

Original research

Functioning of the antioxidant and osmoprotective systems of *Triticum aestivum* cultivars growing under soil drought conditions

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Abstract

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In recent decades, the frequency of autumn droughts has increased in the southern and eastern regions of Ukraine and many other countries. This has negatively affected the growth of winter cereal crops, including wheat, during the early stages of development. We studied the response to soil drought in five- to nine-day-old plants of seven cultivars of *Triticum aestivum* L. (bread wheat) of different ecological and geographical origins. Under drought conditions, cultivars with low and medium drought tolerance showed signs of oxidative stress and a decrease in chlorophyll content. In resistant cultivars, leaf sugar content increased under drought conditions, while in non-resistant cultivars, sugar content decreased or did not change. Superoxide dismutase activity decreased in all cultivars, with a much stronger decrease observed in non-tolerant cultivars. Guaiacol peroxidase activity remained stable in drought-tolerant cultivars under stress conditions, but significantly decreased in cultivars with low and medium tolerance. A strong positive correlation was found between plant growth inhibition and the content of malonic dialdehyde, a marker of oxidative stress. Conversely, a high negative correlation was observed between growth inhibition and stress-induced changes in superoxide dismutase activity and sugar content. No significant correlation was found between growth parameters and proline and anthocyanin content changes. The contribution of components of stress protection systems to the manifestation of drought tolerance in wheat cultivars and possible functional relationships between them are discussed.

Keywords: antioxidant enzymes, drought tolerance, growth, proline, sugars, water deficit, wheat.

INTRODUCTION

Triticum aestivum L., bread wheat, is the staple food for more than 35% of the world's population (Ashfaq

et al., 2022). However, drought as a stressor is a significant threat limiting global wheat production (Din et al., 2020). According to an analysis of data from field experiments published in peer-reviewed publications be-

tween 1980 and 2015, a reduction in available water by 40% of the optimum causes a reduction in wheat yield of more than 20% (Daryanto et al., 2016).

Drought tolerance in plants depends on the regulation of thousands of genes and multiple metabolic pathways associated with the functioning of stress protection systems (Fang & Xiong, 2015; Singh et al., 2021). The primary defence responses of plants to drought stress include stomatal closure, accumulation of various osmolytes in cells, activation of aquaporins, and enhanced functioning of the antioxidant system (Mahmood et al., 2020; Vignesh & Palanisamy, 2021; Auler et al., 2022).

Drought leads to increased generation of reactive oxygen species (hereafter, ROS) by plants (Foyer & Shigeoka, 2011). One of the reasons for this phenomenon may be the excessive reduction of components of the chloroplast electron transport chain caused by a decrease in carbon dioxide fixation due to stomatal closure (Kolupaev et al., 2023a). Excessive ROS can cause oxidative damage to cell structures and even lead to cell death (Mattos & Moretti, 2015; Hasanuzzaman et al., 2020). Therefore, the relationship between the functioning of the ROS-neutralising antioxidant system and the drought tolerance of cereals, including wheat, has been studied for many years (Kolupaev et al., 2023a). Many low-molecular-weight components of the antioxidant system are multifunctional compounds involved in the maintenance of osmotic pressure, stabilisation of biomacromolecules and cellular structures (Joseph et al., 2015; Gangola & Ramadoss, 2018; Mukarram et al., 2021). In this regard, knowledge of the specific metabolic strategies that support the functioning of these systems in particular cultivars and lines can improve the efficiency of breeding programmes aimed at improving drought tolerance (Abid et al., 2018).

Drought negatively affects wheat plants at different stages of ontogeny, both during grain filling and juvenile stages (Hasanuzzaman et al., 2018). At the same time, juvenile winter wheat plants in Ukraine and many other countries are exposed to drought both at the earliest stages (germination of seeds in soil and growth of etiolated seedlings before they reach the soil surface) and later at the 1–2 leaf stage. This is due to the constant recorded drought in September, especially in the southern and eastern regions of Ukraine (Romanenko et al., 2018). Drought stress at the early stages of plant development can lead to

growth suppression at later stages of ontogeny and, ultimately to significant yield losses (Hasanuzzaman et al., 2018). In many cases, this problem is exacerbated by introducing cultivars developed in other countries, particularly in Central Europe, where autumn droughts are less likely to occur.

Previously, we have shown the relationship between growth performance and parameters characterising the state of antioxidant and osmoprotective systems in etiolated seedlings of bread winter wheat cultivars of different ecological and geographical origin under model drought conditions created by polyethylene glycol (Kolupaev et al., 2022). However, drought tolerance refers to traits manifested at the level of the whole organism (Cruz de Carvalho, 2008). Drought tolerance in green plants depends mainly on the state of stomata, the photosynthetic apparatus and other systems regulated by the transduction of signal waves (including stress signals) that integrate root and shoot growth (Considine & Foyer, 2021). The formation of adaptive responses of a plant as a whole organism involves mechanisms of interstitial signalling in leaves and interorgan signal transduction from roots to shoots (Kuromori et al., 2022). In this respect, the response of green plants exposed to drought cannot be unambiguously predicted from data obtained from etiolated seedlings. Varietal peculiarities of drought adaptation strategies of cereals at the stage of one or more first leaves require particular study. However, as far as we know, such studies with a sufficiently large number of wheat cultivars differing in the level and strategies of drought tolerance have not yet been conducted in Ukraine and neighbouring countries.

The study aimed to investigate the relationships between the growth response and the state of antioxidant and osmoprotective systems under soil drought conditions in plants of bread winter wheat cultivars of different ecological and geographical origin at the age of 5–9 days.

