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INDICES OF CARBOHYDRATE METABOLISM AND ANTIOXIDANT SYSTEM STATE DURING GERMINATION OF AGED WHEAT AND TRITICALE SEEDS TREATED WITH H_2S DONOR

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Hydrogen sulfide is a gasotransmitter molecule involved in the realization of many functions of the plant organism, including seed germination. Aging of seeds is shown to be accompanied by oxidative stress and reduced germination. The effect of exogenous hydrogen sulfide on the germination of aged cereal seeds has not been studied. The aim of the work was to estimate the effect of priming with NaHS as an H_2S donor on wheat and triticale seeds previously subjected to natural aging. Seeds of winter wheat (Triticum aestivum) and winter ×Triticosecale were stored indoors for 4 years at fluctuating temperature and humidity. Aged seeds were treated with 0.2-5 mM NaHS solution for 3 h and germinated in Petri dishes for 3 days. The hydropriming treatment was used as a control. Amylase activity in grains, the biomass of shoots and roots, the content of total sugars, H_2O_2, lipid peroxidation products and anthocyanin, and the activity of antioxidant enzymes in seedlings were determined. It was shown that after the treatment with H_2S donor, the activities of catalase and guaiacol peroxidase, as well as the content of anthocyanins were increased only in triticale seedlings. Nevertheless, treatment of seeds of both cereal species was followed by enhanced growth of shoots and roots, increase in amylase and superoxide dismutase activities, decrease in H_2O_2 and MDA contents, and elevated accumulation of sugars in shoots. It is concluded that the increase in germination of aged cereal seeds under the influence of H_2S donor is caused by increased mobilization of reserve carbohydrates and modulation of antioxidant system activity. Such treatment can be considered as an effective tool to improve seedling growth.

K e y w o r d s: seeds aging, Triticum aestivum, Triticosecale, hydrogen sulfide, total sugar, amylase, oxidative stress, antioxidant system.

At present, hydrogen sulfide (H_2S) is considered one of the key signaling molecules-gasotransmitters not only in animal cells but also in plants [1, 2]. Information has been obtained about its involvement in the regulation of basic functions of the plant organism: growth processes, fruit ripening and senescence, seed germination, and adaptation to the action of stressors of various nature [3-6]. The signaling effects of hydrogen sulfide, which account for its participation in key physiological processes of different groups of organisms, are associated with post-translational modification (PTM) of target proteins, namely their persulfidation–conversion of the cysteine-thiol group (-SH) into the corresponding persulfide (-SSH) [7, 8]. In particular, cysteine persulfide (CysSSH), glutathione persulfide (GSSH), and peroxiredoxins are recognized as important redox regulators [9]. At the same time, data from bioinformatic methods indicate that up to 5% of the plant cell proteome can be persulfidized [10]. As a result of PTM, target proteins change their stability, biochemical activity, conformation, and subcellular localization [11]. Persulfidated proteins may include enzymes of glycolysis, tricarboxylic

acid cycle, Calvin cycle, starch biosynthesis, antioxidant enzymes, and enzymes synthesizing reducing agents [12]. Persulfidation is also likely to be part of the gene expression regulation toolbox [12, 13]. For example, a study of tomato gene expression when roots were treated with the hydrogen sulfide donor NaHS showed that 5349 genes were activated and 5536 genes were repressed [14].

The effects of hydrogen sulfide are also realized through its functional interaction with other important mediators, reactive oxygen species (ROS) and nitric oxide (NO) [15]. These signaling mediators may compete with each other for targets in protein PTMs and may also engage in direct chemical interactions with each other [16, 17]. Furthermore, the effects of hydrogen sulfide on ROS and nitric oxide synthesis are known to be realized by altering the gene expression of enzymes involved in these processes and by direct interaction of H_2S with target proteins [18, 19]. Thus, hydrogen sulfide appears at the center of signaling processes in different groups of organisms.

It is known that the start of seed germination is usually accompanied by an increase in the generation of ROS, which are involved in the generation of redox signals necessary for seedling growth [6]. For example, ROS production in the apoplast of germinating seeds entails a decrease in cell wall density [20], and carbonylation of Arabidopsis seed reserve proteins increases their susceptibility to proteolytic cleavage [21]. In sunflower seeds, protein carbonylation is a prerequisite for dormancy interruption [22]. At the same time, during seed germination, especially under unfavorable conditions, an imbalance may occur between the generation and neutralization of ROS and the occurrence of oxidative stress [6, 23]. It is known that normal seed germination is only possible if ROS levels are maintained at the signaling level, i.e. below the critical threshold (within the so-called “oxidative window” for germination) [24].

It is known that initiation of seed germination requires not only changes in the levels of key signaling mediators, but also significant changes in the balance of plant hormones, primarily a decrease in abscisic acid and an increase in the levels of ethylene and gibberellins [25]. Exogenous treatment with gibberellins is used to enhance seed germination in several plant species [26, 27].

