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Effect of melatonin and hydropriming on germination of aged triticale and rye seeds

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Abstract

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The formation of reactive oxygen species, which initiate lipid peroxidation of membranes and disruption of all membrane-associated processes, is one of the critical factors causing seed senescence and loss of germinability. Melatonin is currently considered one of the powerful antioxidant bioregulators that influence many plant functions, including their resistance to stress factors and seed germination. However, the effect of melatonin on the germination of old triticale and rye seeds has not been specifically investigated. The work aimed to study the effect of melatonin treatment and the well-known procedure of hydropriming (soaking in water with subsequent drying of seeds) on the germination of seeds of triticale 'Raritet' and rye 'Pamyat Khudoerka' with low germination capacity and the state of antioxidant system in seedlings. Hydropriming had a small but significant effect on the germination of triticale seeds at $p \leq 0.05$ and significantly increased the germination of rye seeds. Melatonin treatment positively impacted both crop seed germination and significantly exceeded the effects of hydropriming. The most significant effect was observed when melatonin was used at a concentration of 20 μM. The accumulation of shoot and root biomass also increased under hydropriming and (to a greater extent) melatonin. Melatonin treatment led to a significant decrease in the generation of superoxide anion radical and the content of hydrogen peroxide and malonic dialdehyde, a product of lipid peroxidation, in seedlings of both crops. Additionally, melatonin increased the activity of superoxide dismutase and catalase in both crops. The anthocyanin content increased in the seedlings of both cereals under the influence of melatonin. The effect of hydropriming on indicators characterising the prooxidant and antioxidant balance was insignificant. A conclusion was drawn on the ability of exogenous melatonin to enhance the germination of old triticale and rye seeds and the connection of this effect with the regulation of the antioxidant system.

Keywords: antioxidant system, melatonin, oxidative stress, priming, seed ageing, *Secale cereale*, ×*Triticosecale*.

INTRODUCTION

A decrease in seed germinability during storage is a well-known fact. The intensity of the seed ageing process and loss of germination capacity depends on the conditions of seed collection, drying, and storage (Hong & Ellis, 1996). In this regard, searching for methods to increase seed germination is an important practical task. In Ukraine, its relevance is currently intensified by the ongoing war activities, which often make it impossible to maintain the technological requirements for seed storage.

One of the leading causes of reduced seed germinability with improper storage and accelerated ageing is the disruption of membrane integrity due to the formation of intracellular reactive oxygen species and the consequent activation of lipid peroxidation (Pinzino, 1999; Kurek et al., 2019).

Over the past few decades, insights into the physiological mechanisms that regulate seed maturation, resistance, and germination have significantly increased, including through the development of transcriptomics, proteomics, and phytohormonology (Ibrahim, 2019). The new information has provided the basis for technologies to regulate seed germination. Priming technologies have been increasingly utilised in the past decade to improve the speed and uniformity of seed germination (Paparella et al., 2015; Sako et al., 2020). Seed priming is a physiological method of controlled moistening and drying of grains to improve pregerminative metabolic processes (Waqas et al., 2019). This procedure creates conditions for the activation of enzymes hydrolysing biopolymers of endosperm, which is necessary for the improvement of respiration and energy metabolism and synthesis of nucleic acids and proteins. In addition to the simplest priming technique, hydropriming, seeds are exposed to osmotically active substances, high temperatures, salts of macro- and microelements, and a variety of compounds with hormonal and regulatory activity (Ibrahim, 2019; Sen et al., 2021; Chipilski et al., 2023).

Several studies have drawn parallels between grain germination processes and plant stress responses (Hoekstra et al., 1999; Kranner et al., 2010; Hamdini et al., 2021). The similarity between these processes is that they involve the perception of external signals (mainly changes in temperature and humidity), activation of the signalling network, and transduction of signals to the genetic apparatus (Paparella et al., 2015). It is also known that rapid water uptake can be accompanied by an abrupt transition of membranes from a gel state to a liquid-crystalline state and act as a stressor (Hoekstra et al., 1999). Seed germination processes are accompanied by increased reactive oxygen species generation, which is involved in generation redox signals necessary for seedling growth (Kranner et al., 2010). However, as mentioned above, increased reactive oxygen species generation in seeds may be one of the main reasons for their senescence (Kurek et al., 2019; Zhang et al., 2021). This process is responsible for the protein carbonylation typical of ageing seeds (Rajjou et al., 2008). Reactive oxygen species-induced oxidative stress can also damage DNA (Kurek et al., 2019; Afzal, 2023). Ageing seeds are usually characterised by an imbalance between the formation of reactive oxygen species and their neutralisation by the antioxidant system (Zhang et al., 2021). In this regard, it is believed that using antioxidants as priming agents can mitigate oxidative stress and enhance the germination of old seeds (Deng et al., 2017).

