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RESPONSE OF WHEAT SEEDLINGS WITH DIFFERENT DROUGHT TOLERANCE TO MELATONIN ACTION UNDER OSMOTIC STRESS

SUMMARY

Melatonin is a multifunctional molecule whose biological activity has been intensively studied in recent years in the context of plant adaptation to abiotic stresses, including drought. However, comprehensive studies on the effect of melatonin on the status of antioxidant and osmoprotective systems in wheat varieties with different drought adaptation strategies have been lacking. We studied the effect of melatonin on the growth and functioning of stress protection systems of wheat (*Triticum aestivum* L.) seedlings of Tobak (drought-tolerant) and Doskonala (drought-sensitive) varieties. Two-day-old etiolated seedlings were transferred to a 15% PEG 6000 solution for 2 days to create a model drought. The addition of melatonin to the incubation medium of seedlings at concentrations in the range of 0.2-5.0 μM significantly alleviated the inhibition of root and shoot growth of seedlings caused by drought. The effects of melatonin were more pronounced in the drought-sensitive cultivar Doskonala. Melatonin treatment almost completely eliminated the effect of increased hydrogen peroxide content under drought conditions in both varieties. Also in Doskonala variety, melatonin treatment reduced stress-induced accumulation of lipid peroxidation products. Drought caused a decrease in superoxide dismutase activity in both varieties, while melatonin treatment helped to maintain the enzyme activity at a level close to that of the control variants. In both varieties, melatonin treatment also reversed the stress-induced decrease in guaiacol peroxidase activity. Under

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the influence of melatonin, proline accumulation was significantly enhanced in Doskonala under drought conditions. Drought caused a decrease in sugars and flavonoid compounds, while melatonin mitigated these effects in both varieties. It is concluded that melatonin significantly modulates the adaptive responses of etiolated wheat seedlings to drought and that its effects depend on cultivar characteristics.

INTRODUCTION

A global analysis of published results from studies conducted over the past four decades has shown that wheat yield losses due to water stress are at least 20% (Daryanto *et al.,* 2016). Drought stress causes a number of biochemical, morphophysiological, and anatomical disturbances in plants that occur at all stages of plant development, from seed germination to the final stages of the generative phase (Cui *et al*., 2018; Tiwari *et al.,* 2021). Drought tolerance in plants is the result of numerous interrelated mechanisms that operate at the molecular, cellular, and organismal levels. Among them, changes in the state of the stomatal apparatus, accumulation of various osmolytes in cells, activation of aquaporins, increased functioning of the antioxidant system are currently considered to be the key ones (Nawaz *et al*., 2019; Pržulj *et al*., 2020; Singh *et al.*, 2021; Vignesh, Palanisamy 2021; Kolupaev *et al.,* 2023a). The regulation of these processes involves numerous groups of biomolecules that exhibit signaling and hormonal activity (Yemets *et al*., 2019; Kosakivska *et al*., 2022).

In addition to the classical "drought hormone" abscisic acid (ABA) (Jogawat *et al*., 2021), the role of other groups of hormones—salicylic acid (Singh *et al*., 2021; Rouhnezhad *et al.,* 2023), jasmonates (Kolupaev *et al*., 2023b), and brassinosteroids (Ribeiro *et al*., 2019) - in drought adaptation has been intensively studied over the past two decades. Also in recent years, knowledge of the plant functions of neurotransmitters, which were previously discovered and studied in mammals, has been intensively accumulated. The main of these compounds are acetylcholine, biogenic amines (dopamine, noradrenaline, adrenaline, histamine), and indolamines (melatonin and serotonin) (Akula, Mukherjee, 2020). Melatonin (N-acetyl-5-methoxytryptamine) is now recognized as a pleiotropic signaling molecule that plays an important role in regulating responses to various stresses (Buttar *et al.,* 2020). It was first discovered in plants only in 1995 (Dubbels *et al*., 1995). In recent years, however, melatonin has become a popular topic in plant biology (Ayyaz *et al*., 2022).