However, in general, priming technologies have been employed to enhance the rate and uniformity of seed germination in both normal and unfavorable conditions. These technologies involve the exposure of seeds to exogenous plant hormones and signaling molecules [28-32]. The latter include hydrogen sulfide [8, 33]. For example, it was shown that priming tobacco seeds with solutions of the hydrogen sulfide donor NaHS promoted their germination under normal conditions and enhanced seedling growth [34]. Concurrently, an increase in the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase, as well as an increase in the synthesis of endogenous hydrogen sulfide, was observed in the seedlings. A number of works showed normalization of seed germination when exposed to hydrogen sulfide under stress conditions. For example, the treatment of wheat seeds with NaHS improved their germination and also alleviated the manifestation of oxidative stress under conditions of copper toxicity [35]. In Zhou et al. [36], priming of maize seeds with NaHS increased the germination percentage and seedling size when exposed to high temperature. In addition, the treatment with hydrogen sulfide donor stimulated enzymatic and non-enzymatic antioxidant systems and osmolytes accumulation in maize seedlings. The beneficial effect of hydrogen sulfide donors on wheat seed germination is also associated with an increase in amylase and esterase activity, which contributes to the mobilization of reserve polymers of the grain [37]. However, data on the effect of exogenous hydrogen sulfide on seed germination of different species are not so clear. For example, no effect of H_2S on Arabidopsis seed germination under normal conditions has been reported [38]. Treatment with gaseous hydrogen sulfide under optimal conditions showed an increase in germination energy of pea, bean, and maize seeds, but not wheat [39].

Improving the germination of aged seeds is a separate issue [40]. It is known that in such seeds, there is usually an imbalance between the formation of ROS and their neutralization by the antioxidant system [23], which may be the cause of oxidative damage to the structures of the emerging seedlings. Improper seed storage (especially at elevated temperature and humidity) causes accelerated seed senescence. Lipid peroxidation (LPO) is usually activated in aging seeds, eventually leading to the disruption of membrane integrity [40]. The development of oxidative stress also causes carbonylation of proteins [41] and, in some cases, DNA damage [40, 42]. Given the significant contribution of oxidative stress to the destructive processes of seed aging, the use of antioxidants or compounds that activate the antioxidant system is being considered to nor-

malize seed germination. Such data have been obtained, for example, for reduced glutathione, ascorbic acid, and melatonin [43, 44].

Hydrogen sulfide, as a signaling molecule that activates plant stress protection systems as well as mobilization of seed endosperm reserve substances [35, 37], can be considered an effective priming agent that promotes seed germination of cultivated plants, including one of the most important cereals, wheat. However, data on the effect of hydrogen sulfide donors on the germination of aged wheat seeds and metabolic processes in them are not yet available in the literature. However, as noted above, there is information about strengthening the antioxidant system and germination of wheat seeds under unfavorable conditions by hydrogen sulfide. Along with wheat, triticale, a hybrid species obtained by crossing wheat and rye, is an economically valuable cereal in Ukraine. However, triticale seeds are characterized by a rapid decrease in seed germination when stored under non-optimal conditions [44]. We are not aware of any data on the effect of exogenous hydrogen sulfide on any physiological and biochemical parameters in triticale plants.

In connection with the above, the aim of the work was to study the effect of priming with the hydrogen sulfide donor sodium hydrosulfide on the germination of wheat and triticale seeds subjected to natural aging and the relationship between the physiological effects of H_2S and changes in carbohydrate metabolism and the state of the antioxidant system.

Materials and Methods

Plant material. Seeds of bread winter wheat (*Triticum aestivum* L.) of the Scorpion cultivar (Czech Republic, Austria) and winter triticale (×*Triticosecale* Wittmack) of the Raritet cultivar (Ukraine) of the 2020 generation were used. The wheat cultivar Scorpion has blue grains with increased content of polyphenolic compounds [45]. However, there are reports that the seeds of this cultivar, even when stored under optimal conditions, easily shrink and shrivel, resulting in reduced germination [45]. Triticale seeds of the cultivar Raritet, as evidenced by the data obtained earlier, also show a significant loss of germinability after being stored for a number of years [44]. Prior to the experiments, wheat and triticale seeds were stored indoors for four years under uncontrolled conditions (in summer, the temperature periodically reached 30–32°C, and in winter, it dropped to -6...-8°C; relative humidity during storage repeatedly changed from 25–30 to 80–85%), which led to a significant decrease in germination.

Seeds of all experimental variants were disinfected with 5% sodium hypochlorite solution for 15 min and washed repeatedly with sterile distilled water. Some seeds were then kept in glasses with distilled water for 3 h (hydropriming). This procedure alone has been shown to increase seed germination by about 10% [44]. In this regard, the hydropriming treatments were used as a control.

In the hydrogen sulfide donor treatments, seeds were kept for 3 h in tightly closed glasses with NaHS solutions at concentrations of 0.2, 0.5, 1, 2, and 5 mM. At the end of the exposure, the seeds were dried in a thermostat at 24°C and about 40% humidity for one day. The seeds were then germinated in Petri dishes on two layers of moistened filter paper in a thermostat at 24°C for 3 days.

After 2 days from the beginning of seed germination, their germination was evaluated (seeds with shoot lengths not less than half of the grain length were considered germinated). The biomass of shoots and roots was also determined. Amylase activity in grains was assessed both on the first and second days of germination. In addition, total sugar content was determined in shoots of 2-day-old seedlings. ROS generation, antioxidant enzyme activity and secondary metabolite content were determined in shoots of 3-day-old seedlings.

