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RESPONSE OF TRITICUM AESTIVUM SEEDLINGS OF DIFFERENT ECOLOGICAL AND GEOGRAPHICAL ORIGIN TO HEAT AND DROUGHT: RELATIONSHIP WITH RESISTANCE TO OXIDATIVE STRESS AND OSMOLYTE ACCUMULATION

SUMMARY

The phenomenon of plant cross-tolerance to various stressors, particularly heat and drought, has been studied in considerable detail. However, there are no data on the relationship between resistance to these stressors in *Triticum aestivum* cultivars of different ecological and geographical origins at the stage of etiolated seedlings. At the same time, they are used for an accelerated assessment of the heat and drought resistance of breeding samples (separately for each factor). This work compared the response of seedlings of seven winter common wheat cultivars to heat stress (4-hour heating at 45°C) and a model drought (action of 12% PEG 6000). A correlation was found between the inhibition of seedling biomass accumulation as a whole ($r=0.55$) and separately for shoots ($r=0.66$) under heat and osmotic stress. A high correlation was shown between inhibition of shoot growth and accumulation of hydrogen peroxide and lipid peroxidation (LPO) products in shoots during heating ($r=0.91$ and 0.76 , respectively) and a much lower correlation between the values of these markers of oxidative stress and inhibition of shoot growth during drought. A significant inverse correlation was found between the accumulation of sugars in the shoots and inhibition of shoot growth under drought ($r=-0.85$), and moderately high under heat stress ($r=-0.60$). At the same time, only a positive medium correlation ($r=0.49$) was observed between proline content and growth inhibition under both types of

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stress. However, a high positive correlation was found between proline and LPO products under drought conditions ($r=0.91$). It is concluded that the resistance of wheat seedlings to oxidative stress is more closely related to heat tolerance than to drought tolerance. The results also indicate a significant contribution of sugars, but not proline, to the resistance of wheat seedlings to drought and heat stress.

Keywords: *Triticum aestivum*, heat resistance, drought resistance, cross-tolerance, oxidative stress, sugars, proline

INTRODUCTION

The effects of drought and high temperatures are major factors limiting the productivity of most crops, especially cereals (Dudziak *et al.*, 2019; Kolupaev and Blume, 2022). For example, each degree increase in temperature causes a reduction in wheat yields of around 6.0% (Itam *et al.*, 2020). Drought causes an even greater drop in productivity: a 40% drop in available water from optimum results in a 20-40% drop in cereal yields (Daryanto *et al.*, 2016).

In general, drought and high temperatures are considered the main abiotic stressors affecting wheat production (Aberkane *et al.*, 2021). There is evidence of greater sensitivity of *Triticum aestivum* than *T. durum* to these factors (Kavita *et al.*, 2016). And it is known that resistance to the combination of drought and high temperatures is genetically different from tolerance to each of these factors (Keleş and Öncel, 2002). In turn, the properties of drought resistance and heat resistance are interrelated but far from identical (Cairns *et al.*, 2013; Pržulj *et al.*, 2020).

To date, a number of statements have been formulated to explain the mechanisms of cross-resistance in plants to various stressors, including the effects of high temperatures and drought. These include the universal principles of activating the expression of protective genes by a limited set of signaling mediators (Bowler and Fluhr, 2000; Zhang *et al.*, 2020), control by the same transcription factors of a number of genes important for resistance (Pastori and Foyer, 2002), common reasons for cell damage caused by stressors of different nature (Iseki *et al.*, 2014; Kolupaev *et al.*, 2020b), and polyfunctionality of some components of stress-protective systems (Szabados and Savoure, 2009; Kolupaev *et al.*, 2020b). The latter refers to important stress metabolites such as proline and sugars, which can act as osmolytes and simultaneously exert antioxidant, membrane-protective, and anti-denaturation effects on cellular structures (Morelli *et al.*, 2003; Laxa *et al.*, 2019).