A number of studies conducted on plants of different species have found an increase in endogenous melatonin levels in response to stressors (Buttar *et al*., 2020), including the effects of drought (Arnao, Hernández-Ruiz, 2013). A large body of evidence has also been accumulated on the positive effects of exogenous melatonin on plant tolerance to abiotic stressors (Ciu *et al*., 2018; Li *et al.,* 2020). However, the phenomenology rather than the mechanisms of melatonin's stress-

protective effects have been predominantly studied. At least one reason for the protective effects of melatonin is related to the modification of redox homeostasis. In particular, direct antioxidant effects of melatonin have been hypothesized, given its ability to interact with ROS and its presence in significant amounts in plants (Arnao, Hernández-Ruiz, 2015; Fan *et al*., 2018; Ahamad *et al.,* 2021). It has been reported that one molecule of melatonin can bind up to 10 free radicals, which may be higher than the efficiency of superoxide dismutase (SOD) action (Ye *et al*., 2016). However, the effects of melatonin on redox homeostasis are not limited to direct antioxidant effects. Many studies have reported the induction of antioxidant enzyme gene expression by melatonin (Martinez *et al*., 2018; Sun *et al.,* 2018; Zhao *et al*., 2018) and its effect on the synthesis of low-molecular-weight antioxidants (Ahmad *et al*., 2019; Awan *et al*., 2023).

A hallmark of the plant response to drought stress is the accumulation of various osmolytes, mainly proline (Joseph *et al.,* 2015) and sugars (Mukarram *et al.,* 2021), as well as a number of secondary metabolites (Ma *et al*., 2014). All of these compounds have antioxidant activity and may have membrane-protective and antidenaturing effects (Liang *et al.,* 2013; Kolupaev *et al*., 2019; Deryabin, Trunova, 2021). The pattern of accumulation of stress metabolites in plants is known to change under the influence of melatonin (Shi *et al*., 2015; Jiang *et al.,* 2016; Tiwari *et al.,* 2021; Zafar *et al*., 2020; Sattar *et al*., 2023). However, the reported effects can vary. For example, both increases (Antoniou *et al.,* 2017; Ahmad *et al*., 2021; Awan *et al*., 2023) and decreases (Zamani *et al.,* 2019; Buttar *et al.,* 2020) in proline content under the influence of melatonin have been reported in plants of different species under drought. In addition to the multiple effects of increased sugars in plants when treated with melatonin under stress conditions, soybean plants were found to have decreased soluble carbohydrates under drought conditions (Imran *et al*., 2021).

The effects of melatonin may depend not only on the species but also on the varietal characteristics of the plants. Thus, melatonin was shown to enhance proline accumulation under drought in non-resistant wheat cultivars and to attenuate it in resistant cultivars (Li *et al*., 2020). It is known that the adaptive strategies of wheat varieties and other cereal crops can differ significantly, which can be manifested in the different contribution of enzymatic and non-enzymatic components of the antioxidant system to adaptation to oxidative stress (Kolupaev *et al*., 2023a; 2023c).

Despite the importance of wheat as a food crop, there has been very little research on the effect of melatonin on wheat drought tolerance (Cui *et al*., 2018; Li *et al*., 2020; Zhang *et al*., 2023). The information on the peculiarities of the manifestation of stress-protective effects of melatonin in varieties with different drought adaptation strategy is particularly fragmentary. In particular, there are no works that have simultaneously studied the effect of melatonin on the functioning under stress conditions of antioxidant and osmoprotective systems in etiolated seedlings of wheat varieties differing in drought tolerance.

Meanwhile, the study of melatonin regulation of adaptive processes in winter wheat at the earliest stages of development has not only theoretical but also applied significance, as severe droughts in autumn are a typical phenomenon in Eastern European countries, including Ukraine (Romanenko *et al.,* 2018). In this regard, the aim of the work was to compare the reactions of stress-protective systems of 2-4-day-old wheat seedlings of Tobak (drought-tolerant) and Doskonala (sensitive) varieties to melatonin action under model drought conditions (treatment with 15% PEG 6000).