Measurement of amylase activity. Total amylase activity was determined in grains by the amount of reducing sugars formed as a result of starch hydrolysis, modifying known protocols [46, 47]. A sample of plant material (500 mg) was homogenized in 0.2 M acetate buffer (pH 5.6), the homogenate was centrifuged in an MPW 350R centrifuge (MPW MedInstruments, Poland) at 8000 g for 15 min at a temperature of not more than 4°C to prepare the supernatant, which was then assayed. The supernatant was diluted as needed with the same buffer and used for analysis. The enzyme extract was mixed with 5 ml of 2% starch solution and incubated in a thermostat at 25°C for 30 min, after which the reaction was stopped by precipitating the proteins by adding 0.1 ml of 10% lead acetate and 0.1 ml of 16% sodium sulfate. In the control samples, these compounds were added prior to the addition of starch. The reducing sugar content of each sample (including control with inactivated enzyme) was determined by reaction with Fehling's reagent. After the addition of

5 ml of Fehling's reagent, the samples were boiled in a water bath for 10 min, cooled, and centrifuged at 6000×g for 10 min. Supernatant absorbance was determined using a UV-1280 spectrophotometer (Shimadzu, Japan) at a wavelength of 670 nm. D-maltose was used as the standard.

In addition to quantitative determination, amylase activity was also assessed by starch degradation on agar plates [37]. To estimate total amylase activity, halves of cut grains were spread on plates containing 1% agar supplemented with 0.2% starch and incubated for 2 h. The plates were then treated with diluted Lugol's solution (0.04% I_2 in 0.1% KI), and the diameter of starch-free spots was determined. The activity of β-amylase was assessed separately. For this purpose, 2 mM EDTA, which inhibits α-amylase, was added to starch-containing agar [37].

Determination of total sugar content. The total sugar content of the plant material was determined by the Morris-Roe method, based on anthrone reagent, in a modification described by [48]. The plant material was subjected to a boiling water bath for 10 min to facilitate the extraction of sugars. The resulting extract was clarified by adding equal volumes (0.3 ml) of 30% zinc sulfate and 15% blood yellow salt. The samples were then filtered through a paper filter and, if necessary, diluted several times with distilled water before measurement. The reaction tubes were filled with 3 ml of anthrone reagent and 1 ml of the filtered solution, while distilled water was added to the control sample instead of the filtered solution. After boiling for 7 min in a water bath, the samples were cooled, and the absorbance at 610 nm was determined in relation to the control solution. D-glucose was used as the standard.

Determination of hydrogen peroxide content. To determine H_2O_2 content, seedling shoots were homogenized in cold with 5% trichloroacetic acid (TCA). The samples were centrifuged at 8000 g for 10 min at 2–4°C. The ferrothiocyanate method [49] with slight modifications was used to determine the concentration of H_2O_2 in the supernatant. The tubes were filled with 0.5 ml of 2.5 M ammonium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ferrous ammonium sulfate. After mixing, the absorbance of the samples was determined on a spectrophotometer at 480 nm.

Measurement of LPO content. To analyze the amount of LPO products reacting with 2-thiobarbituric acid (mainly malonic dialdehyde, MDA), shoots were homogenized in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% TCA. The homogenate was placed in foil-covered tubes in a boiling bath for 30 min. After cooling, the samples were 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) [50].

Evaluation of antioxidant enzyme activity. Seedling samples were homogenized in cold 0.15 M K, Na-phosphate buffer (pH 7.6) supplemented with EDTA (0.1 mM) and dithiothreitol (1 mM). The homogenate was centrifuged at 8000×g for 15 min at a temperature of no more than 4°C to prepare the supernatant, which was then assayed [48]. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined at pH 7.6 by a method based on the enzyme's ability to compete with nitroblue tetrazolium for superoxide anions generated by aerobic NADH/phenazine methosulfate interaction. Catalase (EC 1.11.1.6) was evaluated by the amount of H_2O_2 decomposition per unit of time. The activity of guaiacol peroxidase (EC 1.11.1.7) was estimated at pH 6.2 using guaiacol as a hydrogen donor and H_2O_2 as substrate.

Measurement of phenolic and anthocyanin content. For the determination of the total amount of phenolic compounds and anthocyanins, the seedlings (300 mg) were homogenized in 6 ml of 80% ethanol. They were extracted for 20 minutes at room temperature and then centrifuged at 8000 g for 15 min. To evaluate the content of phenolic compounds, 0.5 ml of the supernatant, 8 ml of distilled water and 0.5 ml of Folin's reagent were added to the reaction tubes and stirred. After 3 min, 1 ml of 10% sodium carbonate was added. After 1 hour, the reaction mixture absorbance was measured at 725 nm [51]. The content of phenolic compounds was expressed as μmol gallic acid/g fresh weight.

Prior to the determination of the anthocyanin content, the supernatants were acidified with HCl to a final concentration of 1%. The absorbance was measured at 530 nm [52]. The results were expressed as A_{530}/g fresh weight.

Replication of experiments and statistical analysis of data. In order to determine the effects of seed treatment with NaHS on seed germination and seedling biomass, each experimental design was replicated at least three times. Each replicate consisted of 75 seeds. For biochemical analyses, each sample consisted of at least 12 seedlings analyzed in three or more replicates.

Statistical analysis of results was performed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and table present the means of three biological replicates with their standard errors. Different letters indicate values that are significantly different at the $P \leq 0.05$ level.