Among plant antioxidants, melatonin (N-acetyl-5-methoxytryptamine) has recently received special attention. This compound is considered an antioxidant and a pleiotropic signalling molecule that plays an essential role in regulating the responses of living organisms to various stressors (Buttar et al., 2020). Data have been obtained on the positive effect of melatonin on the germination process of aged seeds of oats (Yan et al., 2020), maise (Deng et al., 2017; Su et al., 2018), and various legume species (Yu et al., 2021). In addition, many studies have demonstrated melatonin-induced enhancement of seed germination of various crops under the influence of adverse factors, especially osmotic and salt stress (Jiang et al., 2016; Li et al., 2020; Guo et al., 2022), heavy metals (Wang et al., 2022).

Rye is an economically important crop, primarily for functional nutrition (Popović et al., 2022). Triticale is a hybrid species obtained by crossing wheat and rye, combining the properties of a food and fodder crop (Lalević et al., 2019). However, seeds of rye and triticale are sensitive to the effects of adverse factors (Hong & Ellis, 1996). For example, rye seed embryos are susceptible to changes in humidity,

which can cause membrane fragmentation (Sargent et al., 1981).

However, the effect of melatonin priming on the germination of old triticale and rye seeds and the state of their stress protection systems have not been specifically studied. This work aimed to study the effect of melatonin treatment on the germination of low-germinating triticale and rye seeds and the state of the antioxidant system in seedlings. Hydropriming was used as a reference treatment to increase seed germination.

MATERIALS AND METHODS

Plant material and its treatment

Seeds of hexaploid ×*Triticosecale* 'Raritet' and *Secale cereale* 'Pamyat Khudoerka' (the originator of both cultivars is the Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine, Kharkiv) of the generation 2020 were used for the experiments. The seeds were stored indoors for three years under uncontrolled conditions (in summer, the temperature periodically reached 30°C–32°C, and in winter, it dropped to -6 ... -8°C, and the relative humidity during storage repeatedly changed from 25%–30% to 80%–85%). As a result, seed germination during three years of storage decreased to about 40% for ×*Triticosecale* and 30% for *Secale cereale*.

The experimental variant seeds were disinfected using a 3% sodium hypochlorite solution for 15 minutes and then washed eight times with sterile distilled water. Following this, some seeds were placed in glasses with distilled water for 3 hours (hydropriming). In the variants treated with melatonin, seeds were incubated for 3 hours in melatonin solutions with concentrations of 5 μM, 20 μM, and 50 μM in a dark thermostat at 24°C. Subsequently, the seeds that underwent hydropriming or melatonin treatment were dried in a thermostat at 24°C and 40% humidity for two days. Afterwards, seeds were placed in Petri dishes with two layers of sterile filter paper moistened with distilled water and germinated in a dark thermostat at 24°C for three days. Seeds of the control variant were disinfected with 3% sodium hypochlorite solution for 15 min immediately before germination.

After two days of germination, the relative number of germinated seeds and the mass of shoots and roots of seeds with normal germination were evaluated. On the third day of germination, shoots from normally germinated seeds were used for biochemical analyses.

Superoxide anion radical generation

Superoxide anion radical ($O_2^{\bullet-}$) generation by shoots was estimated by nitroblue tetrazolium 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% nitroblue tetrazolium, 10 μM ethylenediaminetetraacetic acid, and 0.1% Triton X-100 for one hour (Kolupaev et al., 2023a). At the end of the exposure, the shoots were removed from the incubation solution and assessed for the intensity of $O_2^{\bullet-}$ generation. The incubation solution's absorbance was measured at 530 nm using an SF 46 spectrophotometer (LOMO, Russia).

Evaluation of hydrogen peroxide content

Seedling shoots were homogenised in 5% trichloroacetic acid at a low temperature to assess the H_2O_2 content. The samples were centrifuged at 8000 g for 10 minutes at 2°C to 4°C using an MPW 350R centrifuge (MPW MedInstruments, Poland). The concentration of H_2O_2 in the supernatant was measured using the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this purpose, 0.5 ml of 2.5 M ammonium thiocyanate, 0.5 ml of 50% trichloroacetic acid, 1.5 ml of supernatant, and 0.5 ml of 10 mM ferrous ammonium sulphate was added to the tubes. The samples were mixed, and the absorbance at 480 nm was determined at the end.

Measurement of lipid peroxidation product content

The amount of lipid peroxidation products, mainly malonic dialdehyde, reacting with 2-thiobarbituric acid was analysed by homogenising the shoots in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% trichloroacetic acid. The homogenate was placed in tubes covered with foil lids and boiled for 30 minutes in a water bath. After cooling, the samples were centrifuged at 10000 g for 15 minutes. The supernatant's absorbance was measured at

532 nm, malonic dialdehyde's maximum light absorption, and at 600 nm to correct for non-specific light absorption (Kolupaev et al., 2021).