MATERIALS AND METHODS

Plant material and its treatment and determination of physiological parameters

Plants of *Triticum aestivum* L. cultivars intended for cultivation in different climatic zones were used for an investigation (Kolupaev et al., 2022). Five of

the cultivars studied were developed in Ukraine (Table 1). The cultivar ‘Doskonala’ was developed mainly for cultivation in the forest-steppe. ‘Lira Odeska’ and ‘Antonivka’ cultivars are characterised by ecological plasticity necessary for cultivation in the dry Steppe zone. Cultivars ‘Darynka Kyivska’ and ‘Bogdana’ are designed to be cultivated in different climatic zones with high and moderate moisture. ‘Tobak’ cultivar (Table 1), intended for cultivation in Central Europe but can maintain productivity at high temperatures (Urban et al., 2018), was also used for research. In addition, we used the ‘Avgustina’ cultivar, which was developed for growing in the Belarusian Polesie and was characterised by excessive humidity.

The plants were grown at 25/20°C (day/night), illuminated at 12 kLx for 14 h daily, in plastic containers with a typical heavy loamy black soil (70% of total water holding capacity). Drought was induced from the 5th day of cultivation by reducing the irrigation rate and lowering soil moisture to 25–30% of total water holding capacity by the end of the experiment (9th day). In the control variants, soil moisture was maintained at 70% of the total water-holding capacity. Growth suppression of the above-ground part, leaf water deficit and biochemical parameters were measured in 9-day-old plants. Each replicate consisted of at least 30 plants to determine seedling length and biomass. To determine water deficit and biochemical parameters, a replicate was an average sample of 12 plants.

Linear growth inhibition was calculated using the following formula:

$$I = [(C_2 - C_1) - (E_2 - E_1)] / (C_2 - C_1) \times 100\%,$$

where I is growth inhibition (%); C_1 and C_2 are plant lengths of control variants on days 5 and 9 of the ex-

periment, respectively; E_1 and E_2 are plant lengths of experimental variants (drought) on days 5 and 9, respectively.

The biomass of the above-ground part of 9-day-old plants was also determined, and the drought-induced inhibition of its accumulation was calculated using the formula:

$$I = 100 - (E/C \times 100\%),$$

where I is growth inhibition (%); C and E are the fresh weight values of the above-ground part in the control and experimental (drought) variants, respectively.

To assess water deficit, the first leaves were cut and weighed, then placed in cuvettes with distilled water at 20°C, covered with several layers of filter paper, for 24 h to saturate (Tambussi et al., 2005). At the end of the exposure, excess water was carefully removed from the leaves, and they were weighed. The dry weight of these leaf samples was also determined by drying them to a constant weight at 103°C. The water deficit value was calculated according to the following formula:

$$WD = [(MT - MF) / (MT - MD)] \times 100\%,$$

where WD is water deficit (%); MT is leaf raw weight after water saturation; MF is leaf raw weight before water saturation; MD is leaf dry weight.

Evaluation of biochemical parameters

Measurement of lipid peroxidation product content. The analysis of the amount of lipid peroxidation products, mainly malonic dialdehyde, reacting with 2-thiobarbituric acid was conducted by homogenising the shoots in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% trichloroacetic acid. The resulting homogenate was placed in tubes covered with foil lids and boiled for 30 minutes in a water bath.

Table 1. Origin of wheat cultivars used in experiments

Cultivar	Country	Originator
‘Antonivka’	Ukraine	Plant Breeding and Genetics Institute of the National Academy of Agrarian Sciences of Ukraine
‘Avgustina’	Belarus	Scientific and Practical Centre of the National Academy of Sciences of Belarus
‘Bogdana’	Ukraine	Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine
‘Darynka Kyivska’	Ukraine	Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine
‘Doskonala’	Ukraine	Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine
‘Lira Odeska’	Ukraine	Plant Breeding and Genetics Institute of the National Academy of Agrarian Sciences of Ukraine
‘Tobak’	Germany	Saaten-Union GmbH, Isernhagen HB

After cooling, the samples were centrifuged at 10000 g for 15 minutes using an *MPW 350R* centrifuge (*Med-Instruments*). The absorbance of the supernatant was measured at 532 nm, the maximum light absorption of malonic dialdehyde. Additionally, the absorbance was measured at 600 nm to correct for non-specific light absorption (Kolupaev et al., 2021). Malonic dialdehyde content was expressed in nmol/g fresh weight.

Photosynthetic pigment content determination.

Photosynthetic pigments were extracted from leaves with ethanol and quantified by spectrophotometry (Shlyk, 1971). Their contents were expressed as mg/g dry weight of the leaf.

Assessment of sugar, proline and anthocyanin content.

The total sugar content in the plant material was determined by the Morris-Roe method based on the anthrone reagent (Zhao et al., 2003) in our modification. Sugars were extracted using distilled water and heated in a boiling water bath for 10 min. The extract obtained was clarified by adding equal volumes (0.3 ml) of 30% zinc sulphate and 15% blood yellow salt to the tubes, then filtered through a paper filter and, if necessary, diluted several times with distilled water before measurement. We added 3 ml of anthrone reagent and 1 ml of filtrate to the reaction tubes and distilled water to the control sample instead of the filtrate. After boiling for 7 min in a water bath, the samples were cooled, and the absorbance was determined at 610 nm relative to the control solution. D-glucose was used as a standard.

The proline content in the shoots was determined according to Bates et al. (1973) with modifications. Proline was extracted from the plant material using distilled water and boiling for 10 min. The extract was then filtered and mixed with equal volumes of ninhydrin reagent and glacial acetic acid, and the samples were boiled in a water bath for 1 hour. The absorbance of the coloured reaction product was determined at 520 nm using L-proline as a standard.

To determine anthocyanins, leaf samples were homogenised in 10 mL of a solution of 1% HCl in methanol (Nogues & Baker, 2000). After centrifugation of the homogenate for 15 min at 8000 g, the absorbance of the supernatant was read at 530 and 657 nm on an SF-46 spectrophotometer (LOMO, Russia). The anthocyanin content was calculated considering the non-

specific absorbance at 657 nm (Pietrini & Massacci, 1998). Anthocyanin contents were expressed as arbitrary units ($(A_{530} - 0.25A_{657})/g$ dry weight).