Results

Effect of an H_2S donor on the germination of wheat and triticale grains and seedling growth. Priming of wheat seeds with NaHS at concentrations in the range of 0.2–2 mM caused a significant ($P \leq 0.05$) increase in seed germination (Table). The maximum effect was observed when 0.5 and 1 mM concentrations were used. At the same time, there was a marked inhibition of the germination of wheat seeds under the influence of 5 mM NaHS. The positive effect of hydrogen sulfide donor on the germination of triticale seeds was observed in the whole range studied: from 0.2 to 5 mM with a maximum effect at the concentration of 1 mM.

Treatment of wheat seeds with hydrogen sulfide donor at concentrations of 0.5, 1, and 2 mM enhanced accumulation of root biomass (Table). However, a higher concentration of NaHS (5 mM) inhibited root growth. In triticale seedlings, an increase in root biomass accumulation was observed with sodium hydrosulfide at concentrations ranging from 0.5 to 2 mM. At the same time, concentrations of 0.2 and 5 mM had no discernible effect on triticale root growth.

Priming of wheat seeds with NaHS at concentrations of 0.5, 1, and 2 mM significantly enhanced shoot biomass accumulation (Table). The lower concentration of hydrogen sulfide donor (0.2 mM) had no significant effect on shoot growth at $P \leq 0.05$, while the highest concentration used (5 mM) caused inhibition of shoot biomass accumulation in wheat seedlings. In triticale seedlings, a significant enhancement of shoot biomass accumulation was observed when seeds were primed with NaHS at concentrations of 0.5 and 1 mM. The effect of lower and higher concentrations of hydrogen sulfide donor was insignificant (Table).

The total biomass of wheat seedlings increased under the influence of seed priming with NaHS at concentrations of 0.5, 1, and 2 mM. The effects of 0.2 mM NaHS were not significant, while seed treatment with 5 mM sodium hydrosulfide had a negative effect on the accumulation of total biomass of wheat seedlings (Table). The positive effect of triticale seed treatment with NaHS on the accumulation of total biomass of seedlings showed a wider range (0.2 to

Table. Concentration dependence of the effect of H_2S donor priming on seed germination and organ biomass of wheat and triticale seedlings

Treatment	Seed germination rates, %	Shoot mass (mg)	Root mass (mg)	Seedling mass (mg)
		Triticum aestivum L.		
Control	38.6 ± 1.8^{g}	18.1 ± 0.6^{d}	10.5 ± 0.4^{e}	28.6 ± 0.9^{e}
NaHS (0.2 mM)	44.0 ± 1.0^{f}	19.5 ± 0.7^{cd}	11.9 ± 0.9^{e}	31.3 ± 1.5^{e}
NaHS (0.5 mM)	50.2 ± 1.8^{e}	24.2 ± 0.7^{b}	13.8 ± 0.3^{d}	38.0 ± 0.3^{d}
NaHS (1.0 mM)	50.0 ± 1.2^{e}	23.9 ± 1.2^{b}	14.7 ± 0.7^{d}	38.6 ± 1.0^{cd}
NaHS (2.0 mM)	42.7 ± 1.2^{f}	22.8 ± 0.9^{b}	14.3 ± 0.8^{de}	37.1 ± 1.6^{d}
NaHS (5.0 mM)	30.8 ± 1.4^{h}	12.6 ± 0.7^{e}	8.1 ± 0.4^{f}	20.7 ± 0.8^{f}
		×*Triticosecale*		
Control	55.7 ± 2.2^{d}	20.9 ± 0.8^{c}	20.3 ± 1.1^{c}	41.2 ± 0.3^{c}
NaHS (0.2 mM)	60.2 ± 1.2^{c}	23.6 ± 0.6^{b}	22.8 ± 0.7^{b}	47.9 ± 2.5^{b}
NaHS (0.5 mM)	65.7 ± 1.2^{b}	29.0 ± 0.9^{a}	24.1 ± 0.4^{a}	53.8 ± 1.0^{a}
NaHS (1.0 mM)	76.7 ± 1.8^{a}	28.4 ± 0.8^{a}	25.0 ± 1.2^{a}	53.4 ± 0.4^{a}
NaHS (2.0 mM)	67.0 ± 2.0^{b}	27.7 ± 1.0^{a}	20.2 ± 0.7^{c}	47.7 ± 1.5^{b}
NaHS (5.0 mM)	65.1 ± 1.7^{b}	19.8 ± 1.0^{cd}	19.3 ± 0.3^{c}	39.1 ± 1.2^{cd}

Note. *Different letters indicate values with significant differences ($P \leq 0.05$)

2 mM). Higher concentration of NaHS (5 mM) had no significant effect on the total biomass of triticale seedlings.

In general, the most significant positive effect on seed germination and growth of wheat and triticale seedlings was exerted by NaHS treatment at concentrations of 0.5 and 1 mM (Fig. 1). Notably, increasing the NaHS concentration to 5 mM resulted in reduced germination and growth inhibition of wheat seedlings but not of triticale seedlings (Table). In subsequent experiments, NaHS was used at concentrations of 0.5 and 1 mM to evaluate the effect of seed priming with hydrogen sulfide donor on parameters of carbohydrate metabolism, ROS generation, and antioxidant system function in both cereal species.

Effect of priming with hydrogen sulfide donor on amylase activity and sugar content in wheat and triticale grains. Total amylase activity in triticale grains after 24 and 48 h of germination was higher than in wheat grains (Fig. 2, *A*). In wheat, the amylase activity increased significantly after 48 h of germination compared to the values recorded after 24 h of germination. In triticale, the activity values after 48 h of germination differed little from those observed after 24 h. Seed treatment with 0.5 and 1 mM NaHS resulted in increased enzyme activity in seedlings of both species. This effect was observed both at 24 and 48 h after seed germination, but the differences between the treatments were more significant at the end of the first day of observation.