Evaluation of antioxidant enzyme activity

Seedling samples were homogenised with the addition of ethylenediaminetetraacetic acid (0.1 mM) and dithiothreitol (1 mM) in cold 0.15 M K, Na-phosphate buffer (pH 7.6). The homogenate was centrifuged at 8000 g for 15 minutes in an MPW 350R centrifuge (MedInstruments) at 4°C to prepare the supernatant, which was then assayed (Kolupaev et al., 2022a). Superoxide dismutase (enzyme code 1.15.1.1) activity was determined at pH 7.6 using a method based on the enzyme competing with nitroblue tetrazolium for the superoxide anion produced by the aerobic interaction of phenazine methosulfate with nicotinamide adenine dinucleotide (reduced). Catalase (enzyme code 1.11.1.6) was evaluated by measuring the amount of H_2O_2 decomposed per unit time. Guaiacol peroxidase (enzyme code 1.11.1.7) activity was estimated using guaiacol as a hydrogen donor and H_2O_2 as a substrate.

Analysis of anthocyanin content

Samples of shoots were homogenised in 1% HCl (hydrochloric acid) in methanol. The supernatant's absorbance was estimated at 530 nm after centrifugation of the homogenate at 8000 g for 15 min (Nogués & Baker, 2000). The results were expressed as absorbance per unit of fresh plant material weight.

Replication of the experiment and statistical analysis

Each replicate consisted of 50 seeds, and each experimental variant had at least three replicates to determine the effect of hydropriming and seed treatment with melatonin on seed germination and seedling biomass. Each sample consisted of at least 12 seedlings for biochemical studies, and analyses were performed in 3–4 replicates.

First, we checked the normality of sample distribution using the Shapiro-Wilk test method. Statistical analysis of the results was performed using a two-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and table show the means of three independent experiments and their standard errors.

RESULTS

Germination of triticale and rye seeds

Seed germination of triticale and rye seeds in the control group was low, with only 39.3% and 29.7% germination rates, respectively (Fig. 1). Hydropriming resulted in an increase in seed germination for both crops, while melatonin treatment caused an even more significant increase in the number of normally germinated seeds. The most significant positive effect was observed when seeds were primed with a melatonin solution at a concentration of 20 μM for both cereal species (Fig. 1).

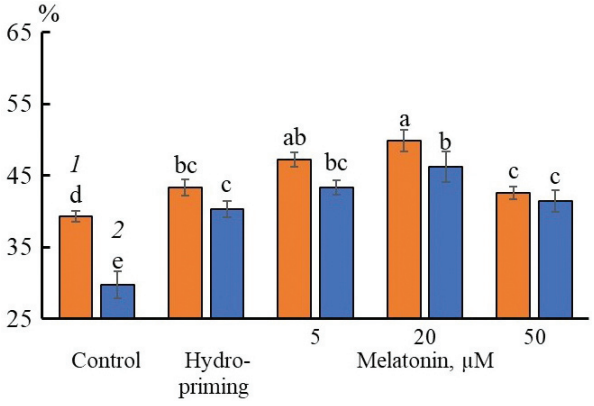

Fig. 1. Effect of melatonin priming and hydropriming on seed germination (%) of triticale (*1*) and rye (*2*). Note. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

Organ biomass of triticale and rye seedlings

Hydropriming resulted in a significant increase in shoot biomass for both cereal species. When triticale seeds were primed with melatonin at all concentrations tested, a more significant increase in shoot biomass was observed compared to hydropriming (Table 1). In rye, a significant increase in shoot biomass at $p \leq 0.05$ was observed in the treatments with melatonin at concentrations of 20 and 50 μM.

Under the influence of hydropriming, only rye showed a significant increase in root mass (Table 1).

Table 1. Effect of melatonin priming and hydropriming on shoot, root, and whole seedling biomass of triticale and rye seedlings

Variant	Shoot mass (mg)	Root mass (mg)	Seedling mass (mg)
×*Triticosecale*			
Control	8.65 ± 0.20	21.8 ± 0.7	30.1 ± 1.6
Hydropriming	11.2 ± 0.33	22.9 ± 0.3	34.1 ± 1.6
Melatonin, 5 µM	13.3 ± 0.59	23.3 ± 0.4	36.6 ± 0.9
Melatonin, 20 µM	14.2 ± 0.47	24.6 ± 1.0	38.8 ± 1.9
Melatonin, 50 µM	13.2 ± 0.64	22.8 ± 0.5	36.0 ± 0.6
Secale cereale			
Control	7.55 ± 0.24	12.5 ± 1.7	20.1 ± 2.3
Hydropriming	8.70 ± 0.12	15.3 ± 0.5	24.0 ± 0.7
Melatonin, 5 µM	8.90 ± 0.07	15.9 ± 2.0	24.8 ± 2.8
Melatonin, 20 µM	11.0 ± 0.28	20.9 ± 2.1	31.9 ± 2.5
Melatonin, 50 µM	10.4 ± 0.60	15.8 ± 0.4	26.2 ± 0.3
$LSD_{0.05}$	0.97	1.6	1.9

