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GROWTH RESPONSES OF WHEAT SEEDLINGS OF DIFFERENT VARIETIES TO HEAT-STRESS AND THEIR RELATION TO THE ANTIOXIDANT SYSTEM STATE AND OSMOLYTES ACCUMULATION

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Background. In recent decades, Ukraine has been experiencing abnormally high temperatures and droughts in different seasons, including autumn. This creates stressful conditions for winter cereals, especially wheat, at the very beginning of ontogeny. A comprehensive study of the functioning of antioxidant and osmoprotective systems in the early stages of development for wheat varieties of different ecological and geographical origins has not been conducted yet. This study aimed to investigate the effect of heat stress on the growth of etiolated seedlings of seven varieties of winter wheat (*Triticum aestivum* L.) and the indicators characterizing the functioning of antioxidant and osmoprotective systems.

Materials and Methods. Wheat grains of different varieties were germinated at 24 °C for three days in the dark. Subsequently, they were subjected to 4 h of heating at 45 °C in an air thermostat. Immediately after stress, the generation of superoxide anion radical ($O_2^{\bullet-}$) by seedlings, the content of hydrogen peroxide (H_2O_2), lipid peroxidation (LPO) products, catalase and guaiacol peroxidase activity, and also proline and soluble carbohydrates were analyzed. One day after heating, the relative inhibition of shoot and root growth was determined.

Results and Discussion. The Antonivka and Tobak varieties demonstrated the highest ability to maintain growth after exposure to high temperatures; the Darynka Kyivska and Lira Odeska varieties were medium resistant. In the Doskonala, Bogdana and Avgustina varieties, a strong inhibition of shoot and root growth after heat stress was noted. In Antonivka, after high-temperature exposure, the $O_2^{\bullet-}$ generation increased slightly, while the content of H_2O_2 and LPO products did not change. In Tobak, Darynka Kyivska and Lira Odeska, the stress-induced increase in ROS formation and accumulation of LPO products was moderate. The hyperthermia-sensitive Doskonala, Bogdana and Avgustina showed a significant development of oxidative stress. Heat-resistant varieties had high catalase activity and increased total soluble carbohydrate content under heat stress. After exposure to high temperature, the proline content increased in all studied varieties, but no positive correlation was recorded between its amount and their heat resistance. Likewise, no significant correlation was found between the guaiacol peroxidase activity and the ability of varieties to maintain growth after heat stress.

Conclusion. The high-level inverse correlation between catalase activity, soluble carbohydrate content, and the manifestation of oxidative stress effect indicates a significant contribution of these stress-protective system components to the adaptation of seedlings to heat stress. Indicators of the oxidative stress intensity and the functioning of individual components of the antioxidant system can be used to assess the heat resistance of wheat varieties at the early stages of development.

Keywords: heat resistance, antioxidant system, osmoprotective system, *Triticum aestivum* L.

INTRODUCTION

Cereals (bread and durum wheat, rye, barley and others) can be exposed to adverse weather factors at almost all phases of ontogenesis (Schoppach & Sadok, 2013). High temperatures are now considered one of the main factors affecting global plant distribution, crop growth and yields globally (Ali *et al.*, 2020; Kiriziy & Stasik, 2022). Under Ukrainian conditions, hyperthermia and drought have a detrimental effect on plants not only in summer, but also in early autumn period. In particular, over the past two decades in the Steppe zone of Ukraine, the average temperature in August and September increased by 2.8 and 1.9 °C, respectively, and on particular days the temperature can reach critical values (Romanenko *et al.*, 2018). As a result, winter cereals can be subjected to heat damage even at the seed germination stage.

Among the effects induced by hyperthermia, membrane fluidization (Niu & Xiang, 2018), hormonal fluctuations (Kosakivska *et al.*, 2022) and heat shock protein synthesis (Kumar & Rai, 2014; Yadav *et al.*, 2020) are the most actively studied. At the same time, nonspecific resistance mechanisms also contribute to plant resistance. Among them, the antioxidant system plays a special role, since heat stress (Asthir, 2015), like most others (Choudhury *et al.*, 2017), causes an increase in the reactive oxygen species (ROS) content in cells.

In particular, thylakoid membrane fluidity increases and photosystems are disrupted when exposed to high temperatures (Yamamoto, 2016). There is also an increase in the stochastic ROS formation in mitochondria and peroxisomes (Choudhury *et al.*, 2017). In addition, activation of ROS-generating enzymes (primarily NADPH oxidase) and thermal damage to antioxidant enzyme molecules are possible (Gautam *et al.*, 2017). An increased ROS generation and oxidative modification of certain proteins and lipids by them can serve as a signal necessary for the activation of defense systems (Chmielowska-Bąk *et al.*, 2015; Yao *et al.*, 2017). However, disruption of the pro/antioxidant balance and redox regulation processes is considered one of the key causes of cell damage and death (Kumar & Rai, 2014; Gautam *et al.*, 2017). In this regard, the activation of the antioxidant system is considered as an important protective reaction of plants to high temperatures (Kolupaev *et al.*, 2020b).

In recent decades, attempts have been made to link individual indicators of this system functioning with resistance of various cultivated plants genotypes, including wheat. It was shown that heat-resistant wheat varieties, in contrast to non-resistant ones, showed an increase in the superoxide dismutase (SOD) activity during a prolonged exposure to moderately high temperature (Oboznyi *et al*., 2013). A similar effect was found in wheat plants under a relatively short-term exposure at 40 °C (Kumar *et al.*, 2012). An increase in catalase (CAT) activity was also shown in heat-resistant wheat varieties during hyperthermia (Gupta *et al.*, 2013). At the same time, a decrease in CAT activity in wheat plants under severe heat stress has also been reported (Hameed *et al.*, 2012).