Evaluation of antioxidant enzyme activity. The activities of antioxidant enzymes, namely cytosolic superoxide dismutase (SOD, EC 1.15.1.1) and guaiacol peroxidase (GPX, EC 1.11.1.7), were determined as previously described (Kolupaev et al., 2020). Middle fragments of the first leaves were homogenised in cold 0.15 M K, Na-phosphate buffer, pH 7.6, containing 0.1 mM EDTA and 1 mM dithiothreitol. The homogenate was centrifuged at 8000 g for 15 minutes at 4°C to obtain the supernatant, which was then assayed. SOD activity was measured in the supernatant using an assay based on the competition of the enzyme with nitroblue tetrazolium for the superoxide anion radical, which occurs in an aerobic interaction of NADH with phenazine methosulphate.

The activity of guaiacol peroxidase was determined using guaiacol as a hydrogen donor and hydrogen peroxide as substrate at pH 6.2 of the reaction mixture, adjusted with K, Na phosphate buffer. Enzyme activities were expressed as arbitrary units/(g dry weight min).

Replication and statistical processing of experimental results.

Each experiment consisted of at least three independent replicates. The Shapiro-Wilk test was used to determine that the sample was normally distributed. The results were analysed using a two-factor analysis of variance (ANOVA) and Fisher's least significant difference (LSD). The figures show the means of three independent experiments and their standard errors. Pearson's correlation coefficients were estimated using the R programming language, version 4.1.1 (R Core Team).

RESULTS

Inhibition of plant growth under the influence of drought

The most significant inhibition of linear growth of the above-ground part was observed in cultivars 'Avgustina' and 'Doskonala' (Figs 1, 2A). More minor but significant inhibition of linear growth was observed in 'Darynka Kyivska', 'Bogdana' and 'Lira

Odeska'. Only in cultivars 'Antonivka' and 'Tobak' did the 4-day drought cause an insignificant (less than 10%) inhibition of the growth of the leaf length.

The distribution of cultivars by inhibition of above-ground biomass accumulation was similar, but not quite identical. The most significant drought-induced biomass reduction was observed in cultivars 'Doskonala' (by almost 70%) and 'Avgustina' and 'Bogdana' (by more than 40%) (Figs 1, 2 B). Biomass accumulation was inhibited by about one-

third in the cultivar 'Darynka Kyivska'. Finally, the least significant decrease in biomass accumulation (by 20–27%) was observed in cultivars 'Antonivka', 'Tobak' and 'Lira Odeska'.

Considering two growth inhibition indices (linear growth and biomass), the cultivars can be arranged in the order of decreasing drought tolerance as follows: 'Antonivka' > 'Tobak' > 'Lira Odeska' > 'Darynka Kyivska' ≥ 'Bogdana' > 'Avgustina' > 'Doskonala'. In other words, we can distinguish a group of highly

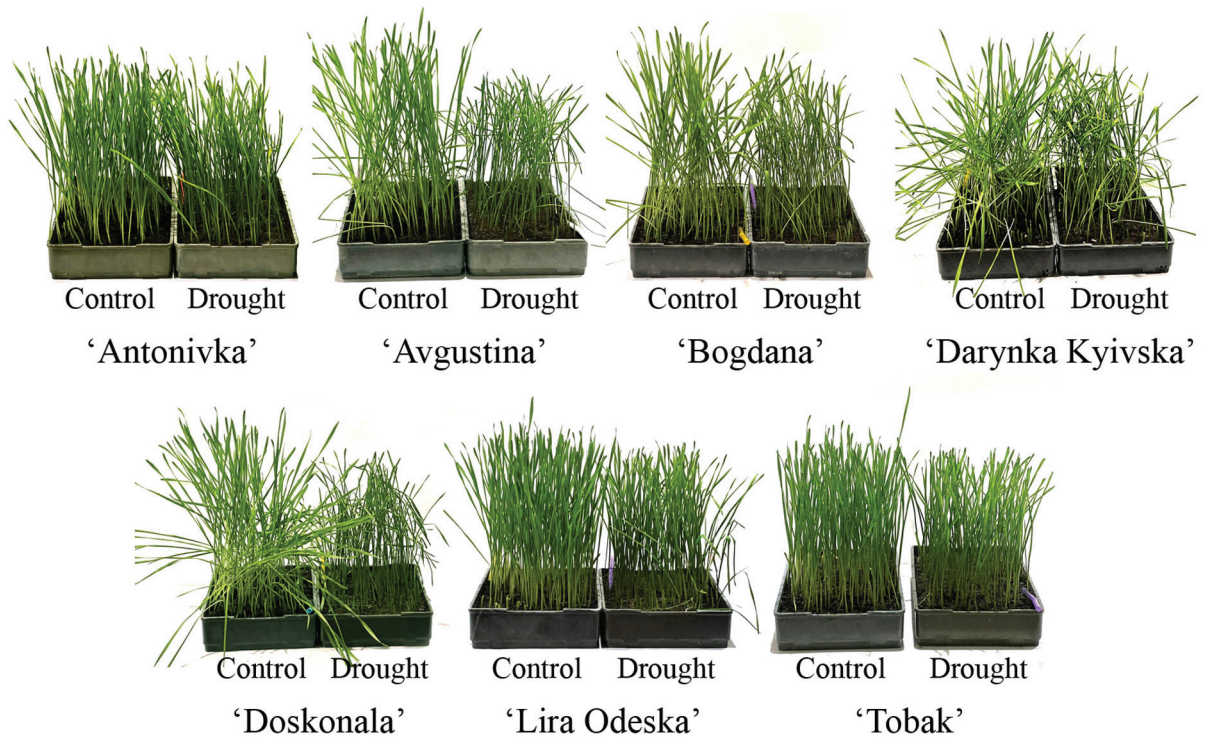


Fig. 1. Condition of wheat plants after four days of drought exposure.