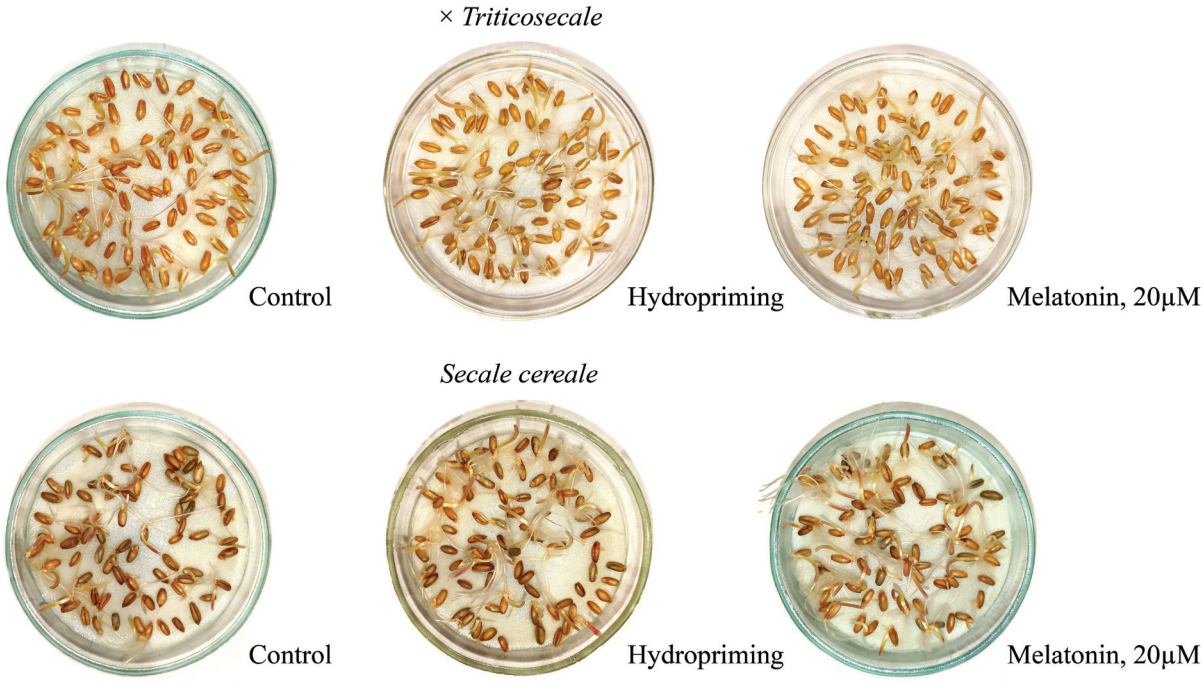

Fig. 2. State of 3-day-old triticale and rye seedlings obtained from aged seeds.

Rye seeds primed with 20 µM melatonin showed a significant increase in root biomass compared to the control and hydropriming variants. The same concentration of melatonin (20 µM) also effectively promoted root growth in triticale seedlings (Table 1).

When evaluating the effect of hydropriming and seed priming with melatonin on seedling biomass, it can be stated that there was a significant increase in both cereal species with all presowing treatments. In addition, triticale seeds primed with melatonin at all concentrations showed more significant effects than those of hydropriming. At the same time, the effect of priming rye seeds with melatonin on the total biomass of rye seedlings was substantially different from the effect of hydropriming when using concentrations of 20 and 50 µM, but not 5 µM (Table 1). Overall, however, the effect of melatonin at a concentration of 20 µM was most effective on both seed germination and seedling formation from normally germinated seeds for both cereal species (Fig. 2). In this regard, for further evaluation of indicators characterising the state of antioxidant system in seedlings

formed from normally germinated seeds, melatonin was used only at 20 μM concentration.

Reactive oxygen species generation and lipid peroxidation levels in seedlings

The basal level of superoxide anion radical generation was found to be higher in rye than in triticale (Fig. 3A). In shoots of seedlings derived from hydroprimed seeds, $O_2^{\bullet-}$ generation did not differ significantly from the control in both crops. Meanwhile, in triticale and rye seedlings grown from seeds primed with melatonin, the generation of superoxide anion radicals was 13% and 32% lower than in the corresponding controls (Fig. 3A).