An important cause of plant damage, when exposed to heat, is thought to be an excessive increase in reactive oxygen species (ROS) in cells (Asthir, 2015). The oxidative stress effect that develops under heat stress is a consequence of increased fluidity of chloroplast and mitochondrial membranes, leading to impaired electron transport in chloroplasts and mitochondria (Yoshioka, 2016; Choudhury *et al.*, 2017). The effects of the activation of ROS-generating enzymes (primarily NADPH oxidase) under heat stress have also been shown (Gautam *et al.*, 2017). Drought has a similar effect on plants. Restriction of CO₂ intake into cells due to the closing of stomata has an effect of over-reduction of

the electron-transport chain of chloroplasts and increases the probability of ROS formation (de Carvalho, 2008). Mitochondria are also known to contribute significantly to the development of oxidative stress under drought conditions. Thus, in wheat leaves under severe drought conditions, the content of carbonylated proteins in mitochondria was an order of magnitude higher than in chloroplasts (Bartoli *et al.*, 2004). A correlation was shown between the stability of membranes of different rice genotypes under the action of osmotic stress agent polyethylene glycol (PEG) and the oxidative stress inducer methyl viologen (Iseki *et al.*, 2014).

Despite many years of research on the tolerance of wheat to the combined adverse effects of high temperatures and drought (Schmidt *et al.*, 2020; Tyagi and Pandey, 2022), data on the correlations between heat-stress and drought tolerance in varieties of different ecological and geographical origin at the seedling stage are still lacking. At the same time, results were obtained indicating a link between the resistance of etiolated seedlings to osmotic and heat stress and the field resistance of adult plants to these factors (Oboznyi *et al.*, 2013; Pykalo *et al.*, 2020). With this phenomenon in mind, methods have been developed for evaluating the drought tolerance of wheat and other cereal varieties using the indicator of growth inhibition in the presence of osmotic agents (Pykalo *et al.*, 2020). There is also a method for assessing the heat resistance of wheat by inhibiting the growth of etiolated seedlings after exposure to elevated but not lethal temperatures (Pat. 45879 UA, 2002).

In this connection, studying the correlation between heat and dehydration resistance of etiolated wheat seedlings of different varieties can help to improve methods of evaluating samples of genetic collections and breeding material for cross-tolerance to these stress factors. A simultaneous study of the oxidative stress development in seedlings exposed to heat or model drought will make it possible to assess its role in damage development and to gain a deeper understanding of plant adaptation to these two most common natural stressors.

The purpose of this work was to compare the growth response of seedlings of seven common wheat cultivars of different ecological and geographical origin to heat stress and model drought created using PEG 6000. In addition, a comparative study of the development of oxidative stress and osmolyte accumulation under these adverse conditions was included in the scope of the work.

MATERIAL AND METHODS

Plant materials and treatments

Seven cultivars of common winter wheat (*Triticum aestivum* L.) of different ecological and geographical origin were used for the research. Five of these cultivars have been developed in different soil and climate zones in Ukraine. The Antonivka and Lira odeska (originated by Plant Breeding and Genetics Institute of the National Academy of Agrarian Sciences of Ukraine, Odesa) have the resistance required for cultivation in the Steppe zone (Khakhula

et al., 2013). The Doskonala cultivar (originated by Yuriev Plant Production Institute of NAAS of Ukraine, Kharkiv) is mainly intended for cultivation in the Forest-steppe and has a low heat and drought tolerance (Karpets *et al.*, 2016). The Darynka kyivska and Bogdana (originated by Institute of Plant Physiology and Genetics of NAS of Ukraine, Kyiv) are characterized as moderately resistant and designed for cultivation in different zones (Chernobai *et al.*, 2013, 2019; Khomenko, 2020). The cultivar Tobak (originator: Saaten-Union GmbH, Isernhagen HB, Germany), intended for cultivation in Central Europe but capable of maintaining productivity under conditions of drought and high temperatures, was also used in the research (Hlaváčová *et al.*, 2018; Urban *et al.*, 2018). In addition, the Avgustina cultivar, developed for cultivation in Belarusian Polesie, which is characterized by high humidity (originator: Scientific and Practical Center of NAS of Belarus), was used.

The experiments were carried out in the Laboratory of Plant Physiology and Biochemistry of the Yuriev Plant Production Institute of NAAS of Ukraine. Seed samples were provided by the National Center for Genetic Resources of Plants of Ukraine. Seeds of 2020 and 2021 reproduction years were used for the research.