In response to heat stress, heat-resistant wheat varieties showed an increase in peroxidase (POX) activity, but this effect was not typical of all studied resistant genotypes (Gupta *et al.*, 2013).

Along with antioxidant enzymes, numerous low-molecular-weight antioxidants, as well as multifunctional compounds (Kiriziy & Stasik, 2022), in particular proline (Laxa *et al.*, 2019), are involved in plant defense responses to hyperthermia. An increase in proline content has been shown in wheat of various genotypes in response to the temperature of 35 °C (Ahmed & Hassan, 2011). On another set of wheat varieties, an increase in proline content was found in resistant genotypes after a short-term heating of seedlings at 42 °C (Gupta *et al.*, 2013). However, the effects of a decreased proline content in wheat leaves after heat stress were recorded in the later developmental phases (Kumar *et al*., 2012). The contribution of different sugars to maintaining the heat resistance property of plants has also been considered (Tarkowski & Vanden, 2015). Thus, the key components of the osmoprotective system are involved in plant adaptation to hyperthermia.

The role of the antioxidant system in heat resistance of cultivated cereals has been studied mainly on adult green plants (Hameed *et al.*, 2012; Gupta *et al*., 2013). However, the functioning of the antioxidant system in etiolated seedlings differs significantly from that in green vegetative plants. In particular, young cereal seedlings are characterized by a higher constitutive content of low-molecular-weight compounds that can perform protective functions (proline, sugars, etc.) (Kolupaev *et al.*, 2020a). At the same time, a comprehensive study of the components of antioxidant and osmoprotective systems of etiolated wheat seedlings under hyperthermia using a sufficient set of varieties contrasting in resistance has not been conducted yet. Our work aimed to study a complex of indices characterizing these systems' functioning under moderate heat stress on etiolated seedlings of seven wheat varieties differing in heat resistance.

MATERIALS AND METHODS

Plant material and treatments. Plants of bread winter wheat (*Triticum aestivum* L.) varieties, intended for cultivation in different climatic zones, were used for the study.

Five of the studied varieties were created in Ukraine. The Doskonala variety (originator: Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine, Kharkiv) designed mainly for cultivation in the Forest-Steppe zone (Karpets *et al.*, 2016). The Lira Odeska and Antonivka varieties (originator: Plant Breeding and Genetics Institute of the National Academy of Agrarian Sciences of Ukraine, Odessa) are distinguished by ecological plasticity necessary for cultivation in the Steppe zone (Khakhula *et al.*, 2013). The Darynka Kyivska and Bogdana varieties (originator: Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine, Kyiv) are designed for cultivation in different climatic zones (Chernobai *et al.*, 2019; Khomenko, 2020). Also the Tobak variety (originator: Saaten-Union GmbH, Isernhagen HB, Germany), intended for cultivation in Central Europe, but capable of maintaining productivity at high temperatures (Urban *et al.*, 2018), was used for research. In addition, we used the Avgustina variety, created for cultivation in Belarusian Polesie (originator: Scientific and Practical Center of the National Academy of Sciences of Belarus).

The seeds were disinfected for 30 min with a 6 % hydrogen peroxide solution and washed with distilled water. Then they were germinated for 3 days in the dark at 24 °C in Petri dishes on filter paper moistened with distilled water. The heat resistance of seedlings was evaluated by the growth response to high temperature using the method proposed by O. I. Zhuk and I. P. Grigoryuk (Pat. 45879 UA, 2002) with our modifications. Three-day-old seedlings of experimental variants were placed in open Petri dishes in a thermostat at 45±1 °C and 45±3 % air humidity (4-hour exposure). To prevent the roots from drying out, the filter paper in the cups was moistened every hour with the same amount of distilled water. At the end of the exposure, one part of the seedlings was used for biochemical analyzes, and the other part was placed in 24 °C thermostat to evaluate the growth response. The seedlings of control variants were kept in the thermostat at 24 °C throughout the experiment. After 24 hours post heat stress, the growth inhibition of shoots, roots and whole seedlings was evaluated according to the formula:

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

where I is growth inhibition (%); C_1 and C_2, E_1 and E_2 are, respectively, the initial and final values of shoot, root or whole seedling (without grains) fresh weight in the control and experimental (heat stress) variants. The stress conditions (temperature and exposure time) were selected based on preliminary experiments in such a way that the inhibition of seedling growth ranged from 15–20 % (for resistant varieties) to 60–65 % (for non-resistant ones).

Generation of superoxide anion radicals (SAR) by the shoots was estimated by nitroblue tetrazolium (NBT) reduction. Ten identical shoots were placed in a tube 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 for 1 hour (Karpets *et al.*, 2012). At the end of the exposure, shoots were gently removed from the incubation solution and photographed to visually assess the intensity of $O_2^{\cdot-}$ generation. The optical density of the incubation solution was also measured at 530 nm on an SF 46 spectrophotometer (LOMO, Russia). The increase in SAR generation under heat stress was determined by the ratio (%) of optical density in the experimental and control variants.