The activity of β-amylase after 24 h of the germination of wheat and triticale seeds was low. However, after 48 h, there was an increase in its activity, especially noticeable in triticale (Fig. 2, *B*). At the same time, treatment with hydrogen sulfide donor promoted the manifestation of higher β-amylase activity. This effect was well visualized in triticale, while it was weaker in wheat.

The amount of sugars in the shoots of 2-day-old wheat and triticale seedlings of the control treatment was almost the same (Fig. 3). Under the influence of 0.5 and 1 mM NaHS, a significant (by 40 and 37%, respectively) increase in the content of soluble carbohydrates was observed in the shoots of wheat seedlings. The same pattern of effects was observed in triticale seeds treated with hydrogen sulfide donor. However, in this case, the increase in the total amount of soluble carbohydrates exceeded the control values by less than 20%, although it was significant at $P \leq 0.05$ (Fig. 3).

Hydrogen peroxide and MDA levels in shoots of wheat and triticale seedlings. The amount of hydrogen peroxide in shoots of control triticale seedlings was higher than in shoots of wheat control seedlings (Fig. 4, *A*). Seed pretreatment with hydrogen sulfide donor at concentrations of 0.5 and 1 mM significantly reduced the hydrogen peroxide content in the shoots of wheat seedlings. The reductions in hydrogen peroxide content in shoots of triticale seedlings grown from seeds treated with hydrogen sulfide donor were somewhat smaller, but the differences were significant at $P \leq 0.05$.

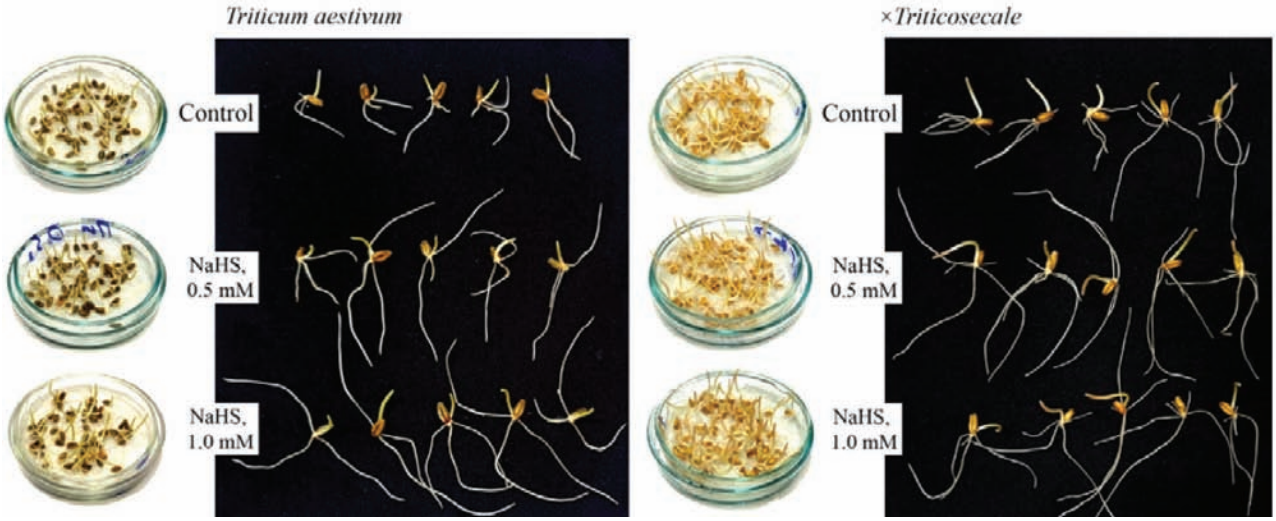

Fig. 1. Condition of 2-day-old wheat and triticale seedlings derived from aged seeds