The seeds were disinfected with a 1% sodium hypochlorite solution for 15 minutes and then washed with distilled water at least 8 times. For experiments on the effect of heat stress on seedling growth and biochemical parameters, seeds were germinated at 24°C for 3 days in Petri dishes on two layers of filter paper moistened with distilled water. Three-day-old seedlings of experimental variants were placed in open Petri dishes in a thermostat at 45±1°C (Pat. 45879 UA, 2002) and an air humidity of 40-45% (4 h exposure). To prevent the roots from drying out, a filter paper in the dishes was dampened with an equal amount of distilled water every hour. After exposure, one part of the seedlings was used for biochemical analyses, and the other part was placed in a 24°C thermostat to evaluate the growth response. The seedlings of control variants were kept in a thermostat at 24°C throughout the experiment. 24 h after heat stress, the inhibition of shoot and root growth of the seedlings was determined 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 seedling organ fresh weights in the control and experimental (heat stress) variants.

In experiments on the effect of model drought (osmotic stress) on seedling growth, seeds of experimental variants were germinated at 24°C in Petri dishes on two layers of filter paper moistened with 12% PEG 6000 solution. The seeds of the control variant were germinated under the same conditions in distilled water. On the 4th day of the experiment, biochemical analyses were carried out

and the shoot and root biomass of the seedlings was assessed. Inhibition of seedling growth under drought conditions was calculated using the formula:

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

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

The stress intensity causing a 20-60% inhibition of seedling growth in different cultivars was selected based on preliminary experiments using PEG 6000 solutions with concentrations in the range of 10-16%.

Determination of water content

The water content of seedling organs was determined gravimetrically by drying a sample of shoots or roots (0.5 g of fresh material) at 103°C to a constant weight.

Evaluation of hydrogen peroxide content

For the determination of H₂O₂ content, seedling shoots were homogenized in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000 g for 10 min at 2-4°C on an MPW 350R centrifuge (MPW MedInstruments, Poland). Supernatant concentration of H₂O₂ was determined by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this, 0.5 ml of 2.5 M potassium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ammonium iron(II) sulfate were added to tubes. After stirring, the samples were poured into cuvettes and the absorbance at 480 nm was determined.

Evaluation of LPO products content

The rate of lipid peroxidation (LPO) in seedling shoots was assessed by its products reacting with 2-thiobarbituric acid (TBA) (mainly malondialdehyde, MDA) (Kolupaev *et al.*, 2020a). Sample material was homogenised in 0.1 M Tris-HCl buffer (pH 7.6); a 0.5% solution of TBA in 20% TCA was then added to the homogenate. Following heating the mixture in a boiling water bath for 30 min, the samples were cooled and centrifuged at 8000 g for 10 min. Afterwards, the absorbance of the supernatant was measured at 532 nm. Also the non-specific absorbance at 600 nm was determined, with the value subtracted from the main result. The measurements were carried out relative to a reagent mixture not containing TBA.

Assessing content of osmolites

The total sugars content in plant material was determined by the Morris-Roe method based on the anthrone reagent (Zhao *et al.*, 2003) in our modification. Sugars from the plant material were extracted with distilled water and heating for 10 minutes in a boiling water bath. The obtained extract was clarified by adding equal volumes (0.3 ml) of 30% zinc sulfate 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. 3 ml of anthrone reagent and 1 ml of the filtrate were added to the reaction tubes, and distilled water was added 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. The standard used was D-glucose.

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

Replicate of experiment and statistical analysis

The experiment had 3 biological replicates. For the assessment of seedling organ weights and water content, each biological replication consisted of 30 seedlings. When measuring biochemical parameters, each sample of plant material was taken from 12-15 seedlings.

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

RESULTS AND DISCUSSION

Seedling growth under heat stress and drought

The most significant growth inhibition after 4 h of high temperature was observed in the seedlings of the cultivars Doskonala, Avgustina, and Bogdana (Table 1). It was lower in Darynka kyivska and Lira odeska. Tobak retained its ability to grow sufficiently well after stress and, finally, Antonivka showed the greatest resistance to heat.

