure 7.** Correlation between the tolerance index (TI) (weight of winter wheat shoots, % to control) and indicators of stress protection system functioning under osmotic stress (12% PEG 6000)

correlation between the sugar content and the shoot growth under osmotic stress (Figure 7).

No relationship between the anthocyanins content and drought tolerance was found in the wheat seedlings. However, under osmotic stress, a significant positive correlation was observed between the shoot growth rate and the content of colourless UV-B absorbing flavonoids (Figure 7).

## Discussion

The winter wheat cultivars studied, created in different natural and climatic zones of Ukraine, Belarus, and Central Europe, differed considerably in their sensitivity to model drought created with PEG 6000 at the etiolated seedling stage. So, based on the accumulation of total seedling biomass under moderate osmotic stress conditions, the 'Tobak' showed the greatest drought resistance. 'Lira Odeska', 'Bogdana', and 'Antonivka' turned out to be relatively resistant. The growth of 'Darynka Kyivska' seedlings was more noticeably inhibited under osmotic stress than in previous cultivars. The seedlings of 'Avgustina' and 'Doskonala' showed the greatest sensitivity to drought. Thus, the relationship between the ability to accumulate the total seedling biomass under osmotic stress and field drought tolerance data was not clear. In particular, the medium-resistant group included cultivars created both in the arid Steppe zone ('Antonivka' and 'Lira Odeska') and in the more humid northern Forest-steppe zone ('Bogdana') of Ukraine (Khakhula et al., 2013; Chernobai et al., 2019; Khomenko, 2020).

It is known that more important characteristics of drought tolerance in wheat cultivars than total mass accumulation are the ability to maintain growth of the above-ground part and the shoot-to-root mass ratio close to the control under stress conditions (Laxa et al., 2019). Indeed, the differentiation of cultivars in terms of resistance to dehydration was clearer in these seedling indices. The highest relative rate of shoot biomass accumulation under the PEG 6000 action as well as the biomass of whole seedlings was noted in the 'Tobak' (Figure 1), characterised by a high field drought tolerance under the European conditions (Hlaváčová et al., 2017; Urban et al., 2018). The same cultivar under osmotic stress conditions retained the shoot-to-root ratio close to the control as well as the water content in tissues (Table).

The other group consisted of medium-resistant cultivars designed in the Steppe – the 'Lira Odeska' and 'Antonivka' as well as the 'Darynka Kyivska', a modern cultivar created in the Forest-steppe. In these three cultivars, the values of the biomass accumulation index of the aerial part of seedlings were almost the same. Also, under drought stress conditions, these cultivars retained a similar shoot-to-root ratio as in the control conditions. In the 'Bogdana' (created in the north of the Forest-steppe of Ukraine), the accumulation of shoot biomass under osmotic stress was significantly less. And, finally, those parameters of 'Avgustina' and 'Doskonala' (created in the north-eastern Ukrainian Forest-steppe and in the Belarusian Polesie, respectively) were the lowest. In general, the most noticeable differentiation of cultivars was observed in the shoot mass (Figure 1B).

One of the indicators of oxidative stress under the influence of external adverse factors is the  $H_2O_2$  accumulation (Hasanuzzaman et al., 2020). In our experiment, a significant increase in the  $H_2O_2$  content was observed in the cultivars with the highest inhibition of shoot biomass accumulation under the PEG 6000 action,

'Doskonala' and 'Avgustina'. However, a significant increase in the  $H_2O_2$  content in shoots was also found in the medium-resistant 'Darynka Kyivska'. Thus, the relationship between changes in  $H_2O_2$  content in response to dehydration stress and varietal resistance was evident, but not sufficiently clear.

The most significant disturbances in the pro-/antioxidant balance in cells usually lead to the accumulation of lipid peroxidation products, mainly MDA (Gruznova et al., 2017; Hasanuzzaman et al., 2020). A significant increase ( $p \leq 0.05$ ) in the MDA content under osmotic stress was recorded in the seedlings of cultivars whose growth was the most significantly inhibited in the presence of PEG 6000, namely, 'Avgustina' and 'Doskonala'. These results indicate quite clearly a relationship between the resistance of cultivars to oxidative stress and their ability to grow under drought conditions. In turn, resistance to oxidative stress is largely determined by the state of the antioxidant system. The activity of key antioxidant enzymes and the content of some low-molecular antioxidants, some of which (proline, sugars) also have osmoprotective, membrane-protective, and chaperone properties, were investigated (Liang et al., 2013; Kolupaev et al., 2019).