The hydrogen peroxide content in the shoots of rye seedlings was significantly lower in the variant with hydropriming compared to the control. However, in triticale, this effect was weakly manifested (Fig. 3B). Treatment of seeds with melatonin decreased hydrogen peroxide content in the shoots of seedlings of both species.

The content of the lipid peroxidation product malonic dialdehyde was higher in rye seedlings (Fig. 3C). Hydropriming did not affect this index in either cereal species. However, seed treatment with melatonin resulted in a significant decrease in malonic dialdehyde content in the shoots of triticale and rye seedlings.

The activity of antioxidant enzymes

The superoxide dismutase activity in the shoots of seedlings from both species did not show any significant difference (Fig. 4A). Hydropriming did not affect superoxide dismutase activity in triticale, but it increased in rye. In both cereal species, the enzyme activity was higher in seedlings grown from melatonin-treated seeds than in the control and hydropriming variants.

The catalase activity in the shoots of triticale and rye seedlings of control variants, as well as the superoxide dismutase activity, did not differ (Fig. 4B). Hydropriming did not affect catalase activity in triticale, but increased it in rye. Priming triticale seeds with melatonin caused an increase in catalase activity compared to the control and hydropriming variants. Meanwhile, the enzyme activity in rye in the melatonin-primed variant was approximately the same as in the hydropriming variant.

In the control variants, rye exhibited more than twice the activity of guaiacol peroxidase compared to triticale (Fig. 4C). Hydropriming did not significantly affect the enzyme activity in triticale, but caused an increase in the shoots of rye seedlings. Melatonin treatment resulted in a slight increase in guaiacol peroxidase activity in triticale. In rye seed-

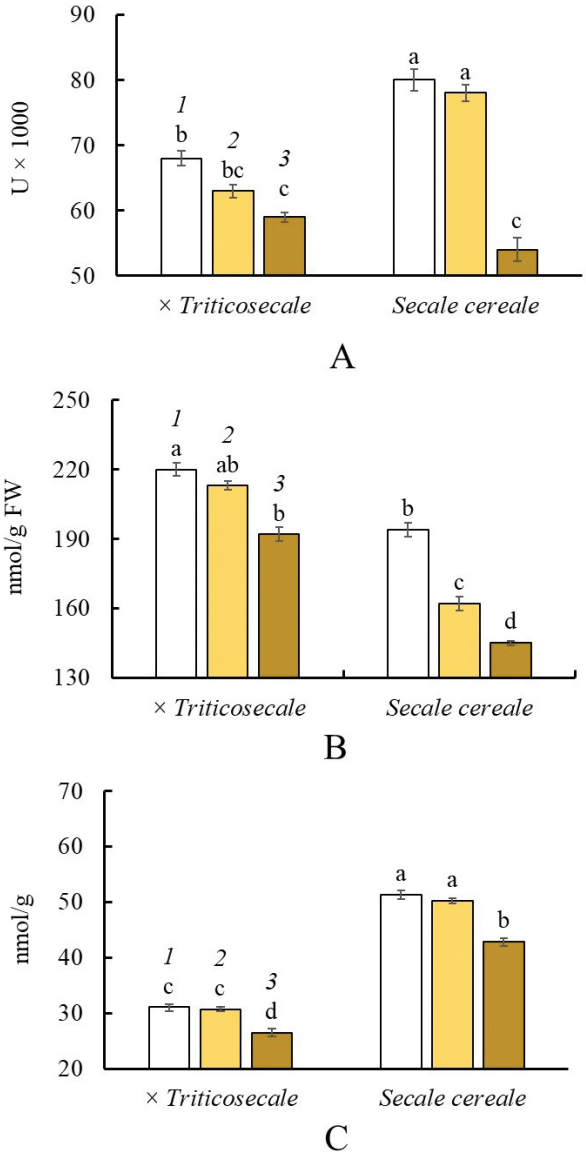

Fig. 3. Superoxide anion radical generation (A), hydrogen peroxide (B) and MDA content (C) in shoots of triticale and rye seedlings. *1* – Control; *2* – Hydropriming; *3* – Melatonin, 20 μM. Note. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

lings, melatonin priming also caused an increase in enzyme activity, but this effect was less significant compared to hydropriming.

Anthocyanin content in shoots of seedlings

Rye seedlings were characterised by a significantly higher anthocyanins content in the control variant compared to triticale seedlings (Fig. 5). Hydropriming increased this indicator in both cereal species. A more significant increase in the content of anthocyanins in the shoots of both triticale and rye seedlings was observed in the variant with melatonin priming.