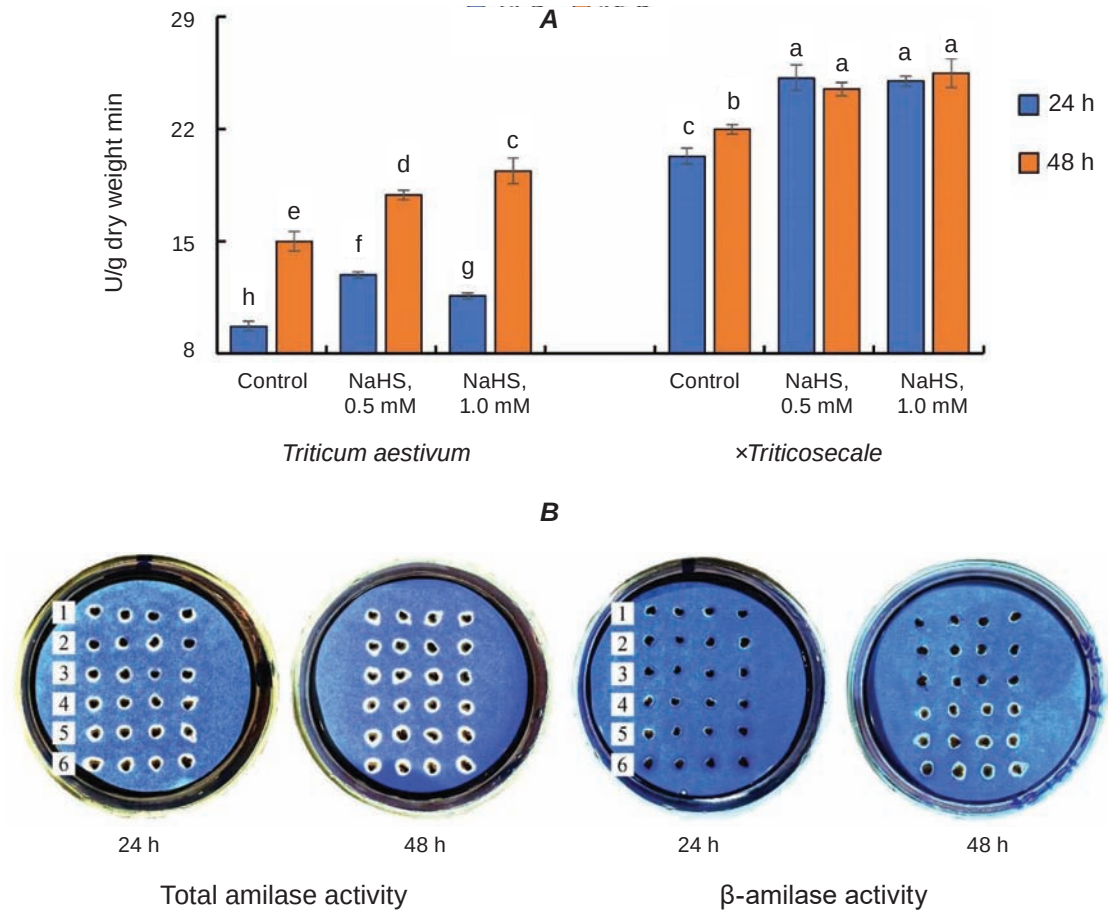

Fig. 2. Effect of hydrogen sulfide donor on amylase activity in wheat and triticale grains. ***A*** *– total amylase activity measured spectrophotometrically;* ***B*** *– visual manifestation of total amylase activity and β-amylase activity in wheat (1-3) and triticale (4-6) grains: 1, 4 – control; 2, 5 – NaHS (0.5 mM); 3, 6 – NaHS (1 mM). The same letters denote quantities between which differences are not reliable for* $P \leq 0.05$

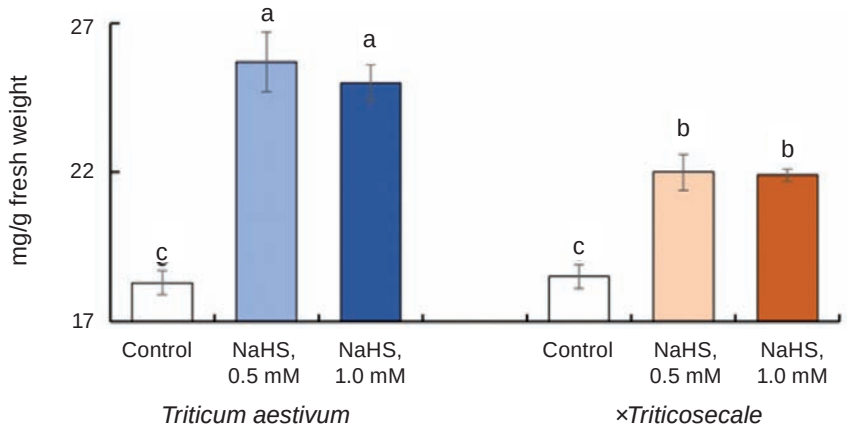

Fig. 3. Soluble carbohydrates content in shoots of wheat and triticale seedlings. The same letters denote quantities between which differences are not reliable for $P \leq 0.05$