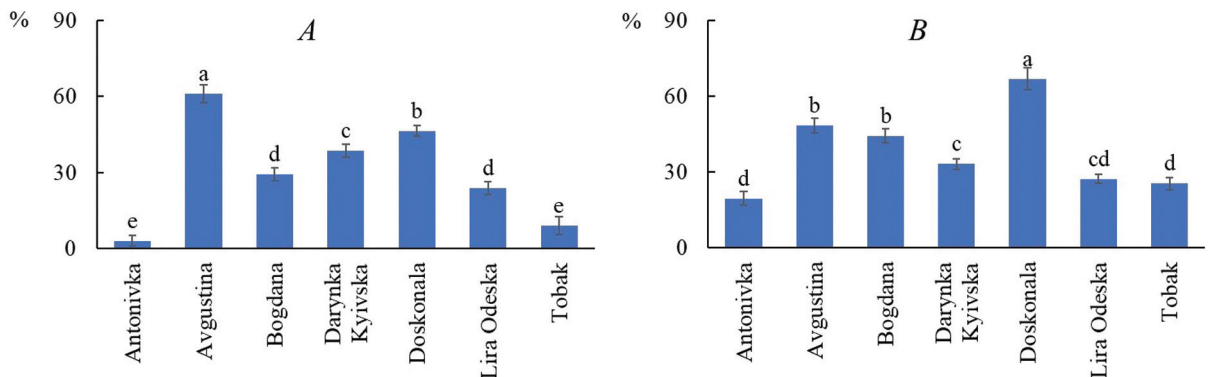


Fig. 2. Inhibition (%) of linear growth (A) and biomass accumulation (B) of wheat leaves after four days of drought. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

drought-tolerant cultivars with growth inhibition up to 30% ('Antonivka', 'Tobak' and 'Lira Odeska'), medium-tolerant (growth inhibition up to 50%) – 'Darynka Kyivska' and 'Bogdana', and non-tolerant with growth inhibition over 50% ('Avgustina' and 'Doskonala').

Water deficit in leaves of wheat plants under drought conditions

Under normal moisture conditions, water deficits were low for all cultivars, ranging from about 2–7% (Fig. 3). Under drought conditions, the water deficit increased up to 20–25% in 'Darynka Kyivska', 'Avgustina' and 'Doskonala'. In 'Bogdana' and 'Antonivka', the water deficit after drought was 12–14% and finally, in 'Tobak' and 'Lira Odeska', this indicator was the lowest and did not exceed 10% (Fig. 3).

Content of lipid peroxidation products in leaves

In the absence of stress, the content of lipid peroxidation products (per malonic dialdehyde) differed slightly according to the cultivar characteristics (Fig. 4). In response to drought, the most significant (twofold) increase in this index was observed in the non-tolerant cultivar 'Doskonala'. Minor but significant ($p \leq 0.05$) increases in malonic dialdehyde content after drought were observed in 'Avgustina', 'Darynka Kyivska', 'Lira Odeska' and 'Bogdana'. At the same time, in the most resistant cultivars, 'Antonivka' and 'Tobak', the content of lipid peroxidation products after drought was not significantly different from the corresponding values in the control (Fig. 4).

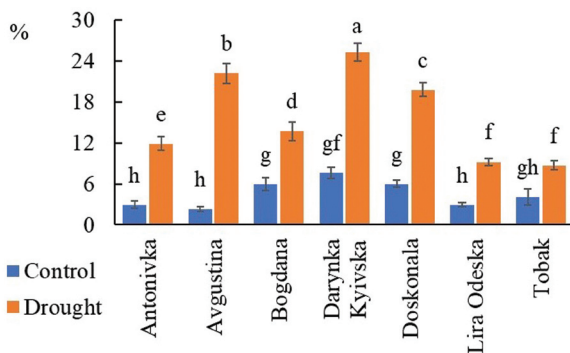


Fig. 3. Water deficiency (%) in the leaves of wheat plants under soil drought. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

Photosynthetic pigment content in wheat leaves under drought conditions

The total chlorophyll content in leaves of different cultivars in plants grown under physiologically normal conditions differed insignificantly. Only in the cultivar 'Tobak' was this index significantly higher than in the other cultivars (Fig. 5, A). After exposure to drought, chlorophyll content did not change significantly in the resistant cultivars 'Tobak', 'Lira Odeska' and 'Antonivka'. At the same time, four other cultivars, classified as medium and low tolerant in terms of growth inhibition, showed a significant decrease in leaf chlorophyll content ($p \leq 0.05$).

The carotenoid content in the control variants also differed insignificantly, although the values in 'Darynka Kyivska' and 'Avgustina' were slightly higher than in the others (Fig. 5, B). Exposure to drought for four days caused a significant decrease in carotenoid content in the leaves of all cultivars except the most resistant 'Antonivka' and 'Tobak'.

Content of low-molecular-weight protective compounds in wheat leaves under drought conditions

The proline content in control seedlings differed significantly. It was highest in cultivars 'Tobak' and 'Avgustina' and lowest in 'Darynka Kyivska' (Fig. 6, A). Under the influence of drought, the proline content increased significantly in all cultivars except 'Tobak'. This effect was most pronounced in 'Darynka Kyivska'. The highest absolute values of proline content against drought were observed in the

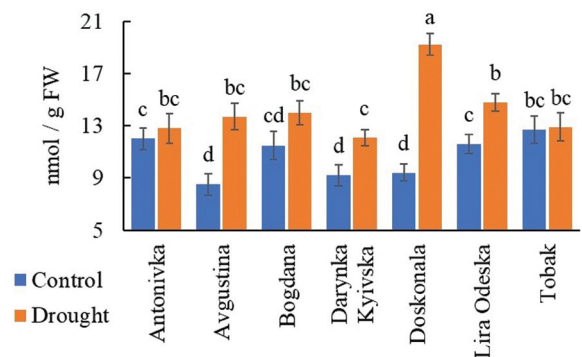


Fig. 4. Malonic dialdehyde content in leaves of wheat plants under soil drought conditions. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