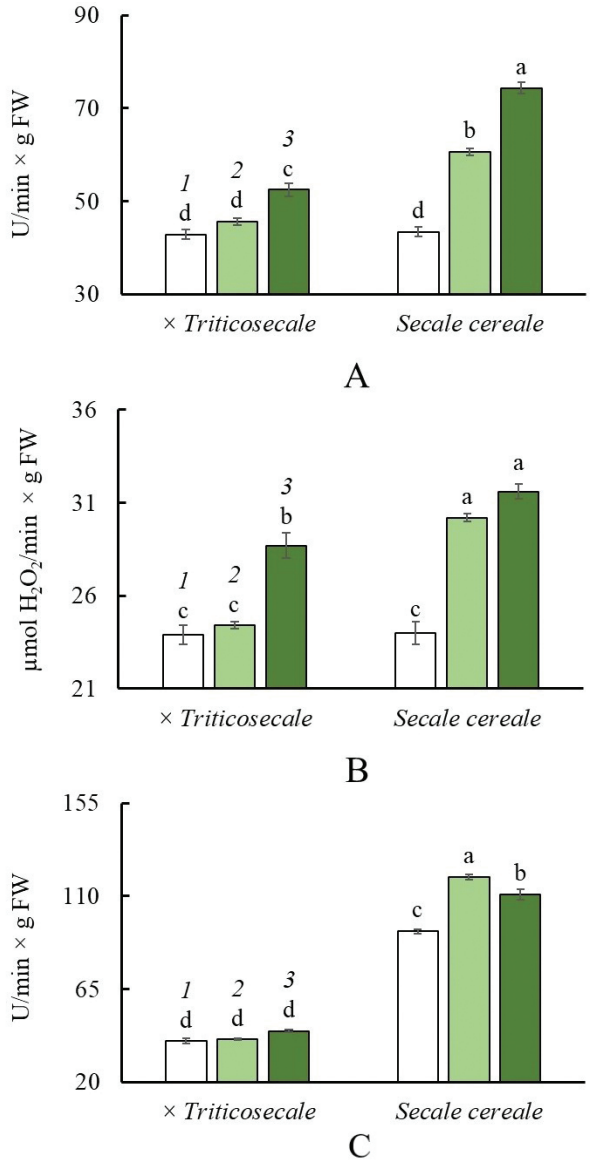

Fig. 4. Activity of superoxide dismutase (A), catalase (B), and guaiacol peroxidase (C) in shoots of triticale and rye seedlings. *1* – Control; *2* – Hydropriming; *3* – Melatonin, 20 µM. Note. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

DISCUSSION

The present study showed that melatonin priming can improve the germination of triticale and rye seeds with impaired germination ability and seedling growth. The impact of melatonin treatment was significantly greater than that of hydropriming, which is considered one of the simplest methods for enhancing seed germination in various crops and is used as a reference for evaluating the effectiveness of other methods of inducing seed germination (Paparella et al., 2015; Waqas et al., 2019).

It should be noted that the manifestation of any physiological activity of melatonin on triticale and rye has hardly been studied. In triticale, only one work is known, which shows an increase in the germination of seeds with normal germinability against the background of drought (Guo et al., 2022). In the case of rye, the only information available is the increase in heat tolerance of seedlings grown from melatonin-treated seeds (Kolupaev et al., 2023b). At the same time, the ability of melatonin to induce the germination of seeds, including those with low germination, has been demonstrated in several other plant species (Dawood, 2018). Thus, melatonin priming increases the germination of oat seeds previously reduced by accelerated ageing caused by prolonged high tem-

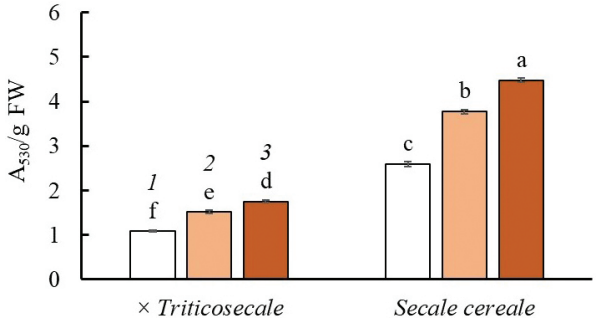

Fig. 5. Anthocyanins content in shoots of triticale and rye seedlings. *1* – Control; *2* – Hydropriming; *3* – Melatonin, 20 µM. Note. Mean values and their standard errors for three replicates are presented; different letters above columns indicate significant differences ($p \leq 0.05$).

peratures (Yan et al., 2020). In this case, melatonin treatment reduces oxidative stress indicators, such as H_2O_2 and malonic dialdehyde content. It prevents the decrease in superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase activities in seed embryos induced by senescence. It has also been shown that melatonin treatment resulted in significant changes in the proteome of germinating oat seeds. Specifically, there was an increase in the content of 96 proteins. In addition, amino acid metabolism and phenylpropanoid synthesis were activated in melatonin-primed oat seeds (Yan et al., 2020). Similar results have been obtained in experiments with maise seeds whose germination was impaired by short-term exposure to 70°C temperature and high humidity (Deng et al., 2017). This work also documented the positive effect of melatonin on superoxide dismutase and catalase activity. Treating rice seeds with melatonin increases their germination rate, promotes enhanced plant growth during early developmental phases, and increases overall plant productivity during ontogenesis (Tyagi et al., 2023).