Table 1. Effects of heat stress and drought on wheat seedling growth

Cultivar	Growth inhibition, %					
	Heat stress (45°C, 4 h)			Drought (12% PEG 6000)		
	Seed-lings	Shoots	Roots	Seed-lings	Shoots	Roots
Tobak	27.6±2.2d*	38.6±2.9c	11.6±0.4f	32.5±0.4d	37.9±0.5c	27.7±1.0c
Antonivka	16.4±1.3e	15.1±1.5d	17.8±2.1e	39.7±2.1bc	44.7±2.1b	35.6±2.2b
Lira odeska	40.7±1.2c	48.5±1.9b	29.0±2.2d	38.0±1.6c	44.0±2.2b	32.3±1.5b
Darynka kyivska	39.5±0.7c	42.7±1.8c	29.3±1.3d	41.1±1.3b	44.9±1.2b	37.1±1.6a
Bogdana	56.0±2.5b	61.1±2.5a	51.0±3.2b	39.2±0.7bc	50.6±1.3a	26.2±0.3c
Avgustina	51.6±2.7b	60.0±3.2a	43.7±2.5c	46.4±0.4a	52.9±1.0a	38.9±1.8a
Doskonala	61.2±1.0a	60.9±1.5a	62.6±2.1a	43.2±2.2ab	54.1±2.5a	32.0±2.8b

*Different letters in the same column denote statistically significant difference at $P \leq 0.05$

Shoot growth after heat stress was inhibited more severely than root growth in most cultivars (except Antonivka). This may in part be due to the loss of moisture by the shoots under heat stress, whereas no such effect was observed in the roots in any of the cultivars (Table 2). Variety differences in heat stress inhibition of shoot biomass growth were virtually indistinguishable from those of seedlings as a whole (Table 1).

Table 2. Water content (%) in the organs of wheat seedlings under heat stress and drought

Cultivar	Heat stress (45°C, 4 h)				Drought (12% PEG 6000)			
	Shoots		Roots		Shoots		Roots	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tobak	88.0 ± 0.2b*	87.8 ± 0.3bc	90.9 ± 0.3ab	90.8 ± 0.4ab	88.6 ± 0.4bc	87.8 ± 0.4c	91.2 ± 0.1ab	87.2 ± 0.2d
Antonivka	89.6 ± 0.3a	88.9 ± 0.4ab	90.2 ± 0.4b	90.2 ± 0.3b	90.0 ± 0.3ab	87.2 ± 0.3cd	90.5 ± 0.2b	85.9 ± 0.2f
Lira odeska	89.2 ± 0.2a	88.6 ± 0.3ab	90.2 ± 0.2b	90.0 ± 0.4b	89.5 ± 0.3b	87.8 ± 0.5c	90.6 ± 0.5b	86.8 ± 0.2c
Darynka kyivska	89.9 ± 0.3a	88.6 ± 0.3ab	90.4 ± 0.3b	90.1 ± 0.4b	90.7 ± 0.2a	88.1 ± 0.2c	90.7 ± 0.6b	87.8 ± 0.2d
Bogdana	88.8 ± 0.2 ab	87.4 ± 0.3bc	90.0 ± 0.2b	90.2 ± 0.2b	89.2 ± 0.6b	87.3 ± 0.7d	88.8 ± 0.1c	82.6 ± 0.2g
Avgustina	89.7 ± 0.3a	87.9 ± 0.2bc	91.3 ± 0.3a	91.1 ± 0.4a	90.1 ± 0.2ab	87.6 ± 0.1d	91.6 ± 0.2a	86.9 ± 0.1e
Doskonala	89.2 ± 0.2a	86.6 ± 0.3c	91.1 ± 0.4a	90.7 ± 0.3ab	89.8 ± 0.1ab	87.1 ± 0.5d	91.0 ± 0.3ab	85.6 ± 0.1f

*Different letters denote statistically significant difference at $P \leq 0.05$

Root growth was found to be the least sensitive to heat stress in Tobak and Antonivka cultivars. The inhibition of root growth was more pronounced in Lira odeska and Darynka kyivska. Strong suppression of root growth was observed in Doskonala, Bogdana, and Avgustina (Table 1).