MATERIAL AND METHODS

Plant materials and treatments

Wheat seedlings of the varieties Tobak (characterized by high drought tolerance under laboratory (Kolupaev *et al.,* 2023c) and field conditions (Urban *et al.,* 2018), originator—Saaten-Union GmbH, Isernhagen HB, Germany) and Doskonala (sensitive to drought (Kolupaev *et al*., 2023c), originator—Yuriev Plant Production Institute of NAAS of Ukraine) were used for research. Seeds of reproduction of 2022, obtained in the National Center for Plant Genetic Resources of Ukraine (Kharkiv, eastern forest-steppe of Ukraine), were used in this work.

Seeds were disinfected in 70% ethanol for 2 min, then transferred to 2% sodium hypochlorite solution for 15 min, washed 10 times with sterile distilled water, and germinated on water in Petri dishes in a thermostat at 24°C without light for 2 days. After that, seedlings of approximately the same length were transferred to Petri dishes with two layers of filter paper moistened with 15% PEG 6000 solution. Control seedlings were transferred to Petri dishes with filter paper moistened with distilled water. Melatonin was dissolved in a small volume of ethanol, the solutions were diluted with distilled water and added to Petri dishes to obtain working solutions with concentrations in the range of 0.2-5.0 μM. In special experiments it was found that ethanol in concentrations not higher than 0.001% applied with melatonin solutions did not affect the growth of wheat seedlings and their resistance to osmotic stress.

After two days of exposure of seedlings to PEG 6000 and/or melatonin solutions, shoot and root biomass of seedlings were evaluated and biochemical parameters in shoots were determined.

Measurement of hydrogen peroxide content

To determine H_2O_2 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). The concentration of H_2O_2 in the supernatant was determined by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this purpose, 0.5 ml of 2.5 M NH_4SCN, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ammonium ferrous sulfate were added to tubes. After mixing, the samples were transferred to cuvettes and the absorbance at 480 nm was determined.

Evaluation of LPO products content

To analyze the amount of lipid peroxidation products (LPO) reacting with 2-thiobarbituric acid (mainly malonic dialdehyd-MDA), shoots were homogenized in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% TCA, the homogenate in tubes covered with foil lids was placed in a boiling bath for 30 min. The samples were then cooled and centrifuged at 10000 g for 15 min. The absorbance of the supernatant was determined at 532 nm (maximum light absorption of MDA) and 600 nm (to correct for non-specific light absorption) (Kolupaev *et al.,* 2021).

Analysis of antioxidant enzyme activity

To determine the activity of the antioxidant enzymes SOD, catalase, and guaiacol peroxidase, shoots were homogenized on cold in 0.15 M K, Na-phosphate buffer (pH 7.6) containing EDTA (0.1 mM) and dithiothreitol (1 mM) (Kolupaev *et al.,* 2022). Enzyme activity was determined in the supernatant after centrifugation of the homogenate at 8000 g for 10 min at 4°C. Since the water content in shoots of seedlings of different variants differed during sampling, the studied parameters were calculated per gram of dry weight.

SOD activity (EC 1.15.1.1.1) was determined at pH 7.6 by a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions formed by aerobic interaction of NADH and phenazine methosulfate; absorbance was determined at 540 nm (Kolupaev *et al*., 2012). The activity of catalase (EC 1.11.1.6) was analyzed at pH 7.0 by the amount of hydrogen peroxide decomposed per unit time. The activity of guaiacol peroxidase (EC 1.11.1.7) was determined using guaiacol as hydrogen donor and hydrogen peroxide as substrate. In this case, the pH of the reaction mixture was preliminarily adjusted to 6.2 with K, Na-phosphate buffer (Kolupaev *et al*., 2022). The absorbance of tetraguaiacol was determined at 470 nm.

Estimation of low-molecular-weight protectors

The total sugar content in the plant material was determined by the Morris-Roe method on the basis of the anthrone reagent (Zhao *et al*., 2003) in our modification described earlier (Kolupaev *et al*., 2023c). D-glucose was used as a standard.

Proline content was determined using the ninhydrin reagent (Bates *et al*., 1973). L-proline was used as the standard.