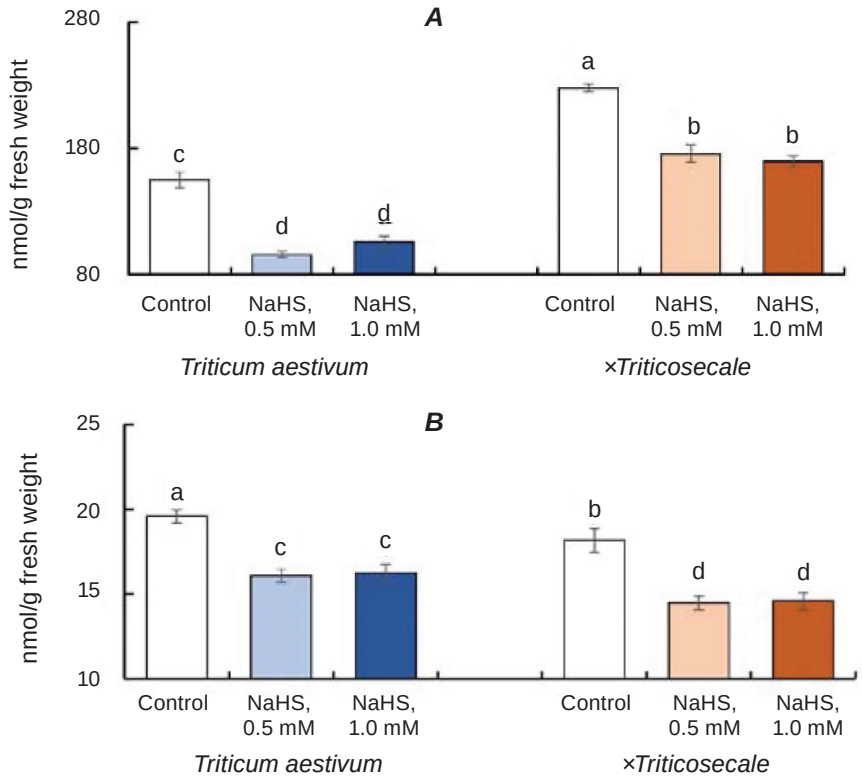

*Fig. 4. Content of hydrogen peroxide (**A**) and malonic dialdehyde (**B**) in shoots of wheat and triticale seedlings. The same letters denote quantities between which differences are not reliable for $P \leq 0.05$*

The content of the LPO product MDA in the shoots of wheat seedlings was higher than in triticale seedlings (Fig. 4, *B*). NaHS treatment of both wheat and triticale seeds significantly (by 17 and 20%, respectively) reduced the MDA content in shoots.

Activity of antioxidant enzymes in shoots of wheat and triticale seedlings. In the control, SOD activity in shoots of wheat seedlings was higher than that of triticale (Fig. 5, *A*). Seed priming with NaHS at concentrations of 0.5 and 1 mM caused an increase in enzyme activity in shoots of both cereal species. The effect of 1 mM NaHS was more significant.

The catalase activity was higher in wheat than in triticale in the control (Fig. 5, *B*). Priming the seeds of the two cereal species with hydrogen sulfide donor resulted in different effects. In wheat, a decrease in catalase activity was observed under the influence of both NaHS concentrations used, whereas in triticale, the enzyme activity increased under the influence of NaHS.

The activity of guaiacol peroxidase in shoots of triticale control seedlings was more than two times lower than in wheat ones (Fig. 5, *C*). Treatment of wheat seeds with hydrogen sulfide donor at both concentrations caused a relatively small, but significant at $P \leq 0.05$, decrease in enzyme activity in shoots. At the same time, in triticale, a slight increase in guaiacol peroxidase activity was observed in the NaHS seed priming treatments, especially when a concentration of 0.5 mM was used.

Content of secondary metabolites in shoots of wheat and triticale seedlings. The total content of phenolic compounds in shoots of wheat seedlings was higher than in triticale (Fig. 6, *A*). Seed treatment with hydrogen sulfide donor did not affect the value of this index in both cereal species.

The anthocyanin content in the shoots of triticale seedlings was more than three times higher than in the shoots of wheat (Fig. 6, *B*). In addition, seed priming with NaHS did not affect the anthocyanin content in wheat, but caused a significant increase in triticale.

Discussion

We studied for the first time the effect of exogenous hydrogen sulfide on metabolic processes during the germination of aged wheat and triticale seeds