As the only enzymatic antioxidant that neutralises radical ROS, SOD is considered a particularly important component of the antioxidant system (Hasanuzzaman et al., 2020). In our experiment, against the background of stress exposure, a high correlation ( $r = 0.93$ ) was noted between the shoot growth rates under stress conditions and SOD activity. Like SOD, CAT does not require additional substrates to be active and neutralises high concentrations of  $H_2O_2$  very effectively (Hasanuzzaman et al., 2020). CAT, especially its molecular form CAT2, has been reported to play an important role in the detoxification of  $H_2O_2$  under severe drought stress conditions (Sofa et al., 2015). In our experiment, a significant correlation between CAT and the ability of cultivars to maintain shoot growth under osmotic stress was found. GPX belongs to stress-induced enzymes; however, along with antioxidant activity, it can also exhibit oxidase activity associated with the electron transfer from reducing agents to oxygen and the formation of superoxide anion radicals and  $H_2O_2$  (Kolupaev et al., 2019). In our experiment, no significant correlation between the activity of GPX and the seedling tolerance index under osmotic stress was found. Thus, the GPX contribution to the antioxidant system in winter wheat seedlings under osmotic stress is difficult to interpret unambiguously. There was evidence that in more drought-resistant genotypes, the gene expression and activity of CAT and ascorbate peroxidase (APX) increased to a greater extent, while GPX was activated in sensitive cultivars (Laxa et al., 2019).

In general, the results of our experiment indicate a significant contribution of SOD and CAT to the antioxidant defence system in drought-resistant winter wheat seedlings. In part, these results are consistent with the data of other authors obtained for winter wheat at later stages of ontogeny. Thus, in the study of Kirova et al. (2021), performed on 10–20-day-old plants of two winter wheat cultivars, it has been shown that the preferred mechanism for preventing the excessive oxidative stress in a tolerant cultivar was the activation of the enzymatic part of antioxidant defence system, while in a sensitive one the accumulation of non-enzymatic antioxidants has been noted. Another study (Nasirzadeh et al., 2021), also conducted on 20-day-old winter wheat plants, has shown that higher activity and genes expression for SOD, CAT, and APX, characteristic of resistant cultivars, manifested only under the stress. In the study of Abid et al. (2018),

which used tillered plants, it was found that under drought stress conditions, the activity of SOD, CAT, and APX in a resistant winter wheat cultivar was also higher. Thus, it can be stated that the key antioxidant enzymes are involved in the adaptation of winter wheat plants to drought at different stages of ontogeny.

As already noted, the accumulation of osmolytes is of great importance for adaptation to drought. The main osmolytes of plant cells also have antioxidant properties; this concerns proline, a polyfunctional stress metabolite with antioxidant, membrane-protective, and chaperone properties (Liang et al., 2013). However, in our experiment, we failed to find a relationship between the proline content in seedling shoots and their drought resistance under stress conditions. A negative correlation between the proline content and drought tolerance was also shown for triticale (Saed-Moucheshi et al., 2019). Despite the importance of proline as a multifunctional protector, a lower increase in its content under moderate stress may indicate a greater resistance of plants (Kuznetsov, Shevyakova, 1999). It is possible that under moderate stresses the accumulation of proline as an adaptive response is switched on in sensitive cultivars, while at the same time under stronger stresses its accumulation is more noticeable in resistant plants. Thus, it was shown on 20-day-old winter wheat plants that the proline content in the leaves of the most drought-resistant cultivar exceeded those in other cultivars only during very severe drought (Nasirzadeh et al., 2021).

Another group of compatible osmolytes accumulating in plants under the action of stress factors are sugars. Their level is important for osmotic adaptation and mainly correlates with drought tolerance (Stagnari et al., 2016). An increase in sugar content in response to drought was shown in a resistant wheat cultivar (Nemati et al., 2018). One of the main functions of carbohydrates accumulated during stress is their anti-denaturation effect on the protein-lipid components of cells that experience dehydration or influence of other altering factors (Bita, Gerats, 2013). Sugars also have antioxidant properties (Kolupaev et al., 2019). Due to the metabolic regulation of the components of antioxidant system, the antioxidant effect of sugars can be either direct (binding free radicals) or indirect. For example, it has been shown that glucose can induce some stress response genes in *Arabidopsis*, genes for glutathione S-transferases and glutathione conjugate transporters (Couee et al., 2006). In our experiment, a high level of correlation ( $r = 0.85$ ) between the sugar content and the resistance index of winter wheat seedling cultivars in the background of stress was found.

Another group of compounds involved in the antioxidant protection of plant cells are flavonoids – polyphenolic compounds. They have an extremely high antioxidant activity. Thus, it has been shown that the efficiency of the interaction of flavonoids with ROS and RNS (reactive nitrogen species) was four times higher than that of ascorbic acid and  $\alpha$ -tocopherol (Khlestkina, 2013). A positive correlation was found between the ability of cultivars to maintain shoot growth under stress conditions and the content of flavonoids that absorb in the UV-B region, although no such relationship was found for anthocyanins that absorb in the visible part of the spectrum. Dugasa et al. (2021) reported an enhanced expression of genes for enzymes of flavonoid metabolism in a wheat cultivar with a high drought and salt tolerance.