For the determination of total phenolics and flavonoids, seedlings were homogenized in 80% ethanol, extracted for 20 min at room temperature, and centrifuged at 8000×g for 15 min. For the analysis of phenolic compounds, 0.5 ml of supernatant, 8 ml of distilled water and 0.5 ml of Folin reagent were added to reaction tubes, stirred and after 3 min, 1 ml of 10% sodium carbonate was added. The absorbance of the reaction mixture was measured at 725 nm after 1 h (Bobo-García *et al*., 2015). The phenolic content was expressed as μmol gallic acid per gram dry weight.

Before determining the content of anthocyanins and UV-B absorbing flavonoids, the supernatant was acidified with HCl to a final concentration of 1%. The absorbance of the solutions was determined at 530 and 300 nm (Nogués, Baker, 2000). Results were expressed as absorbance per dry weight of plant material.

Experimental replication and statistical analysis

Experiments had 3-4 biological replicates. For seedling organ weight determination, each sample consisted of 30 seedlings. Samples for biochemical analyses consisted of 12 seedlings each. Data for each parameter were statistically analyzed using Fisher's analysis (ANOVA and Least Significant Difference (LSD) test at 5% probability level). Figures and tables show means and their standard errors; different letters indicate values whose differences are significant at P≤0.05.

RESULTS AND DISCUSSION

Growth response of wheat seedlings to drought action and melatonin treatment

Under the influence of drought created by PEG 6000, the growth of root and shoot biomass decreased by about 35% in the resistant variety Tobak (Figure 1). At the same time, in the drought-sensitive variety Doskonala, under the conditions of model drought, the indicators of seedling organ biomass decreased by about 53%.

Treatment with melatonin at concentrations of 0.2-5.0 μM significantly attenuated the growth inhibitory effect of osmotic stress (Figure 1). The most effective concentration was 1 μM. At the same time, varietal differences were observed: the positive effect of melatonin on root and shoot growth under osmotic stress was more pronounced in the Doskonala variety than in the Tobak one (Figure 1).

Melatonin modulation of oxidative stress effects under drought conditions

Incubation of seedlings on medium containing PEG 6000 increased the hydrogen peroxide content by about 35% in the resistant cultivar Tobak and by 1.5-fold in the drought-sensitive cultivar Doskonala (Figure 2, A). Melatonin treatment in both varieties under stress conditions reduced the hydrogen peroxide content in the shoots almost to the control level.

Under drought conditions, the content of TBA-active LPO products increased by almost 30% in Tobak and by 70% in Doskonala (Figure 2, B). Under the influence of melatonin treatment, this effect decreased to 35% in the non-tolerant Doskonala variety. At the same time, the decrease in the content of LPO products in the presence of melatonin was less pronounced in the Tobak variety.

Activity of antioxidant enzymes

Basal SOD activity in the resistant variety Tobak was 2 times higher than in the non-resistant variety Doskonala (Table 1). Under drought stress, SOD activity decreased in both varieties. Melatonin treatment alleviated this effect in

Tobak. In Doskonala, however, the enzyme activity increased significantly under the influence of melatonin, exceeding the values not only of the variety treated with PEG 6000, but also of the control.
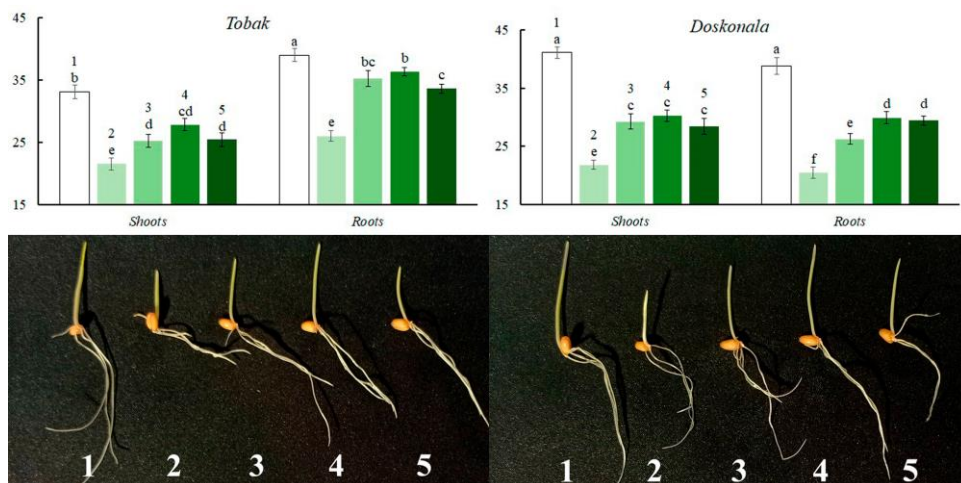