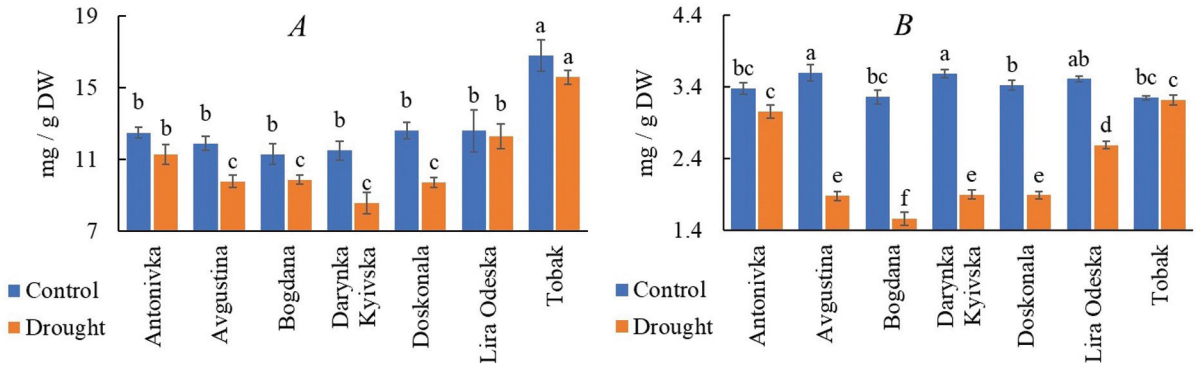


Fig. 5. Content of chlorophylls (A) and carotenoids (B) in leaves of wheat plants under soil drought conditions. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

cultivar ‘Avgustina’, which was also characterised by higher proline content in the control compared to other cultivars. In general, there was no significant relationship between drought tolerance of cultivars and proline content in their first leaves.

The total sugar content in the control varied according to cultivar characteristics. The highest values were recorded in cultivars ‘Bogdana’ and ‘Doskonala’, while the lowest values were characteristic of cultivars ‘Lira Odeska’ and ‘Antonivka’ (Fig. 6, B). Thus, there was no positive relationship between the drought tolerance of the cultivars and the basal sugar content in the first leaves of the plants.

At the same time, the leaf sugar content after drought exposure varied according to the resistance of the cultivars. It increased significantly in the most resistant cultivars, ‘Tobak’ and ‘Antonivka’. In the highly resistant cultivar ‘Lira Odeska’ and the moderately resistant cultivar ‘Darynka Kyivska’, a less pronounced but still significant ($p \leq 0.05$) increase in sugar content was observed. At the same time, the sugar content of ‘Avgustina’ and ‘Bogdana’ remained almost unchanged under drought, while it decreased in ‘Doskonala’ (Fig. 6, B). Thus, the resistant cultivars were characterised by increased leaf sugar content, whereas the moderately and less resistant cultivars showed a decrease or no change.

The anthocyanin content in control was highest in the leaves of ‘Antonivka’ and ‘Doskonala’ and lowest in the leaves of ‘Avgustina’ and ‘Darynka Kyivska’ (Fig. 6, C). In other words, the relationship between the basal anthocyanin content and the drought tolerance of the cultivars was also not observed.

Drought caused a decrease in leaf anthocyanin

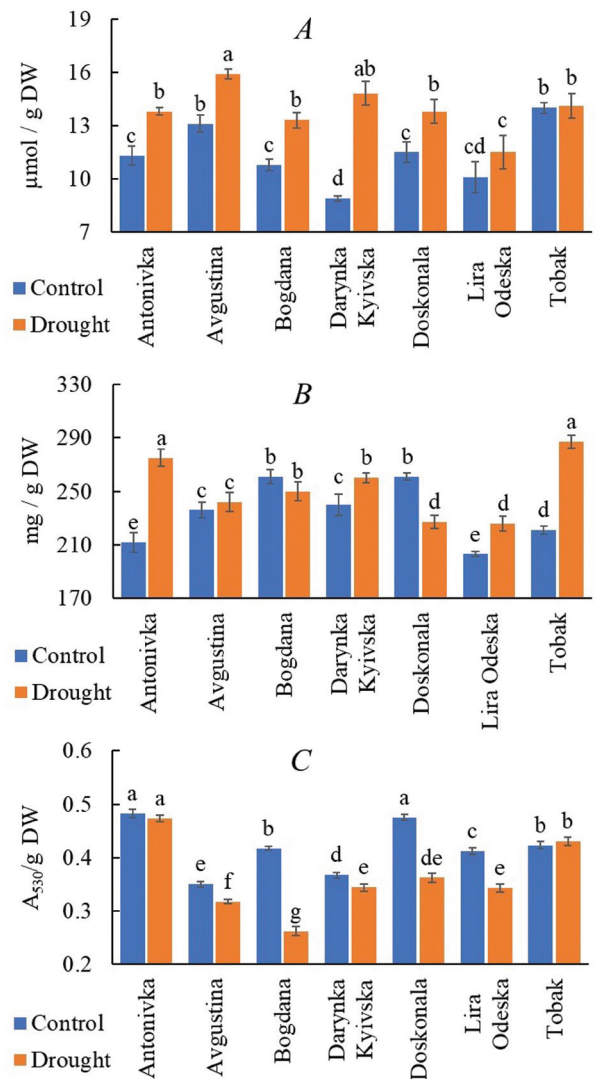


Fig. 6. Content of proline (A), total sugar (B) and anthocyanin (C) in wheat leaves under drought conditions. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

content in most cultivars, but it remained stable in the most drought-tolerant cultivars ‘Tobak’ and ‘Antonivka’ (Fig. 6, C). At the same time, the most significant decrease in anthocyanin content under drought was observed in ‘Bogdana’ and ‘Doskonala’.