Several studies have demonstrated the positive effects of melatonin treatment on seed germination in various plant species under unfavourable conditions. For instance, Jiang et al. (2016) have shown that melatonin-primed maise seeds exhibit enhanced germination under salt stress, which attributes to an increase in relative water and proline content. Melatonin priming enhances soybean seed germination under various unfavourable conditions, including drought created by PEG 6000, salt, cold, and heat stress (Awan et al., 2023). Melatonin treatment also decreases the level of oxidative stress markers in seedlings and increases the activity of catalase, nonspecific peroxidase, and ascorbate peroxidase. Treatment of wheat seeds with melatonin improves seedling growth under osmotic stress (Li et al., 2020). At the same time, under the influence of melatonin, the proline content decreases in seedlings of drought-resistant cultivars, while it increases in drought-sensitive cultivars. The authors have also found that melatonin treatment modulates antioxidant enzyme activity and malonic dialdehyde levels in a cultivar-resistance-dependent manner. Thus, the effect of melatonin priming may vary depending on the characteristics of different cultivars. It is important to note that we also observed differences in the effect of exogenous melatonin on stress-protective systems in two wheat cultivars with varying levels of drought tolerance (Karpets et al., 2023). In this case, more significant protective effects of melatonin were observed in the non-resistant cultivar. Therefore, plant sensitivity to melatonin may be specific to certain cultivars. It is even more likely that plants have a species-specific reaction to melatonin.

The results allow us to classify triticale and rye as crops relatively sensitive to melatonin. Although the effect of melatonin on seed germination and seedling growth of these crops was similar, there were some specific differences. Thus, the effect of melatonin on the germination of triticale seeds was more pronounced than rye seeds (Figs 1 and 6). In triticale, the effect of melatonin even at the minimum concentration, significantly exceeded the effect of hydropriming. At the same time, in rye, a significant effect at $p \leq 0.05$ was recorded only for the concentration of 20 μM (Fig. 1). The positive effect of melatonin on the biomass of triticale seedlings was also significantly manifested at all concentrations. In contrast, in rye, the effect of low melatonin concentration did not differ from that of hydropriming (Table 1).

The effects of melatonin on the indices characterising the level of oxidative stress were generally similar for the two cereals (Figs 3 and 6). For both triticale and rye, the effect of melatonin was more significant than the corresponding effects of hydropriming.

Some specificity of melatonin effect on the activity of antioxidant enzymes in seedlings was also detected. Thus, melatonin was found to increase superoxide dismutase activity in both cereals, with a more pronounced effect in rye (Figs 4A and 6). Additionally, melatonin treatment increased catalase activity in triticale, but did not affect this index in rye (Figs 4B and 6). Conversely, in the melatonin-treated variant, peroxidase activity increased in rye. At the same time, it remained unchanged in triticale (Figs 4C and 6).

Rye plants are distinct from other cultivated cereals due to their intensive secondary metabolism and high content of phenolic compounds and flavonoids, particularly anthocyanins (Kolupaev et al., 2022b). The accumulation of anthocyanins is an essential response to stressors in wheat-rye hybrid triticale (Kolupaev et al., 2020), where the content of anthocyanins is also higher than in other cereals. It is possible that