Figure 1. Weight of roots and shoots (mg) of wheat seedlings of Tobak and Doskonala varieties under the effect of PEG 6000 and melatonin. 1—control; 2—PEG (15%); 3—PEG (15%)+melatonin (0.2 μM); 4—PEG (15%)+melatonin (1 μM); 5—PEG (15%)+melatonin (5 μM).
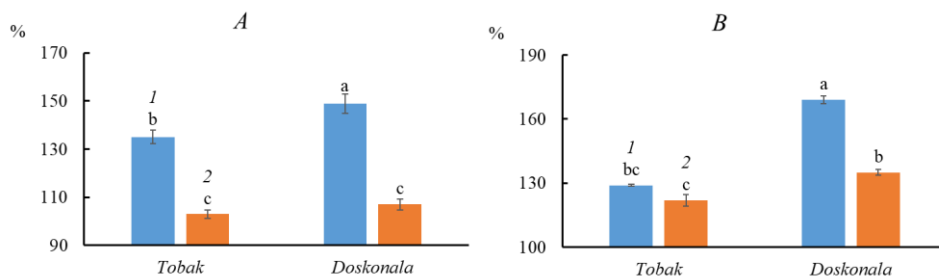

Figure 2. Content (% of control) of hydrogen peroxide (A) and MDA (B) in shoots of wheat seedlings. 1—PEG 6000 (15%); 2—PEG 6000 (15%)+melatonin (1 μM).

The catalase activity of the resistant variety Tobak did not change significantly under model drought, nor did melatonin treatment significantly affect this index (Table 1). At the same time, the non-resistant variety Doskonala showed a significant decrease in catalase activity under stress; concurrent treatment with melatonin slightly alleviated this effect.

The activity of guaiacol peroxidase in response to osmotic stress decreased both in Doskonala and (to a lesser extent) in Tobak varieties. Under the influence of melatonin, a tendency towards stabilization of the enzyme activity was observed in both varieties tested (Table 1).

Table 1. Activity of SOD, catalase, and guaiacol peroxidase in the shoots of wheat seedlings

Experimental variant	SOD activity (U/g DW min)	Catalase activity (mmol H_2O_2/g DW min)	Guaiacol peroxidase activity (U/g DW min)
Tobak			
Control	62.6±1.6 a	10.8±0.2 c	7.64±0.05 c
PEG 6000 (15%)	35.6±1.8 c	10.3±0.2 c	6.81±0.06 e
PEG 6000 (15%) + Melatonin (1 μM)	48.6±1.1 b	11.0±0.2 c	7.43±0.08 cd
Doskonala			
Control	30.1±1.7 d	15.8±0.5 a	9.76±0.08 a
PEG 6000 (15%)	20.4±0.7 e	11.9±0.3 bc	7.26±0.03 d
PEG 6000 (15%) + Melatonin (1 μM)	44.4±1.0 b	12.7±0.2 b	8.62±0.06 b