Activity of antioxidant enzymes in leaves of wheat plants exposed to drought

The basal activity of superoxide dismutase in the leaves of different cultivars varied considerably. The highest values were characteristic of ‘Doskonala’ and ‘Avgustina’, and the lowest were characteristic of ‘Antonivka’ (Fig. 7, A).

Drought caused a decrease in enzyme activity in all cultivars. However, the most drought-tolerant cultivars, ‘Tobak’ and ‘Antonivka’, maintained relatively high levels of superoxide dismutase activity. At the same time, there was more than a twofold decrease in superoxide dismutase activity in the least drought-tolerant cultivars ‘Avgustina’ and ‘Doskonala’. In other cultivars, the enzyme activity decreased by 40–50% under drought conditions.

Guaiacol peroxidase activity varied significantly in different cultivars (Fig. 7, B). In the control, it was highest in ‘Tobak’, ‘Antonivka’ and ‘Lira Odeska’. It was several times lower in ‘Bogdana’ and ‘Darynka Kyivska’.

Drought caused a decrease in guaiacol peroxidase activity in most cultivars. The exception was the highly resistant ‘Tobak’, whose leaves showed a significant increase in guaiacol peroxidase activity (Fig. 7, B). In the other two resistant cultivars, ‘Antonivka’ and ‘Lira Odeska’, the enzyme activity

decreased but remained significantly higher than in the different cultivars.

DISCUSSION

The results showed a significant differentiation of the seven cultivars studied, from different ecological and geographical origins, in the growth response to drought (Figs 1, 2). Differences were also observed in other indicators studied. Not surprisingly, they were most pronounced against the background of the stress factor drought. Fig. 8 shows the heat map, where the changes in biochemical indicators are presented as a percentage of the values in the control. With this method of presenting the results, the most significant increase in the content of oxidative stress marker, malonic dialdehyde, occurred in the cultivars whose growth was most inhibited by drought – ‘Doskonala’ and ‘Avgustina’. Conversely, in the cultivars in which growth was weakly inhibited, ‘Tobak’ and ‘Antonivka’, there was almost no increase in malonic dialdehyde content under drought (Figs 3, 8). Calculations showed a close and reliable correlation at $p \leq 0.05$ between the increase in malonic dialdehyde content under drought and the inhibition of linear growth ($r = 0.78$) and especially biomass accumulation ($r = 0.92$) (Fig. 9). It should be noted that there is evidence in the literature for lower malonic dialdehyde levels in drought-tolerant wheat cultivars under drought conditions. For example, such a pattern has been found when this index is compared in four cultivars with different resistances at the grain-filling stage (Upadhyay et al., 2020). Our previous studies on 3–4-day-old etiolated wheat seedlings showed a

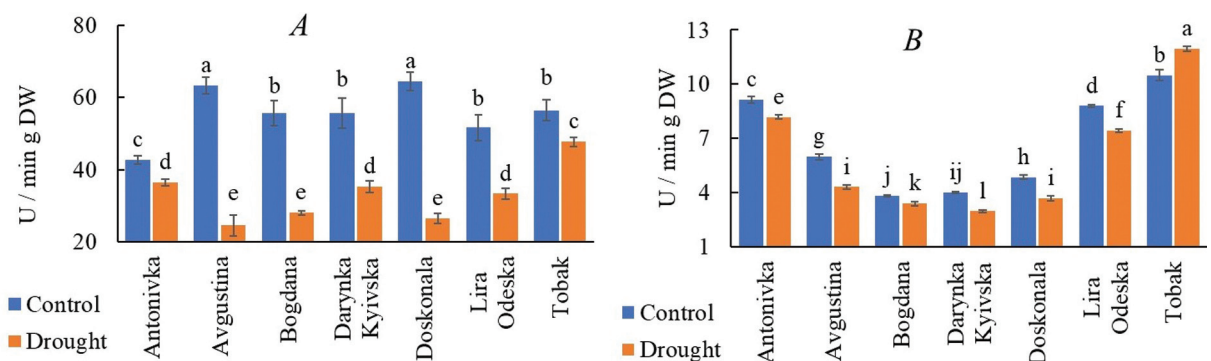


Fig. 7. Activity of superoxide dismutase (A), and guaiacol peroxidase (B) in leaves of wheat plants exposed to drought. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

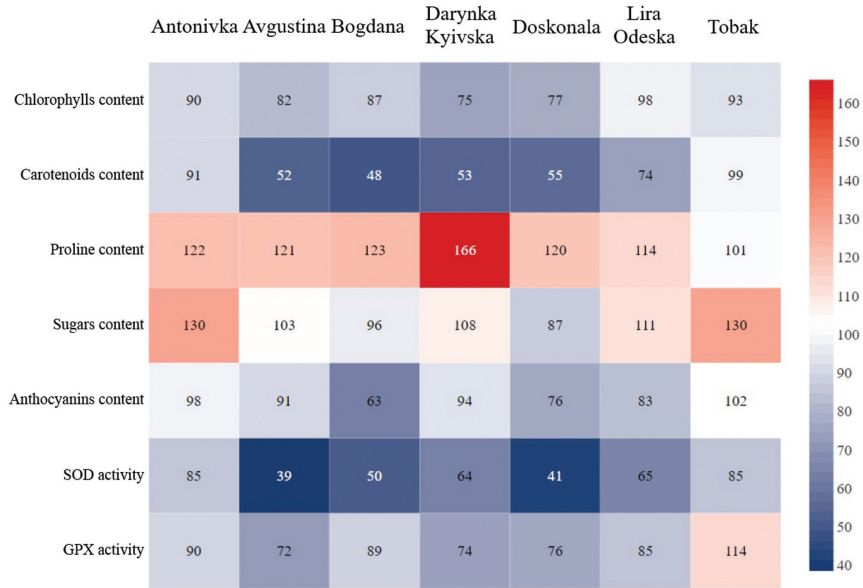


Fig. 8. Heat map of changes in the studied biochemical parameters of wheat leaves under the influence of soil drought. All results are given as percentages of the values in the control.