The pattern of effects of model drought on seedling growth had both significant similarities and differences compared with the impact of heat stress. The most significant inhibition of seedling growth in general was observed in the cultivars Avgustina and Doskonala (Table 1). A lesser growth-inhibiting effect of drought was observed in Antonivka, Lira odeska, Darynka kyivska, and Bogdana. The seedlings of Tobak variety retained the greatest degree of growth capacity under PEG 6000 conditions (Table 1). Approximately the same varietal differences were observed for the shoot growth rate. The pattern of root growth inhibition during drought was slightly different. This effect was most noticeable in the cultivars Avgustina and Darynka kyivska. To a lesser extent root growth was affected by the drought in Doskonala, Lira odeska, and Antonivka. The least significant inhibition of root growth under model drought was observed in Bogdana and Tobak (Table 1).

Water content of seedling organs

As already noted, heat stress had almost no effect on the water content of the seedling roots (Table 2). Its effect on the shoot water content in the Tobak, Antonivka, Lira odeska, and Darynka kyivska cultivars was also insignificant. Some reduction in the water content was recorded in the shoots of Doskonala, Avgustina, and Bogdana.

Drought stress had a more pronounced effect on the water content of the seedling organs. Thus, a significant reduction in root water content was observed in all cultivars (Table 2). It was sharpest in Bogdana cultivar. The water content of the shoots also decreased significantly in all cultivars except the Tobak one.

Correlations between inhibition of seedling growth by heat and drought action.

Under heat stress, root and shoot growth inhibition were closely correlated with each other ($r=0.81$), indicating proportional inhibition of seedling organ growth in different cultivars (Figure 1). At the same time, the correlation between the inhibition of root and shoot growth under osmotic stress was low ($r=0.24$). This is due to the unequal effect of drought on root and above-ground seedling growth in different cultivars.

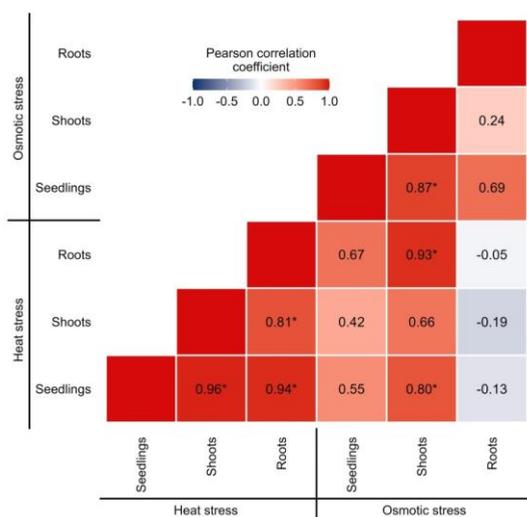


Figure 1. Correlation coefficients between growth inhibition of wheat seedlings and their organs under the effects of high temperature and drought.

*— significant at $P \leq 0.05$

A medium strength correlation ($r=0.55$) was observed between the total inhibition of seedling growth under heat and osmotic stress. Relatively high, although not significant at $P \leq 0.05$, was the correlation between shoot growth inhibition under the two types of stress ($r=0.66$). Herewith a significant correlation was found between the inhibition of root and seedling biomass

accumulation under heat stress and the inhibition of shoot biomass growth under drought ($r=0.93$ and 0.80 , respectively). These values also indicate, to some extent, a cross relation between heat and drought tolerance of seedlings. At the same time, no correlation was found at all between the inhibitions of root growth under these stresses (Figure 1).

The ability to accumulate sufficient above-ground biomass under stress conditions is thought to be a more important characteristic of resistance than root growth (Laxa *et al.*, 2019). In terms of inhibition of shoot growth after heat stress, the cultivars studied were ranked as follows: Antonivka < Tobak < Darynka kyivska < Lira odeska < Avgustina \leq Doskonala \leq Bogdana. At the same time, the order of the cultivars differed somewhat in terms of the magnitude of shoot growth suppression under drought conditions: Tobak < Lira odeska \leq Antonivka \leq Darynka kyivska < Bogdana \leq Avgustina \leq Doskonala. Despite such differences, it can be observed that the cultivars Tobak, Antonivka, Darynka kyivska, and Lira odeska showed significantly greater resistance to both stresses compared to Avgustina, Bogdana, and Doskonala.