Content of low-molecular-weight protective compounds

The basal proline content in shoots of seedlings of the resistant variety Tobak was significantly lower than that of the susceptible variety Doskonala (Figure 3, A). It increased proportionally in both varieties in response to the action of model drought. Melatonin treatment of the Tobak had almost no effect on the character of changes in proline content under stress. At the same time, melatonin treatment of Doskonala under the action of melatonin significantly increased proline content in shoots of seedlings under osmotic stress.
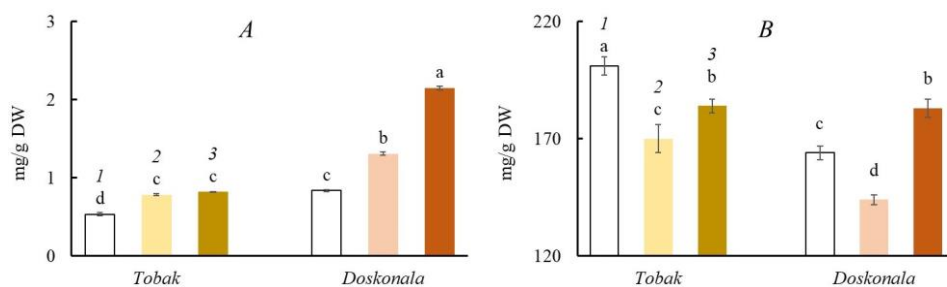

Figure 3. Proline (A) and sugars (B) content in shoots of wheat seedlings. 1—control; 2—PEG 6000 (15%); 3—PEG 6000 (15%)+melatonin (1 μM).

The constitutive sugar content of Tobak was significantly higher than that of Doskonala (Figure 3, B). Under the influence of model drought, both varieties showed a proportional decrease in their amounts.

The melatonin treatment partially offset this effect of drought in Tobak, while its effect was even more pronounced in Doskonala: in the variant with a combination of melatonin and stress, the sugars content exceeded the control values.

The basic total content of phenolic compounds in the shoots of seedlings of the two varieties did not differ significantly (Table 2). Under stress, this index slightly increased in the resistant variety Tobak and did not change in the drought-sensitive variety Doskonala. Melatonin treatment caused a tendency to increase the content of phenolic compounds in Doskonala, but this effect was not significant at $P \leq 0.05$.

Table 2. Content of secondary metabolites in wheat seedlings

Experimental variant	Total content of phenolic compounds (μmoles of gallic acid/g DW)	Anthocyanins content (A_{530}/g DW)	Flavonoids content (A_{300}/g DW)
Tobak			
Control	9.48±0.23 b	0.255±0.003 a	2.05±0.03 b
PEG 6000 (15%)	10.20±0.11 a	0.185±0.003 c	1.55±0.03 d
PEG 6000 (15%) + Melatonin (1 μM)	9.88±0.23 ab	0.220±0.005 b	1.71±0.02 cd
Doskonala			
Control	9.87±0.13 ab	0.250±0.011 a	2.21±0.05 a
PEG 6000 (15%)	9.67±0.25 ab	0.140±0.005 d	1.65±0.05 d
PEG 6000 (15%) + Melatonin (1 μM)	10.30±0.12 a	0.210±0.011 b	1.84±0.02 c

The anthocyanin content under osmotic stress decreased in both varieties, but more significantly in the non-resistant Doskonala. Melatonin treatment largely prevented this stress effect in both (Table 2). Similar changes occurred in the flavonoid content in both varieties: it decreased under osmotic stress, while melatonin treatment mitigated this effect.

DISCUSSION

Under model drought conditions, melatonin treatment had a significant stress-protective effect on seedlings of both wheat varieties studied. These effects were expressed in the alleviation of stress-induced inhibition of root and shoot growth (Figure 1) and in the reduction of the manifestation of oxidative cell damage (Figure 2).

The results obtained and the information available in the literature allow to discuss several mechanisms of melatonin influence on the resistance of seedlings

to osmotic stress. It is known that one of the early negative effects of drought is the excessive formation of ROS associated with the disruption of electron transport processes in chloroplasts and mitochondria. Insufficient water supply increases ROS production not only due to excessive reduction of components of the electron transport chain in chloroplasts, which occurs as a result of stomatal closure due to limited carbon dioxide supply (de Carvalho, 2008), but also due to the significant contribution of mitochondria to oxidative damage processes plants under drought conditions. For example, under stress conditions in wheat leaves, the content of carbonylated proteins in mitochondria was an order of magnitude higher than in chloroplasts (Bartoli *et al.,* 2004). It can be assumed that the mechanisms of oxidative damage unrelated to the functioning of the photosynthetic apparatus were dominant in the model objects we used, etiolated seedlings. It should be noted that under drought conditions it is possible to accumulate ROS due to a decrease in the activity of antioxidant enzymes (de Carvalho, 2008). Under the conditions of our experiments, such effects were manifested with respect to the main antioxidant enzymes, whose activity decreased particularly markedly in the non-tolerant cultivar Doskonala (Table 1).