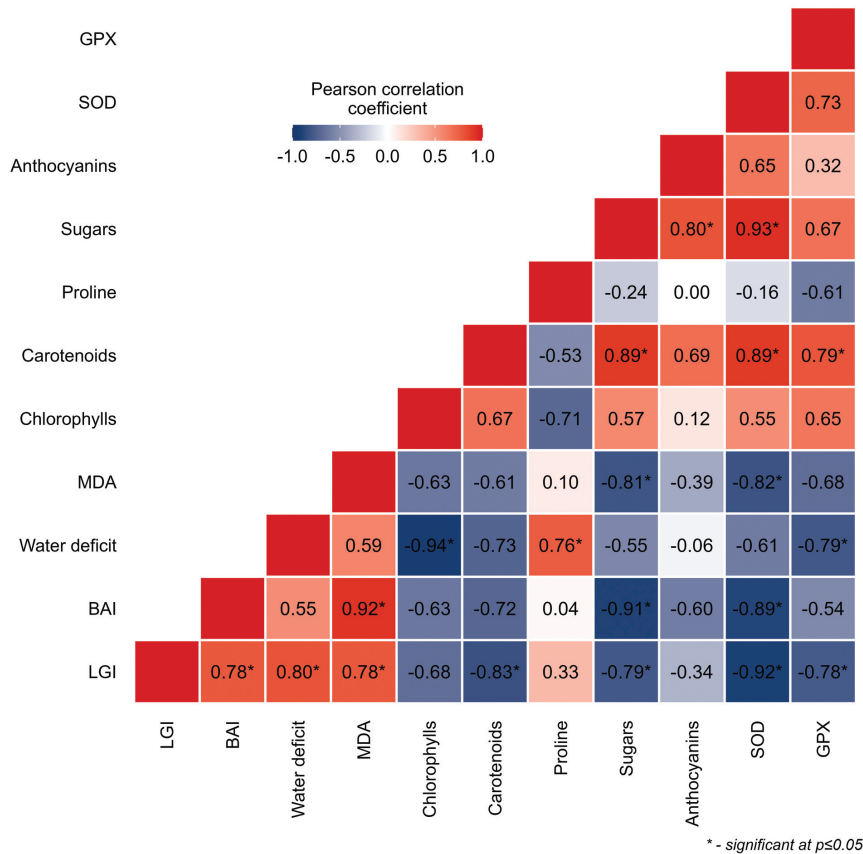


Fig. 9. Correlation coefficients between the studied parameters of wheat plants under soil drought. LGI – linear growth inhibition; BAI – biomass accumulation inhibition. Biochemical parameters used in calculations were expressed as a percentage of the values of the corresponding control variants (Fig. 8).

high degree of inverse correlation between growth inhibition caused by osmotic stress (PEG 6000) or high temperature and an increase in malonic dialdehyde content (Kolupaev et al., 2022, 2023b). Thus, there is reason to believe that the level of malonic dialdehyde can be a sufficiently informative indicator in assessing the drought tolerance of wheat plants at different stages of development.

Together with growth inhibition, the content of photosynthetic pigments is considered an integral indicator that varies depending on the drought tolerance of plants (Ullah et al., 2021). Under the conditions of our experiments, sufficient preservation of chlorophyll pool was observed in the highly resistant cultivars ('Tobak', 'Lira Odeska', 'Antonivka') and the moderately resistant cultivar 'Bogdana'. As expected, a relatively strong inverse correlation was observed between the chlorophyll reduction index and growth inhibition (Fig. 9). However, the values were not significant at $p \leq 0.05$. At the same time, an exceedingly high inverse correlation ($r = -0.94$) was observed between the development of water deficit and the change in the chlorophyll pool. Ullah et al. (2021) have reported a close relationship between chlorophyll content and water use efficiency in wheat cultivars under drought conditions.

In our experiments, a high inverse correlation was observed between linear growth inhibition and carotenoid content (Fig. 9). Carotenoids are essential for the protection of photosynthetic apparatus and, consequently, for the ability of plants to sustain growth under drought (Rao et al., 2020). It should be noted that similar phenomena of reduced carotenoid content in wheat leaves under the model (PEG 6000 effect) or soil drought have been found by Adl et al. (2020) and Rao et al. (2020). However, the decrease in carotenoid content was more pronounced in the non-tolerant cultivars.

Accumulation of proline, one of the important osmolytes with multifunctional stress-protective effects associated with membrane protection and antioxidant effects, is considered a typical plant response to drought stress (Joseph et al., 2015; Liang et al., 2013). However, under the conditions of our experiments, the increase in proline content in leaves of most cultivars on a dry weight basis was relatively small (by 15–20% compared to the baseline value) (Fig. 8). The most significant increase (66%) was observed in the moderate-

ly resistant cultivar 'Darynka Kyivska', while in the most resistant cultivar 'Tobak' the proline content in response to drought did not change practically. Thus, the relationship between the most integral indicator of drought tolerance (value of growth inhibition) and the increase in proline content under drought was not observed, as evidenced by low correlation coefficients (Fig. 9). However, a significant correlation was observed between water deficit and increase in proline content ($r = 0.76$). It can be assumed that the induction of proline synthesis under drought stress occurs when a certain level of water deficit is reached. It should be noted that although the involvement of proline in plant adaptation to drought has been discussed for several decades, the relationship between drought tolerance and proline content remains controversial. Some papers show a positive relationship between proline accumulation and drought tolerance (Anjum et al., 2017; Ayub et al., 2021; Kirova et al., 2021; Nasirzadeh et al., 2021), but many papers state that there is no such relationship. Furthermore, phenomena of inverse correlation between proline content and drought tolerance have also been reported (Altaf, 2021; Kolupaev et al., 2022). The contribution of proline to the adaptation of plants to drought depends on their species characteristics and the strength of the stress effect.