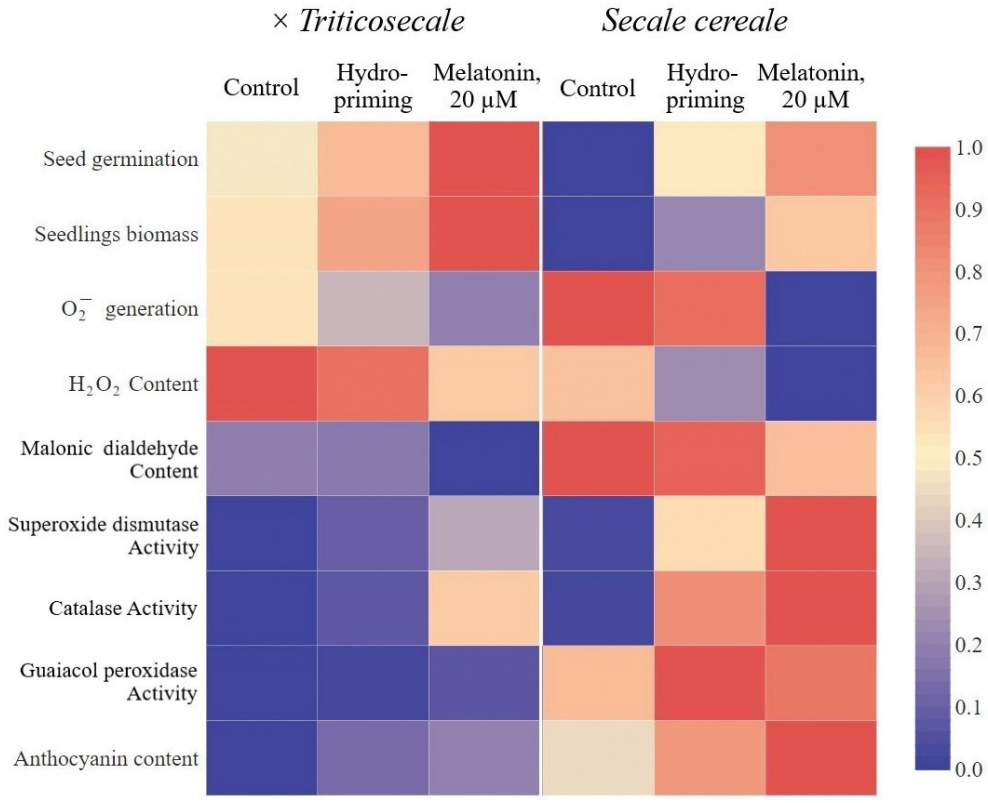

Fig. 6. Heat map of changes in growth indicators and the state of the antioxidant system of triticale and rye seedlings under the influence of hydropriming and melatonin. When constructing the map, each indicator was normalised from 0 to 1.

melatonin priming enhances the antioxidant defence mechanism in the germinating seeds through the accumulation of anthocyanins. This indicator increased significantly in seedlings of both cereals, especially rye (Figs 5 and 6).

Thus, seed treatment with melatonin caused an increase in the activity of antioxidant enzymes in triticale and rye seedlings and an accumulation of anthocyanins in them. Such enhancement of the antioxidant system function significantly reduced oxidative stress, as evidenced by reduced reactive oxygen species generation and malonic dialdehyde content in the seedlings. These processes, in turn, seemed to play an essential role in normalising seed germination. The mechanisms of melatonin improvement of antioxidant system functioning require special studies. Melatonin may increase the activity of antioxidant enzymes by upregulating their gene expression through appropriate signalling. There is evidence for melatonin's ability to activate the signalling network, including ROS generation and calcium entry into the cytosol, which may be a factor in activating gene expression of antioxidant enzymes (Chang et al., 2021; Khan et al., 2023). It has also been demonstrated that priming maise seeds with melatonin increases the activity of phenylalanine ammonia-lyase, a key enzyme for synthesising secondary metabolites (Jiang et al., 2016). This may be one of the reasons for the activation of anthocyanin synthesis.

Undoubtedly, the positive effect of melatonin priming on the germination of old triticale and rye seeds is not limited to its effect on the antioxidant system, as studied in our work. Melatonin appears to affect many regulatory systems, including hormonal regulation (Li et al., 2017), as well as various metabolic processes such as the hydrolysis of reserve biopolymers (Cui et al., 2017; Lei et al., 2021) and the activation of energy metabolism (Turk & Genisel, 2020; Wang et al., 2022), which is vital for seed germination. Further study of species-specific features of the functioning of these systems in different cereals, including triticale and rye, may allow the use of melatonin as a new plant regulatory molecule for targeted adjustment of their functions.

CONCLUSIONS

Priming old triticale and rye seeds with melatonin significantly increased their germination and improved seedling growth. The effect of melatonin significantly exceeded the impact of hydropriming the seeds, which also increased their germination. One of the reasons for the increased germination of triticale and rye seeds under the influence of melatonin may be the reduction of oxidative stress accompanying seed germination. This is indicated by a decrease in the generation of superoxide anion radical and the levels of hydrogen peroxide and malonic dialdehyde in seedlings, as well as an increase in the activity of antioxidant enzymes and the levels of anthocyanins, known for their potent antioxidant activity.

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Author contributions. YEK Writing, formal analysis, project administration and funding acquisition. DAT Methodology, investigation and data curation. AIK Investigations and data curation. TOY Writing and editing. VMP Conceptualisation and supervision. ES Investigation and data curation. YVK Conceptualisation, editing and supervision. All authors have read and agreed to the published version of the manuscript.

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