Evaluation of hydrogen peroxide content was conducted in the shoots homogenized in the cold with 5 % trichloroacetic acid. The samples were centrifuged at 8000 g for 10 min at 2–4 °C using an MPW 350R centrifuge (MPW MedInstruments, Poland). The H_2O_2 concentration was determined in the supernatant using the ferrothiocyanate method (Sagisaka, 1976).

LPO products content evaluation. The rate of lipid peroxidation (LPO) in the shoots of seedlings was assessed by the content of its products reacting with 2-thiobarbituric acid (mainly malondialdehyde – MDA) (Kolupaev *et al.*, 2020a).

Measurement of antioxidant enzymes activity was carried out in seedling shoot samples (200 mg) homogenized in the cold in 10 mL of 0.15 M K, Na phosphate buffer (pH 7.6) with EDTA (0.1 mM) and dithiothreitol (1 mM) (Kolupaev *et al.*, 2020a). The CAT (EC 1.11.1.6) activity was analyzed at pH 7.0 of the reaction mixture evaluating the amount of hydrogen peroxide decomposed per unit of time. The activity of peroxidase (POX, EC 1.11.1.7) was determined using guaiacol as a hydrogen donor and hydrogen peroxide as a substrate at pH 6.2 of reaction mixture adjusted with the K, Na phosphate buffer.

Estimation of low-molecular-weight protectors. Proline content in the seedling shoots was evaluated using ninhydrin reagent (Bates *et al.*, 1973). Total sugar content was assayed using anthrone reagent by the Morris-Roe method with modifications (Kolupaev *et al.*, 2022).

Replication of experiments and statistical processing. The experiments were repeated three times in each variant. Data on every parameter were analyzed statistically by the Analysis of Variance (ANOVA) and Fisher's Least Significant Difference (LSD) test. Data are presented as a mean ± SD. Different letters designate values with differences significant at $P \leq 0.05$.

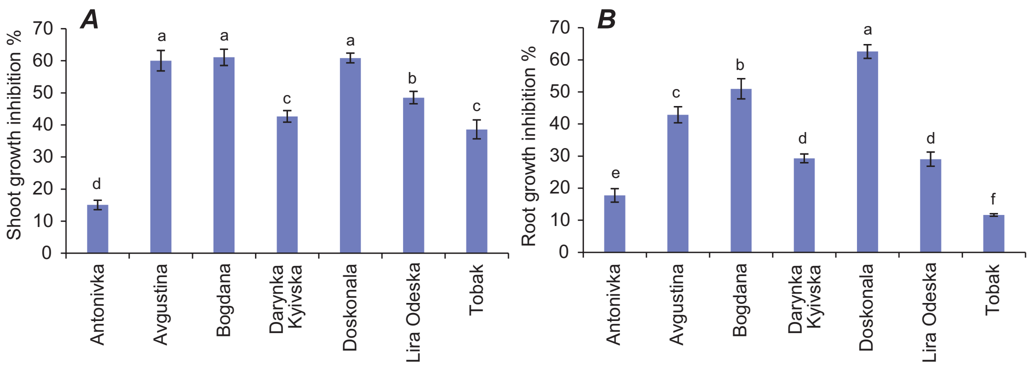
Correlation coefficients were estimated using self-developed software created in the R programming language (version 4.1.1).

RESULTS AND DISCUSSION

Inhibition of seedling growth under heat stress. The least inhibition of shoot biomass accumulation after heating was observed in the Antonivka variety (**Fig. 1*A***). A moderate decrease in shoot biomass growth (approximately by 39–49 %) after exposure to stress was observed in the Tobak, Darynka Kyivska and Lira Odeska varieties. In the Bogdana, Doskonala and Avgustina, the inhibition of shoot growth after heat stress was significant and amounted to 60 % approximately.

Accumulation of root biomass was the least sensitive to heat stress in the Tobak and Antonivka varieties: growth inhibition was 11.6 and 17.8 %, respectively (**Fig. 1*B***). Moderate (approximately 29 %) inhibition of root growth under heat stress was observed in the Darynka Kyivska and Lira Odeska cultivars. Much more significant (from 43 to 63 %) inhibition of the growth of root biomass was observed in the Doskonala, Bogdana and Avgustina varieties.

According to the inhibition of total biomass seedling accumulation, the studied varieties were arranged in a row: Antonivka < Tobak < Darynka Kyivska ≤ Lira Odeska < Avgustina ≤ Bogdana < Doskonala (**Fig. 1*C***). Thus, seedlings of the Antonivka and Tobak varieties are highly resistant, Darynka Kyivska and Lira Odeska are moderately resistant, and Doskonala, Bogdana and Avgustina are sensitive to heat stress.



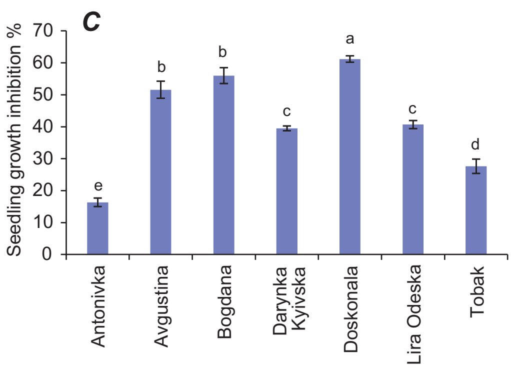


Fig. 1. Growth inhibition of shoots (***A***), roots (***B***) and whole seedlings (***C***) after 4-h exposure to 45 °C

Effects of oxidative stress caused by hot temperature. After a 4-hour exposure to high temperature, an increase in superoxide radical generation by seedlings of all studied varieties was noted. However, in the resistant Antonivka variety, this effect was small (**Fig. 2**). In the Darynka Kyivska, Lira Odeska and Tobak cultivars with highly or moderately resistant growth indices, the increase in $O_2^{\bullet-}$ generation ranged from 30 to 50 % relative to the control. At the same time, in the least heat-resistant Bogdana and Doskonala varieties, the SAR generation increased more than 2-fold. An exception was the effects in the relatively hyperthermia-sensitive Avgustina cultivar, in which the $O_2^{\bullet-}$ generation increased less significantly, by 43 % (**Fig. 2**).