Due to the significant contribution of oxidative stress to the development of drought damage, melatonin, which is a direct and indirect antioxidant (Kobylińska *et al.,* 2018), is considered as a new effective tool for the management of drought tolerance in plants (Ayyaz *et al*., 2022; Gu *et al.,* 2022).

The decrease in the amount of ROS in cells under the influence of melatonin may be related to its direct antioxidant effect (Ayyaz *et al*., 2022). However, the manifestation of the stress-protective effect of melatonin at low (in our case, micromolar) concentrations does not give reason to consider this mechanism as the main one. The mechanisms related to the involvement of melatonin in the complex signal-regulatory network of cells, including signaling processes involving ROS, may be much more likely (Gu *et al*., 2022). Thus, there is evidence for increased expression of genes of the catalytic subunit of NADPH oxidase under the influence of melatonin in tomato (Mukherjee, 2019). The associated increase in H_2O_2 levels may positively regulate the activity of antioxidant enzymes (SOD, catalase, peroxidase) as well as the expression of other stress-induced genes (in particular, mitogen-activated protein kinase 1, thermosperminsintase, and heat shock proteins) (Mukherjee, 2019). In general, melatonin treatment has been found to increase the activity of many antioxidant enzymes (including SOD, peroxidase, ascorbate peroxidase, dehydroascorbate reductase, glutathione S-transferase, glutathione reductase, and others) in plants of different taxonomic groups, including maize, tomato, citrus, and soybean (Gu *et al.,* 2022). Priming of wheat seeds with melatonin caused a significant increase in catalase activity under subsequent heat stress (Kolupaev *et al*., 2023d). Under the same conditions, melatonin reversed the heat stress-induced decrease in guaiacol peroxidase activity. The present work shows stabilization of SOD and guaiacol peroxidase activity in wheat seedlings under osmotic stress by melatonin treatment (Table 1). It should be noted that a more significant modulation of

antioxidant enzyme activity by melatonin was observed in the drought-sensitive cultivar Doskonala.

Other ways to stabilize the homeostasis of wheat seedling cells under the action of melatonin may be related to the activation of the synthesis of various low-molecular compounds that exhibit multifunctional stress-protective effects. Under the conditions of our experiments, melatonin treatment significantly enhanced proline accumulation in Doskonala wheat seedlings under drought conditions (Figure 3, A). At the same time, melatonin had little effect on proline content under stress conditions in Tobak variety. These results are consistent with data from Chinese cultivars (Li *et al.,* 2020). This work showed that melatonin enhanced proline accumulation in a non-drought-tolerant cultivar and attenuated this effect in a drought-tolerant cultivar. A decrease in stress-induced proline accumulation under melatonin treatment was also found in Trig*onella foenum-gracum* plants (Zamani *et al.,* 2019). In general, most of the studies conducted on plants of different species show that melatonin increases proline content under stresses associated with cell dehydration (Buttar *et al.,* 2020; Tiwari *et al*., 2021; Awan *et al.,* 2023). An increase in activity and enhanced gene expression of proline synthesis enzymes under the influence of exogenous melatonin has also been reported (Antoniou *et al*., 2017). However, it should be noted that proline accumulation can be considered as a sign of a rather severe stress effect. For example, a high correlation between the accumulation of proline and the LPO product MDA under heat stress (r=0.91) was found in etiolated seedlings of seven wheat varieties (Kolupaev *et al.,* 2023c). Apparently, the stress used in this work was critical for the non-tolerant variety Doskonala. At the same time, the resistant variety Tobak maintained a relatively stable proline content (Figure 3, A). It is probable that melatonin activates different stress-protective reactions in wheat seedlings, and the extent of its protective effect relies on the adaptive strategies of species and varieties.