Indicators of oxidative stress in shoots under the action of heat and drought

After 4-h heating of the seedlings, a significant increase (1.5-2.1 times) in hydrogen peroxide content was recorded in the cultivars Doskonala, Bogdana, and Avgustina (Table 3). In Lira odeska and Darynka kyivska, this effect was much weaker, while in Tobak and Antonivka, the hydrogen peroxide content after seedling heating was almost unchanged. The content of the LPO product MDA as well as the amount of H₂O₂ increased most markedly (1.3 times) in the cultivars Doskonala, Bogdana, and Avgustina (Table 3). A smaller increase in this index after exposure to high temperature was recorded in Lira odeska and a very slight increase in the cultivars Tobak and Antonivka.

Table 3. Effect of heat and osmotic stress on hydrogen peroxide and MDA content in wheat seedling shoots

Cultivar	Content, % to control			
	Heat stress (45°C, 4 h)		Drought (12% PEG 6000)	
	H ₂ O ₂	MDA	H ₂ O ₂	MDA
Tobak	106±4d	107±2c	103±18b	126±7c
Antonivka	102±3d	103±3c	89±5c	124±2c
Lira odeska	119±5c	115±4b	91±17c	129±4c
Darynka kyivska	112±4cd	108±3bc	158±15ab	123±1c
Bogdana	157±4b	130±4a	109±14bc	131±7c
Avgustina	150±6b	130±2a	131±15b	180±4a
Doskonala	209±2a	130±2a	176±9a	143±1b

*Different letters in the same column denote statistically significant difference at $P \leq 0.05$

Under drought conditions, the H₂O₂ content increased markedly in the cultivars Doskonala, Darynka kyivska, and Avgustina and less so in the Bogdana. In the Tobak, Antonivka, and Lira odeska cultivars, however, the hydrogen peroxide

content did not change significantly. The MDA content increased most significantly in Avgustina and Doskonala under the influence of drought (Table 3).

Osmolyte content in shoots under heat and drought conditions

The initial proline content in 3-day-old seedlings (heat stress experiment) and 4-day-old seedlings (drought experiment) differed slightly, with a tendency to increase in 4-day-old seedlings (Table 4). No significant relationship was observed between its content in the control and resistance to the relevant stressors. For example, the highest proline content was found in the heat and drought-non-resistant cultivar Avgustina. On the other hand, the proline content was almost the same in the Darynka kyivska, which was sufficiently resistant to these stresses, and the least resistant Doskonala. Heat stress caused a 2-4-fold increase in proline content in all cultivars. The highest amounts were recorded in the non-resistant cultivars Bogdana and Avgustina (Table 4). The increase in proline content during drought was less pronounced than during heat stress, with the highest content also found in the Avgustina cultivar.

Table 4. Proline and sugars content in shoots of etiolated wheat seedlings under heat stress and drought

Cultivar	Heat stress (45°C, 4 h)				Drought (12% PEG 6000)			
	Proline (mg/g dry weight)		Sugars (mg/g dry weight)		Proline (mg/g dry weight)		Sugars (mg/g dry weight)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tobak	0.94 ± 0.02g*	1.86 ± 0.05e	210 ± 7f	273 ± 6c	1.12 ± 0.08e	1.59 ± 0.06cd	197 ± 7de	300 ± 22a
Antonivka	0.71 ± 0.01h	2.71 ± 0.09c	215 ± 7f	314 ± 5b	0.85 ± 0.05f	1.04 ± 0.05ef	200 ± 9d	259 ± 10b
Lira odeska	0.79 ± 0.03gh	2.43 ± 0.08d	238 ± 10de	309 ± 6b	0.90 ± 0.08f	1.24 ± 0.11de	199 ± 8de	259 ± 4b
Darynka kyivska	1.23 ± 0.04f	2.46 ± 0.08d	208 ± 7f	363 ± 6a	1.44 ± 0.05d	1.84 ± 0.10bc	211 ± 3d	223 ± 3cd
Bogdana	1.33 ± 0.06f	3.40 ± 0.08a	207 ± 3f	251 ± 7d	1.37 ± 0.10de	1.70 ± 0.15c	182 ± 10e	210 ± 10d
Avgustina	1.89 ± 0.04e	3.23 ± 0.08b	244 ± 7d	252 ± 9d	1.93 ± 0.05b	3.70 ± 0.24a	204 ± 6d	232 ± 9c
Doskonala	1.15 ± 0.01f	2.79 ± 0.12c	206 ± 5f	229 ± 9e	1.49 ± 0.21d	1.61 ± 0.10c	213 ± 9cd	215 ± 14cd