The absolute hydrogen peroxide content in the control varied in the approximate range of 45 to 100 nmol/g fresh mass, depending on the cultivar characteristics (results not shown). After heat stress, hydrogen peroxide content remained stable in two heat-resistant varieties, Avgustina and Tobak (**Fig. 3*A***). A relatively slight increase in H_2O_2 content after heating was observed in the moderately resistant Darynka Kyivska and Lira Odeska. In the sensitive Avgustina and Bogdana cultivars, the amount of hydrogen peroxide increased by 1.5 times or more after exposure to hyperthermia, and in the least resistant Doskonala cultivar, this increase was more than twofold.

The absolute content of the LPO end product MDA in different cultivars was in the range of 9–12 nmol/g fresh mass. The MDA content in the Antonivka, Tobak and Darynka Kyivska cultivars increased insignificantly after heat stress; a slightly greater effect was found in Lira Odeska (**Fig. 3*B***). At the same time, in all three hyperthermia-sensitive varieties (Doskonala, Bogdana and Avgustina), the increase in MDA content was about 30 %.

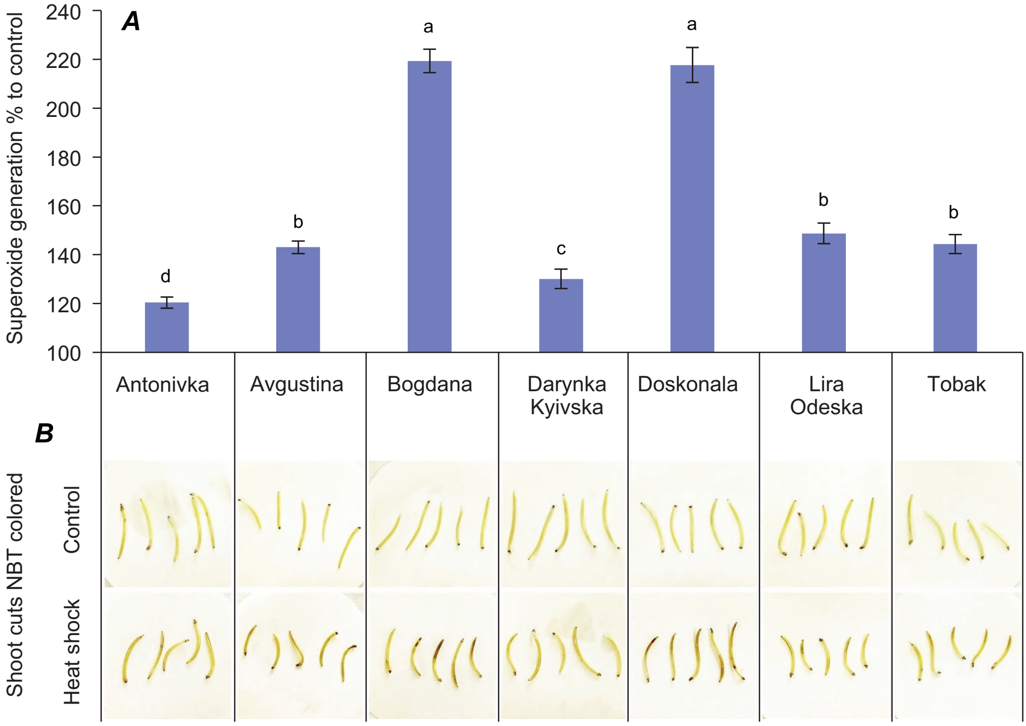


Fig. 2. Generation of SAR by shoots of wheat seedlings, determined by changes in optical density of NBT solution (***A***) and visual effects (***B***). Heat stress – exposure to 45 °C for 4 h

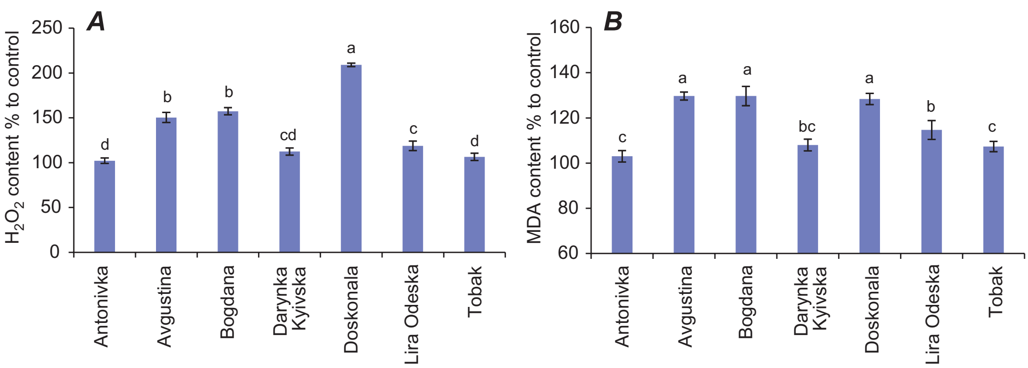


Fig. 3. Hydrogen peroxide (***A***) and MDA content (***B***) in shoots of wheat seedlings after heat stress (45 °C, 4 h)