Drought caused a decrease in sugar content in seedlings of both varieties, apparently due to their increased consumption for respiration and other metabolic processes under stress conditions (Figure 3, B). Melatonin treatment promoted the stabilization of their content under osmotic stress, especially in the sensitive variety Doskonala. Further investigation is required to comprehend the mechanisms of this impact. However, literature suggests that melatonin treatment can affect carbohydrate metabolism in plant cells. For instance, in Bermuda grass plants under drought conditions, sugar content equalization was observed during melatonin treatment (Shi *et al*., 2015). In buckwheat, melatonin-treated plants showed a 60% increase in sugar content compared to untreated plants (Tiwari *et al.,* 2021). It has also been shown that exogenous melatonin under stress conditions can modulate the concentration of sugars in etiolated cells of the mutant (chlorophyll-free) form of tobacco, Bright Yellow 2 (Kobylińska *et al.,* 2018). The authors believe that melatonin shifts cell metabolism along the gluconeogenesis pathway, allowing the synthesis of carbohydrates from precursors that are not sugars. However, in etiolated cereal seedlings, another

mechanism for the enhancement of carbohydrate metabolism by melatonin is more likely to be related to the activation of polysaccharide hydrolysis in the grain and the increased entry of sugars into the shoot (Lei *et al.,* 2021).

Both proline and sugars are not only osmolytes, but also have antidenaturation and membrane-protective properties (Liang *et al*., 2013; Deryabin, Trunova, 2021). In addition, proline and sugars are quite potent antioxidants (Kolupaev *et al.,* 2019).

Melatonin also affects secondary metabolism in plants. Under the conditions of our experiments, treatment of seedlings with melatonin did not significantly affect the total content of phenolic compounds (Table 2). At the same time, such treatment under osmotic stress conditions promoted the preservation of the pool of polyphenols, anthocyanins and flavonoids absorbing in the UV-B region. It should be noted that rapid degradation of polyphenolic compounds under stress conditions was also observed in other works (Zafar *et al*., 2020). At the same time, melatonin has the ability to enhance the accumulation of anthocyanins and other polyphenols with strong antioxidant activity. For example, a ROS-dependent enhancement of anthocyanin synthesis in pear fruit by melatonin has been demonstrated (Sun *et al.,* 2021). It is noteworthy that in Bermuda grass, the function of two genes controlling the expression of phenylalanine ammonia-lyase (a key enzyme for the synthesis of phenolic compounds) is suppressed during drought stress, but increased after treatment with exogenous melatonin (Ayyaz *et al*., 2022). Foliar treatment with melatonin on *Fagopyrum tataricum* plants resulted in a significant increase in the activity of phenylalanine ammonia-lyase and flavonoid content under drought conditions (Hossain *et al.,* 2020). Melatonin treatment also led to the synthesis of polyphenolic compounds in various plant species under UV-B stress conditions (Plokhovska *et al*., 2023).

CONCLUSIONS

Melatonin treatment had a positive effect on biomass accumulation by wheat seedlings under osmotic stress. At the same time, it significantly attenuated oxidative damage by affecting various components of the antioxidant system. Under the effect of melatonin, the activities of key enzymes SOD and guaiacol peroxidase, as well as the amount of sugars, anthocyanins, and flavonoids absorbing in UV-B, stabilized. Melatonin treatment during model drought conditions resulted in a significant increase in proline content in non-tolerant wheat cultivar. In general, melatonin had a more pronounced stress-protective effect on seedlings of drought-sensitive wheat variety. Thus, the stress-protective effect of melatonin is associated with normalization of antioxidant and osmoprotective systems functioning under drought, and it depends to some extent on varietal characteristics of adaptive strategies.

Treatment with melatonin can be considered as a promising technique for increasing the drought resistance of wheat in the early stages of development. However, for the actual use of this technique in plant growing, special research is

required, in particular, an assessment of the sensitivity of varieties to the stress-protective effect of melatonin. Of course, a separate cycle of research is necessary to develop a rational technological method for treating plants or seeds priming with melatonin.

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