Proline is not the only osmolyte necessary for drought adaptation. Sugars are essential for maintaining the native state of proteins and the functional properties of membranes (Mukarram et al., 2021). Under the conditions of our experiments, the most pronounced increase in sugar content per dry weight was observed in the most resistant cultivars, 'Antonivka' and 'Tobak' (Fig. 8). This effect was somewhat weaker in the relatively resistant 'Lira Odeska' and the moderately resistant 'Darynka Kyivska'. No significant increase in sugar content in leaves was found in cultivars belonging to non-tolerant varieties. Moreover, even a substantial decrease in sugar content was observed in 'Doskonala'. The critical role of soluble carbohydrates in the adaptation of wheat cultivars to drought is indicated by the high inverse correlation between their content and indices of biomass accumulation and linear growth ($r = 0.91$ and 0.79 , respectively; Fig. 9). It should be noted that a high correlation between sugar content and preservation of growth capacity on PEG 6000 solutions was previously shown for etiolated wheat seedlings

of different cultivars (Kolupaev et al., 2022). It is known that hydroxyl groups of sugars can replace water molecules to maintain hydrophilic interactions in plant cells, which is crucial for stabilising native biomacromolecules and membrane structure during dehydration (Pukacka et al., 2009; Gangola & Ramadoss, 2018). The accumulation of sugars also reduces the activity of ions in cells under dehydration conditions, preventing the development of toxic effects under stress conditions.

Another component of the protective effect of sugars may be their involvement in signalling processes. For example, it has been shown that an increase in sucrose content can act as a signal that specifically activates gene expression of the key enzyme for the synthesis of secondary metabolites, phenylalanine ammonia-lyase, and several downstream enzymes for anthocyanin synthesis (Solfanelli et al., 2006). It is noteworthy that our experiments showed a high correlation between changes in sugar and anthocyanin content ($r = 0.80$) (Fig. 9). However, it should be noted that drought caused a decrease in anthocyanin content in the leaves of most cultivars. Still, it remained stable in the most resistant ‘Tobak’ and ‘Antonivka’ (Fig. 8). A relatively high inverse correlation was observed between changes in anthocyanin content and growth inhibition under drought ($r = -0.60$). However, this correlation was not significant at $p \leq 0.05$. In general, the data available in the literature indicate a possible contribution of anthocyanins to wheat adaptation to drought. Still, due to the lack of studies on various cultivars with different tolerances, there is no basis for a definite conclusion about a close correlation between anthocyanin content and wheat tolerance. However, information on anthocyanins’ exceedingly high antioxidant activity and membrane-protective effects (Neill & Gould, 2003) suggests that further studies on their role in plant adaptation to drought are advisable.

In addition to multifunctional low-molecular compounds, the enzymatic component of the antioxidant system plays an essential role in plant adaptation to drought (Kolupaev et al., 2023a). Of these, superoxide dismutase, the only enzyme that neutralises the radical form of ROS (superoxide anion radical), has a special place (Kirova et al., 2022). We did not observe an increase in SOD activity in response to drought: in all cultivars the enzyme activity per dry

weight decreased after drought, but this effect was least significant in the most resistant cultivars ‘Antonivka’ and ‘Tobak’ (Fig. 8). An exceedingly high inverse correlation between SOD activity and plant growth inhibition was observed (Fig. 9). It should be noted that the increase in SOD gene expression in response to drought at different stages of development has been found in maize, barley, rice, and other cereals (Kolupaev et al., 2023a). Using the model of etiolated wheat seedlings, a high correlation between the tolerance of cultivars and the change in SOD activity under the action of PEG 6000 has also been shown (Kolupaev et al., 2022).

The present work also revealed differences in the change of guaiacol peroxidase activity under drought in different cultivars. The ‘Tobak’ cultivar showed an increase in enzyme activity compared to all other cultivars. Simultaneously, in other high- and medium-tolerant cultivars, enzyme activity remained above 80% of control. At the same time, a more significant decrease in guaiacol peroxidase activity was observed in less resistant cultivars (Fig. 8). A significant correlation was found between linear growth inhibition indicators and changes in guaiacol peroxidase activity ($r = 0.79, p \leq 0.05$). Phenolic peroxidases move from the cytosol to the apoplast (Minibayeva et al., 2009). They are multifunctional enzymes that are involved not only in hydrogen peroxide neutralisation, but also in the processes of phenolic oxidation and lignin formation (Lee et al., 2013), which may be necessary for strengthening leaf cover tissues under drought (Liu et al., 2018).

Thus, it appears that many components of stress protection systems are involved in the adaptation of wheat plants at early stages of development. However, their contribution may differ depending on the characteristics of the cultivar. The results show that the accumulation of soluble carbohydrates in the leaves and the maintenance of sufficient activity of antioxidant enzymes, superoxide dismutase and guaiacol peroxidase, are the most important in adapting drought tolerant cultivars to soil drought.

CONCLUSION

Plants of wheat cultivars of different ecological and geographical origins at the first-second leaf stage were well differentiated regarding resistance

to soil drought. The relationship between the ability of drought-tolerant cultivars to maintain growth under drought conditions and to resist the development of oxidative stress was demonstrated. Drought-tolerant cultivars showed no significant increase in water deficit and decreased photosynthetic pigments in leaves. The drought-tolerant cultivars were characterised by the absence of a substantial increase in the oxidative stress marker malonic dialdehyde, the maintenance of superoxide dismutase activity at a sufficiently high level and the increase in sugar content under the influence of soil drought. The results also suggest the involvement of phenol peroxidase and secondary metabolites (anthocyanins) in drought adaptation. However, no significant correlation ($p \leq 0.05$) was found between these parameters and the growth of wheat cultivars under drought conditions. Similarly, no close correlation was found between changes in proline content and drought tolerance of wheat cultivars.

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