Activity of antioxidant enzymes. The basal CAT activity in shoots of seedlings of the studied varieties differed markedly (**Fig. 4*A***). It was the highest in Tobak and Lira Odeska. At the same time, the CAT activity in the Avgustina variety was significantly lower than in all other varieties. However, no correlation was found between the basic CAT activity and the heat tolerance of the seedlings. The heating of seedlings caused a marked increase in the enzyme activity in Darynka Kyivska, Antonivka and Tobak; a relatively small increase in CAT activity was recorded in the Avgustina and Bogdana varieties. In Lira Odeska and Doskonala, no significant changes in CAT activity were found after a 4-hour exposure to stress temperature. In general, higher absolute values of the enzyme activity under stress conditions were characteristic of highly and moderately resistant varieties.

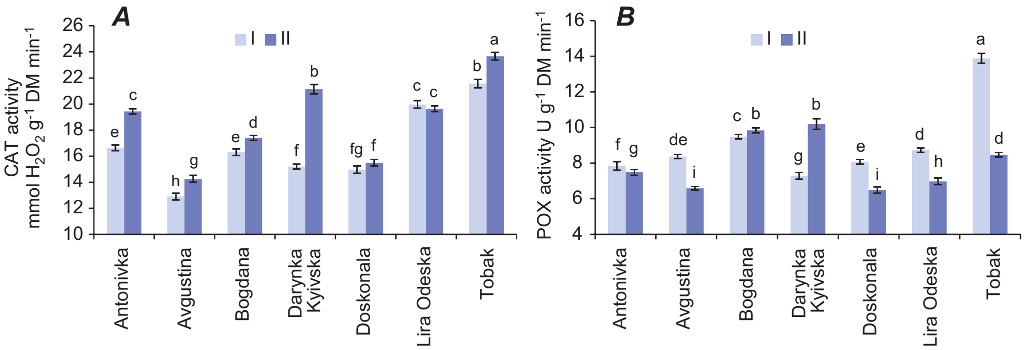


Fig. 4. Activity of CAT (***A***) and POX (***B***) in shoots of wheat seedlings. I – control; II – heat stress (45°C, 4 h)

The basal POX activity was the highest in Tobak (**Fig. 4*B***). Differences were less pronounced between the other studied cultivars. After heat stress, there was a decrease in the enzyme activity in most varieties. However, in Darynka Kyivska and Bogdana, POX activity increased after stress. Thus, no significant relationship with seedling heat resistance was found for both the absolute values of POX activity and its changes under stress.

Content of low-molecular-weight protector compounds. The constitutive proline content in the seedlings differed considerably between the varieties (**Fig. 5*A***). It was the lowest in the seedlings of Antonivka, Lira Odeska and Tobak. Higher values were observed in Darynka Kyivska, Bogdana and Doskonala. The highest proline content in the absence of stress factor was in the Avgustina variety. Heat stress caused a significant increase in proline content in all studied varieties. In varieties with low initial proline content, this effect was more significant. Thus, in the Antonivka and Lira Odeska cultivars, the proline amount increased by 3-4 times after the heating of seedlings. In Bogdana, Darynka Kyivska, Doskonala and Tobak, its content increased by 2.0–2.5 times. In the Avgustina variety, characterized by high basic proline content, its accumulation under stress was lower, 1.7 times. The highest absolute values of proline content after hyperthermia were observed in the non-tolerant varieties (Bogdana, Avgustina and Doskonala), and the lowest in the Tobak variety – one of the heat-resistant.

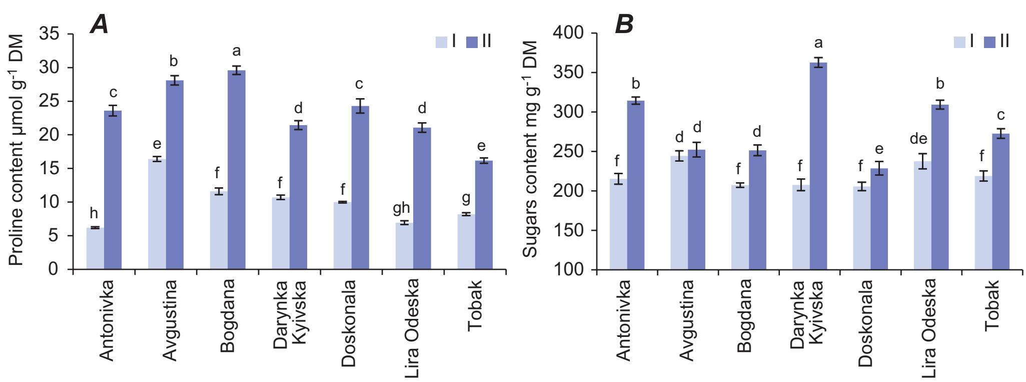


Fig. 5. Proline (***A***) and sugars (***B***) content in shoots of wheat seedlings. I – control; II – heat stress (45 °C, 4 h)

The basic content of sugars in the shoots of seedlings of the varieties differed insignificantly; only in the Avgustina cultivar it was slightly higher than in the others (**Fig. 5*B***). The pattern of change in sugar content in response to heat stress depended on the heat resistance of seedlings. Thus, in the highly resistant (Antonivka and Tobak) and moderately resistant (Darynka Kyivska and Lira Odeska) varieties, an increase in total sugar content after 4 hours of heat stress was noted. An increase in the sugar content was also found in the non-resistant Bogdana and Doskonala, but it was low. In the heat-sensistive Avgustina cultivar, sugar content did not change after the stress.