*Different letters denote statistically significant difference at $P \leq 0.05$

The sugar content of 3-4 day-old seedlings differed slightly depending on age and cultivar (Table 4). Heat stress caused an increase in sugar content of the shoots of all the cultivars studied with the exception of Avgustina. Wherein higher absolute values were recorded for the more heat-resistant cultivars (Darynka kyivska, Antonivka, Lira odeska, and Tobak). Under the drought conditions created by PEG 6000, a significant increase in sugar content was found in the resistant cultivars Tobak, Antonivka, and Lira odeska (Table 4). In the relatively resistant Darynka kyivska, as well as in the weakly resistant

Bogdana and Augustina, this effect was relatively small, while in the cultivar Doskonala it was absent.

Correlations between growth inhibition and the content of oxidative stress markers and osmolytes in shoots

A strong correlation ($r=0.76$ and 0.91 , respectively) was found between inhibition of shoot growth under heat stress and its hydrogen peroxide and MDA content (Fig. 2). At the same time, the corresponding correlation coefficients were much lower for drought conditions (Figure 2). There is therefore reason to believe that under heat conditions the contribution of oxidative stress to damage and inhibition of seedling growth is more significant than under drought conditions.

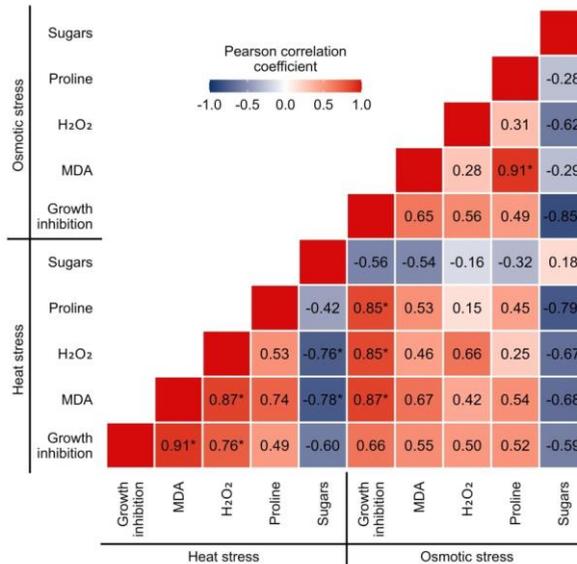


Figure 2. Correlation coefficients between shoot growth inhibition and content of oxidative stress markers and osmolytes under high temperature and drought stress. *—significant at $P \leq 0.05$

A moderate positive correlation ($r=0.49$) was found between the inhibition of shoot growth under heat stress and proline content, but not significant at $P \leq 0.05$ (Figure 2). At the same time, a fairly strong negative correlation ($r=-0.60$) was shown between shoot growth inhibition and sugar content. Also of note is the negative correlation, significant at $P \leq 0.05$, between sugar content and oxidative stress (MDA and H₂O₂ content) after heating the seedlings. This fact may indicate a role for sugars in protecting seedlings against oxidative stress under high-temperature conditions. It was reported that soluble carbohydrates can participate in antioxidant protection under heat stress and are probably involved in antioxidant system regulation (Kolupaev *et al.*, 2023). For example, exogenous glucose increased heat tolerance and reduced ROS in cucumber plants (Huang *et*

al., 2015). This was accompanied by an increase in the number of transcripts and increased activity of Cu/Zn-COD, Mn-COD, catalase, and glutathione reductase. The heat-tolerant chickpea cultivar accumulated more sugars under stress compared to the non-tolerant one (Yadav *et al.*, 2022). It is also known that sucrose can protect membranes by forming bonds with phosphate "heads" of lipids (Tarkowski and Vanden, 2015).