## Conclusion

A significant correlation was found between the drought resistance of winter wheat seedlings of various cultivars and the activity of two antioxidant enzymes (SOD and CAT) and the content of two groups of low-molecular-weight protectors – sugars and UV-B-absorbing flavonoids. These four biochemical parameters in the shoots of stressed seedlings correlated not only with the growth parameter, but also with each other. SOD and CAT closely correlated with the content of flavonoids, and a significant correlation was also noted between the content of sugars and flavonoids. This fact may indicate the presence of a protective system with the connected elements in resistant cultivars. It was established that at the earliest stages of winter wheat development (seed germination and growth of etiolated seedlings), the above-noted components of the stress-protective system play an important role in cell homeostasis maintenance under osmotic stress conditions. It is quite natural that at other stages of ontogeny, the contribution of individual components of the antioxidant and osmoprotective systems to drought resistance may change. The defence processes in etiolated winter wheat seedlings also involve other components of the stress-protective systems not investigated in this experiment, the components of the ascorbate-glutathione cycle. It can be assumed that the activity of antioxidant enzymes functioning without cofactors (SOD and CAT) as well as low-molecular-weight protectors (sugars and flavonoids) can be used to screen drought-resistant samples of winter wheat promising for use in selection.

## Acknowledgement

This research was supported by the Development and Optimization of Methodological Approaches to the Identification of the Gene Pool of Winter Crops by the Level of Adaptability to Abiotic Factors in Ontogenesis, State Budget Project No. 0121U100564.

Received 17 10 2022

Accepted 13 12 2022

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## Įvairių veislių etioluotų žieminių kviečių daigų antioksidacinių ir osmoapsauginių sistemų būklė priklausomai nuo tolerancijos sausrui

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### Santrauka

Dėl klimato kaitos jau ankstyviausiose javų augimo stadijose, iš karto po rudeninės sėjos, kyla žieminių javų tolerancijos sausrui problema. Tirta etioluotų žieminių kviečių daigų antioksidacinių ir osmoapsauginių sistemų reakcija į osmosinį stresą. Septynių veislių žeminio kviečio (*Triticum aestivum* L.) sėklos daigintos 4 dienas Petri lėkštelėse ant filtrinio popieriaus, sudrėkinto distiliuotu vandeniu (kontrolinis variantas) arba uždengtų neprasisakverbiančia osmosine medžiaga PEG 6000, esant 12 % koncentracijai (osmosinis slėgis 0,17 MPa) ir 24° C temperatūrai. Po to buvo nustatyta eksperimentinių ir kontrolinių daigų ūglių bei šaknų masė, taip pat ir biologiniai 4 dienų daigų ūglių rodikliai. Didžiausią gebą išlaikyti ūglių augimą osmosinio streso sąlygomis parodė 'Tobak', vidutinę – 'Antonivka', 'Lira Odeska' ir 'Darynka Kyivska', mažiausią – 'Bogdana', 'Doskonala' ir 'Avgustina' veislių žieminių kviečių daigai. Esant osmosiniam stresui, mažiausiai atsparių veislių 'Doskonala' ir 'Avgustina' ūgliuose žymiai padidėjo lipidų peroksidacijos produkto malondialdehido kiekis. Esant osmosiniam stresui, neatspariose veislėse, taip pat ir vidutiniškai atsparioje 'Darynka Kyivska', buvo nustatytas vandenilio peroksido (H<sub>2</sub>O<sub>2</sub>) kiekio padidėjimas. Kaip atsakas į osmosinį stresą, labai ir vidutiniškai atsparių veislių kviečių daiguose padidėjo superoksido dismutazės (SOD) aktyvumas. Labai ir vidutiniškai atsparių veislių kviečių daigai parodė ir didelį katalazės (CAT) aktyvumą. Osmosinis stresas sukėlė visų veislių kviečių daigų gvajakolio peroksidazės aktyvumo padidėjimą, nepaisant jų tolerancijos sausrui. Prolino kiekis šiek tiek padidėjo visų veislių kviečių daiguose kaip atsakas į osmosinį stresą, tačiau nenustatyta jokio ryšio tarp prolino kiekio ir atsparumo sausrui. Esant osmosiniam stresui, cukraus kiekis padidėjo tik labai arba vidutiniškai sausrui atsparių veislių kviečių daiguose. Sausrai atsparioms veislėms būdingas ir didelis UVB spindulius sugeriančių flavonoidų bazinis kiekis ir jų išlaikymas osmosinio streso sąlygomis. Osmosinio streso sąlygomis stipriausias tarpusavio ryšys ( $p \leq 0,05$ ) nustatytas tarp atsparumo osmosiniam stresui ir SOD aktyvumo ( $r = 0,93$ ), taip pat cukraus kiekio ( $r = 0,85$ ). Be to, nustatytas reikšmingas ryšys tarp daigų tolerancijos sausrui ir flavonoidų kiekio bei CAT aktyvumo. Šių rodiklių kompleksą galima naudoti etioluotų žieminių kviečių daigų įvairių genotipų tolerancijai sausrui įvertinti.

Reikšminiai žodžiai: *Triticum aestivum*, tolerancija sausrui, antioksidaciniai fermentai, prolina, cukrus, ROS.