Correlations between indicators of oxidative stress intensity, functioning of protective systems, and inhibition of seedling growth during hyperthermia. Changes in the main indicators of oxidative stress development (generation of SAR, accumulation of hydrogen peroxide and MDA) significantly correlated with the inhibition of seedling growth under high temperature (**Fig. 6**).

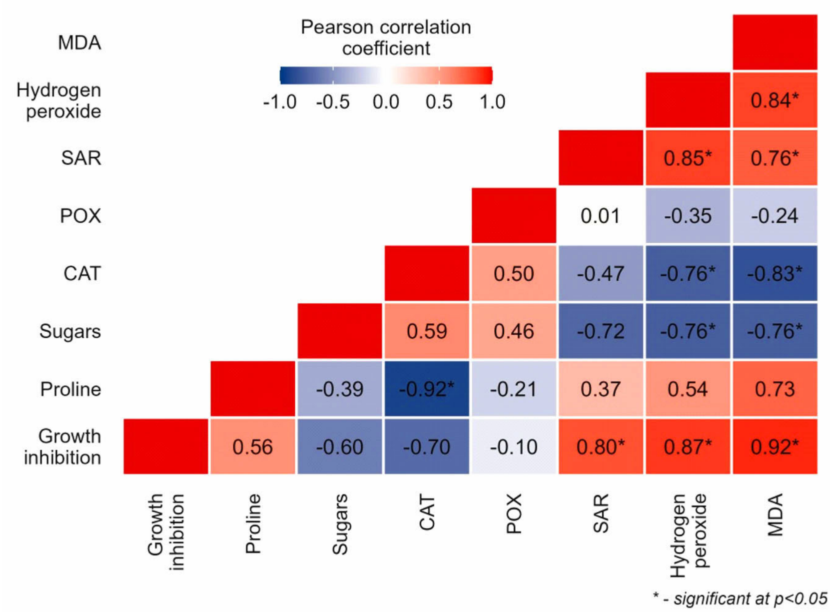


Fig. 6. Correlation coefficients between growth inhibition and biochemical parameters of wheat seedlings of different varieties under heat stress (45 °C, 4 h)

There was a fairly high (but not significant at P ≤ 0.05) negative correlation between CAT activity and sugar content and inhibition of seedling growth. At the same time, a direct correlation of moderate strength was observed between proline content of under stress conditions and inhibition of seedling growth, although it was not significant at P ≤ 0.05. There was almost no correlation between POX activity and seedling heat resistance. The presence of reliable at P ≤ 0.05 inverse correlations between the development of oxidative stress (H_2O_2 and MDA content in seedlings) and such indicators of the functioning of protective systems as CAT activity and sugar content should also be noted (**Fig. 6**).

So, the obtained results point to a significant role of the pro-/antioxidant balance disturbance in the realization of hyperthermia negative effect on wheat seedlings. In the seedlings of wheat varieties, whose growth was most inhibited by high temperature (Doskonala, Bogdana and Avgustina), the most significant increase in all studied oxidative stress parameters was observed (**Fig. 1–3**). A significant high correlation (*r* from 0.80 to 0.92) was found between the inhibition of seedling growth and an increased formation of superoxide radical, as well as an increased content of hydrogen peroxide and LPO product MDA (**Fig. 6**). Hyperthermia-induced effects of an increased ROS generation in various plant cell compartments are described in the literature (Choudhury *et al.*, 2017). Excessive amount of SAR and hydrogen peroxide in cells creates conditions for non-enzymatic formation of other ROS in Fenton and Haber-Weiss reactions, including more aggressive ones, in particular hydroxyl radical (Halliwell & Gutteridge, 2015). Hydroxyl and hydroperoxyl radicals are the main ROS initiating the LPO process (Halliwell & Gutteridge, 2015). Such a pattern of events in wheat seedlings under hyperthermia is indicated by the high correlation between the generation of $O_2^{\bullet-}$ and H_2O_2 and the content of LPO product MDA: 0.76 and 0.84, respectively (**Fig. 6**).

The highly and moderately resistant to hyperthermia Antonivka, Tobak, Darynka Kyivska and Lira Odeska varieties exhibited relatively small effects of hydrogen peroxide accumulation and an increase in LPO intensity due to heat stress (**Fig. 3*B***), indicating a more balanced functioning of their antioxidant system. In particular, the key hydrogen peroxide-depleting enzyme CAT was found to be elevated or maintained at a sufficiently high level in these cultivars (**Fig. 4*A***). This enzyme does not require additional substrates for activity and is very efficient in neutralizing high H_2O_2 concentrations (Hasanuzzaman *et al.*, 2020). The important contribution of CAT to the antioxidant protection of wheat seedlings under heat stress is evidenced by a significant negative correlation between its activity and accumulation of hydrogen peroxide and MDA: 0.76 and 0.83, respectively (**Fig. 6**).