In our experiments under drought conditions, as well as after heat stress, a positive correlation of moderate strength was found between proline content and growth inhibition (Figure 2). Note the very high level of positive correlation between proline content and the main marker of oxidative stress MDA in shoots during drought ($r=0.91$). In this connection, it can be assumed that under these experimental conditions, the proline content correlates not with the resistance of the seedlings to the stress factor but, on the contrary, with the manifestation of oxidative damage. Similar effects have been found in studies of the impact of other stressors on plants. For example, a strong positive correlation has been shown between proline and MDA accumulation when rapeseed plants were exposed to a wide range of toxic copper concentrations (Kholodova *et al.*, 2018). It is difficult to give an unambiguous interpretation of such effects. On the one hand, they may indicate a link between oxidative stress and proline accumulation (Signorelli *et al.*, 2014). On the other hand, this relationship cannot be unequivocally interpreted as proline's involvement in antioxidant defense. High proline concentrations are known in some cases to induce pro-oxidative processes in mitochondria and may induce programmed cell death (Fabro *et al.*, 2004; Miller *et al.*, 2009). To discuss this issue is beyond the scope of the results obtained in this work. It should be noted, however, that the presence of both antioxidant and pro-oxidant effects of proline casts doubt on its use as an indicator of stress tolerance, at least in large-scale experiments to evaluate breeding material (Pykalo *et al.*, 2020).

While proline accumulation in wheat seedlings under drought conditions correlated positively with growth inhibition, the content of other osmolytes, sugars, was in a high negative correlation with growth inhibition ($r=-0.85$). This fact indicates the special role of sugars as osmolytes and probably as multifunctional protective compounds in seedling adaptation to drought conditions.

In general, the results obtained indicate both common and specific features of wheat seedling adaptation to the two stressors that often act simultaneously in natural conditions – high temperature and drought. Seven cultivars of different ecological and geographical origins were used in our experiments. In their example, it was possible to establish a positive correlation between heat and drought resistance of the seedlings. However, the values of the correlation coefficients for this sample were not significant at $P \leq 0.05$. Perhaps if more correlation pairs were used, such a relationship would show up as more reliable. On the other hand, the presence of a significant correlation between oxidative

stress and growth inhibition only under heat stress but not under drought stress (Figure 2) indicates differences in the mechanisms of plant damage under these two stresses. At least within the scope of the model we used, it can be argued that heat damage is more dependent on ROS formation than PEG-induced drought damage. It should be noted that on etiolated seedlings of different cereal species, it was possible to show the relationship between heat tolerance and their resistance to oxidative stress (Kolupaev *et al.*, 2022). The functioning of the enzymatic component of the antioxidant system has not been studied in the present work. However, a fair amount of data on the relationship between heat tolerance in plants and the functioning of their antioxidant defense components is generally available in the literature (Puckette *et al.*, 2007; Kolupaev *et al.*, 2023).

As for drought stress, osmolytes, in particular sugars, seem to play an especially important role in resistance under the conditions of the model we used. Sugars have been shown to be a better substitute for water under drought conditions and better preserve the hydrated state of proteins in drought compared to proline (Tyagi and Pandey, 2022).

Overall, the results show that etiolated wheat seedlings can be used both for accelerated evaluation of breeding material for heat and drought resistance and for studying the mechanisms of damage caused by these factors and adaptation to them. To elucidate the role of the antioxidant system in wheat resistance to the above stress factors, it is of further interest to study the response of varieties of different ecological and geographical origins to the action of direct oxidative stress agents – exogenous ROS or inducers of their formation. Similar studies carried out on clover (Puckette *et al.*, 2007) and *Arabidopsis* (Iseki *et al.*, 2014) plants from different ecological and geographical zones indicate a link between adaptation to unfavorable natural conditions and resistance to pro-oxidants.

CONCLUSIONS

A relationship has been established between the resistance of seven varieties of etiolated wheat seedlings of different ecological and geographical origin to high temperatures and drought. Wherein specific features of the action of each stressor were also found. A close correlation between the inhibition of seedling growth under heat stress and the accumulation of oxidative stress markers, hydrogen peroxide and MDA, in seedlings was shown. In a drought, such a connection was not so prominent. At the same time, the accumulation of osmolytes with multifunctional protective effects appears to be more important for seedling drought resistance. A high negative correlation was shown between sugars accumulation and inhibition of seedling growth in drought conditions. At the same time, proline accumulation was positively correlated with growth inhibition and the development of oxidative stress, which limits the use of this indicator in applied research to assess the stress tolerance of wheat varieties.

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