Both the increased and decreased CAT activity in plants of different species, including wheat, under heat stress has been reported in the literature. Meanwhile, higher values of the enzyme activity were observed in resistant varieties, moreover at different phases of plant development (Hameed *et al.*, 2012; Gao *et al.*, 2021). An increased expression of catalase genes, especially CAT2, under severe drought and hyperthermia has been reported (Laxa, 2019).

On the other hand, our research found no relationship between POX activity and heat resistance of wheat varieties (**Figs. 4*B*, 6**). This result is to some extent consistent with the evidence that POX activity can increase in non-resistant plants in response to drought and hyperthermia (Laxa, 2019). In our experiments, a significant increase in POX activity was recorded only in the moderately resistant Darynka Kyivska variety; in other varieties, the enzyme activity changed slightly or decreased (**Fig. 4*B***). POX is a stress-inducible enzyme, but in addition to its antioxidant activity it can also exhibit pro-oxidant activity due to electron transfer from reducing agents (e.g. NADH) to oxygen and formation of $O_2^{\bullet-}$ and H_2O_2 (Gautam *et al.*, 2017). The negligible contribution of POX to antioxidant defense system of wheat seedlings under heat stress is indicated by low correlation coefficients between its activity and the accumulation of hydrogen peroxide and LPO products under stress conditions (**Fig. 6**).

One of the key antioxidant defense enzymes is considered to be SOD, which converts SAR into hydrogen peroxide (Kolupaev *et al.*, 2020b). We did not directly determine its activity, but the low level of SAR generation in the most heat-resistant Antonivka variety indicates the contribution of this enzyme to antioxidant protection of wheat seedlings under heat stress (**Fig. 2**). There are data on an increased expression of Cu/Zn-SOD and Mn-SOD genes under heat stress in plants subjected to a preliminary action of moderately high (hardening) temperatures (Kumar & Rai, 2014).

Currently, it is believed that not only antioxidant enzymes and such canonical antioxidants as ascorbate and glutathione are involved in antioxidant protection and redox regulation processes (Lou *et al.*, 2018), but also many other low-molecular-weight compounds, in particular sugars (Morelli *et al.*, 2003). Direct and indirect antioxidant effects of exogenous sugars on plants under stress conditions have been shown (Hu *et al.*, 2012). In our experiments, the highest increase in sugar content in response to hyperthermia was recorded in varieties highly and moderately resistant to hyperthermia (Antonivka, Darynka Kyivska and Lira Odeska) (**Fig. 5**). The possible contribution of sugars to the protection of wheat seedlings from damage caused by hyperthermia is indicated by a moderately high negative correlation coefficient ($r = -0.60$) between growth inhibition and sugar content in seedlings of different varieties (**Fig. 6**). The presence of high significant negative correlations (r values ranging from 0.72 to 0.76) between sugar content in seedlings and an increased generation of $O_2^{\bullet-}$ and H_2O_2, as well as MDA accumulation under stress conditions is also remarkable (**Fig. 6**). In this regard, the literature data on the high antiradical activity of sugars *in vitro* (Morelli *et al.*, 2003) is worth noting.

Multifunctional protective metabolites with pronounced antioxidant properties include proline (Laxa *et al.*, 2019). In our experiments, a significant increase in its content was found in all studied varieties in response to hyperthermia. Notably, in the most resistant cultivar, Antonivka, the proline amount increased almost 4-fold in response to heat stress (**Fig. 5**). At the same time, no significant correlation between heat resistance of the varieties and the absolute proline content in seedlings after heat stress was found. Moreover, there was a fairly high, though not significant at $P \leq 0.05$, direct correlation between proline content in seedlings and inhibition of their growth under stress (**Fig. 6**). Thus, it is impossible to unambiguously assess the proline contribution to the protection of wheat seedlings from heat damage based on the results obtained. It is possible that relationship between proline accumulation and varietal resistance may become apparent under other experimental conditions, in particular under stronger stress effects. For example, Nasirzadeh *et al.* (2021) showed that the relationship between proline content in leaves of 20-day-old wheat plants and the drought resistance of varieties was evidenced only under a very strong drought.

When discussing the possible involvement of proline in the response of wheat seedlings to hyperthermia, attention should also be drawn to the negative correlation between its content and catalase activity under stress, which was found to be significant at $P \leq 0.05$. There is evidence in the literature of reciprocal relationships between proline content and gene expression and activity of antioxidant enzymes (Kolupaev *et al.*, 2020b). Probably, in genotypes characterized by high activity of antioxidant enzymes, the contribution of proline to the protective mechanisms is less significant.

In general, the obtained results indicate a close relationship between heat tolerance of wheat seedlings and their ability to resist the development of oxidative stress. This is evidenced by a high level of correlation between an increased ROS generation, accumulation of LPO products, and inhibition of seedling growth under heat stress. Among the studied components of the antioxidant system, CAT activity and sugar content make a significant contribution to the maintenance of redox homeostasis, as indicated by a high level of negative correlation of these indicators with LPO intensity and seedling growth inhibition. It should be noted that the relationship between seedling dehydration tolerance, activity of antioxidant enzymes in seedlings and their field heat and drought resistance (ability to maintain yield in dry years) has previously been shown in three wheat varieties of different ecotypes (Oboznyi *et al*., 2013). In this regard, data on the functioning of stress protection systems in seedlings can be partly extrapolated to characterize their resistance at later stages of development.

Quite naturally, the important role of these components of the antioxidant system should not be overestimated, since the antioxidant system itself is very multicomponent, and its components are in a complex functional interaction with each other (Kolupaev *et al*., 2020b). For a more comprehensive characterization of antioxidant protection of wheat seedlings of different varieties under hyperthermia, it is necessary to further study the contribution of other components of the antioxidant system, in particular ascorbate-glutathione cycle, whose role has been shown in other experimental models (Lou *et al*., 2018).

CONCLUSION

1. Exposure to the temperature of 45 °C (4 hours) had varying, cultivar-dependent effect on the further wheat seedling growth. According to the degree of growth inhibition under stress, the studied varieties were ranked as follows: Antonivka < Tobak < Darynka Kyivska ≤ Lira Odeska < Avgustina ≤ Bogdana < Doskonala.
2. Seedlings of highly (Antonivka and Tobak) and moderately (Darynka Kyivska and Lira Odeska) resistant varieties were characterized by a less significant increase in SAR generation and rise in content of hydrogen peroxide and MDA in seedlings after heat stress. The high level of correlation between growth inhibition and these oxidative stress indicators shows the possibility of their use in characterizing the heat resistance of *Triticum aestivum* varieties at the early development phases.
3. A high level of inverse correlation between catalase activity, sugar content, and manifestation of the oxidative stress (accumulation of hydrogen peroxide and MDA) indicates a significant contribution of these parameters to the protective processes that develop in wheat seedlings in response to heat stress.
4. Heat stress caused a significant increase in proline accumulation in wheat seedlings of all studied varieties. However, no relationship between its content and the seedlings ability to maintain growth at high temperatures was found.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Animal rights: This article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [Y.E.K.]; methodology, [N.I.R., Y.E.K.]; validation, [T.O.Y.]; formal analysis, [M.A.S.].; investigation, [Y.E.K., B.E.M., O.B.]; resources, [O.B., V.M.T.]; data curation, [Y.E.K.]; writing – review and editing, [Y.E.K., B.E.M., T.O.Y.]; visualization, [T.O.Y.] supervision, [N.I.R., Y.E.K.]; project administration, [Y.E.K.]; funding acquisition, [N.I.R.].

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РОСТОВА РЕАКЦІЯ ПРОРОСТКІВ ПШЕНИЦІ РІЗНИХ СОРТІВ НА ДІЮ ВИСОКИХ ТЕМПЕРАТУР ТА ЇЇ ЗВ'ЯЗОК ЗІ СТАНОМ АНТИОКСИДАНТНОЇ СИСТЕМИ І НАКОПИЧЕННЯМ ОСМОЛІТІВ

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Вступ. В останні десятиліття в Україні спостерігають аномально високі температури і посухи у різні сезони включно з осіннім. Це створює стресові умови для озимих злаків, передусім пшениці, на самому початку онтогенезу. Комплексного дослідження функціонування антиоксидантної та осмопротекторної систем на ранніх фазах розвитку для сортів пшениці різного еколого-географічного походження дотепер не проводили. Метою роботи було вивчення впливу теплового стресу на ріст етіольованих проростків семи сортів пшениці озимої (*Triticum aestivum* L.) та показники, що характеризують функціонування антиоксидантної та осмопротекторної систем.

Матеріали та методи. Зернівки пшениці різних сортів пророщували за температури 24 °C протягом трьох діб у темряві. Надалі їх піддавали 4-годинному прогріванню за 45 °C у повітряному термостаті. Одразу після стресу аналізували генерацію супероксидного аніон-радикала ($O_2^{\bullet-}$) проростками, вміст у них пероксиду водню (H_2O_2), продуктів пероксидного окиснення ліпідів (ПОЛ), активність каталази та гваяколпероксидази, кількість проліну і розчинних вуглеводів. Через добу після прогріву визначали відносне інгібування росту пагонів і коренів.

Результати. Найбільшою здатністю зберігати ріст після впливу високих температур відзначилися сорти Антонівка і Тобак; сорти Даринка київська та Ліра одеська виявилися середньостійкими. У сортів Досконала, Богдана й Августина відзначено сильне пригнічення росту пагонів і коренів після теплового стресу. У сорту Антонівка після високотемпературного впливу генерація $O_2^{\bullet-}$ зростала незначно, а вміст H_2O_2 та продуктів ПОЛ не змінився. У сортів Тобак, Даринка київська і Ліра одеська стрес-індуковане стресом збільшення утворення АФК та накопичення продуктів ПОЛ було помірним. Чутливі до гіпертермії сорти Досконала, Богдана й Августина характеризувалися значним розвитком окиснювального стресу. Теплостійкі сорти показали високу активність каталази та підвищений сумарний вміст розчинних вуглеводів за теплового стресу. У відповідь на дію високої температури вміст проліну зростав у всіх досліджуваних сортів, однак позитивної кореляції його кількості з теплостійкістю сортів не зареєстровано. Так само не виявлено вірогідної кореляції між активністю гваякопероксидази і здатністю сортів зберігати ріст після дії теплового стресу.

Висновки. Встановлений високий рівень зворотної кореляції між активністю каталази, вмістом розчинних вуглеводів і проявом ефекту окиснювального стресу вказує на істотний внесок цих складових стрес-протекторної системи в адаптацію проростків до теплового стресу. Показники інтенсивності окиснювального стресу та функціонування окремих компонентів антиоксидантної системи можуть бути використані під час оцінювання теплостійкості сортів пшениці на ранніх фазах розвитку.

Ключові слова: теплостійкість, антиоксидантна система, осмопротекторна система, *Triticum aestivum* L.

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