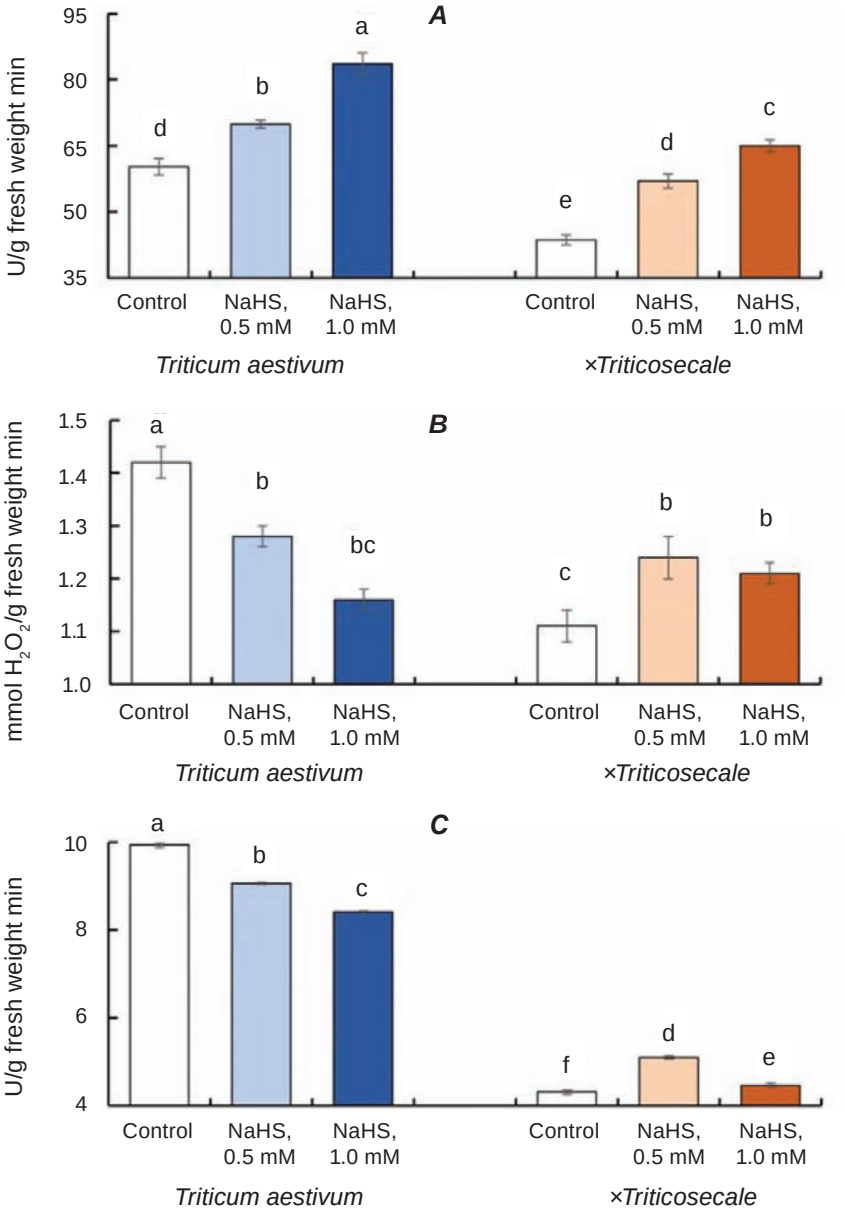

*Fig. 5. Activity of SOD (**A**), catalase (**B**), and guaiacol peroxidase (**C**) in shoots of wheat and triticale seedlings. The same letters denote quantities between which differences are not reliable for $P \leq 0.05$*

characterized by low germination. It was found that priming seeds of both cereals with H_2S donor increased their germination and promoted the growth of seedling organs (Table, Figs. 1, 7). The range of effective NaHS concentrations for the two different cereal species was comparable. The most significant positive effect on both seed germination and biomass accumulation of wheat and triticale seedlings was exerted by the hydrogen sulfide donor at concentrations of 0.5 and 1 mM. It is noteworthy that priming with 5 mM NaHS had a pronounced inhibitory effect on seed germination, root and shoot growth in wheat but not in triticale. Moreover, the treatment of triticale seeds with 5 mM NaHS had no significant effect on seedling biomass accumulation, but it still significantly increased the percentage of seed germination (Table). Thus, the ranges of stimulating and toxic concentrations of hydrogen sulfide donor were slightly different for the two cereal species. Apparent1ly, wheat is more sensitive to the effect of elevated concentrations of exogenous hydrogen sulfide, which is known to act not only as a signaling molecule-gasotransmitter, but also as a gaseous toxicant [6]. Nevertheless, at physiological concentra-

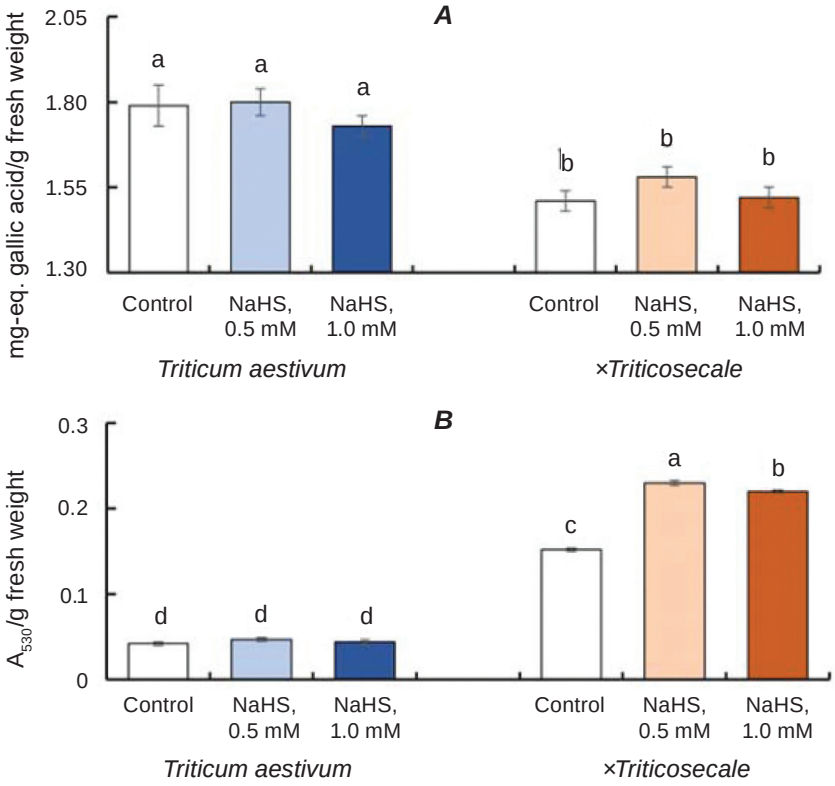

*Fig. 6. Content of phenolic compounds (**A**) and anthocyanins (**B**) in shoots of wheat and triticale seedlings. The same letters denote quantities between which differences are not reliable for $P \leq 0.05$*

tions, hydrogen sulfide has beneficial effects on seed germination. Such effects have been described in the literature for species whose seeds do not have a very high natural germination rate, such as maize, peas, and beans [39]. Conversely, as previously stated, the treatment with a hydrogen sulfide donor did not enhance the germination of wheat seeds with a normal germination rate (approximately 90%) and germinating under optimal conditions [50]. At the same time, however, a number of studies have recorded an increase in the germination of wheat seeds under the influence of priming NaHS on the background of stress factors–toxic doses of copper [35], aluminum [53], and chromium [54] salts. The possible causes of such phenomena are the effects of the increase in the activity of amylase under the influence of hydrogen sulfide, which ensures the mobilization of reserve starch necessary for seedling growth, as well as the effects of the activation of various components of the antioxidant system [33, 53, 54].

There is a reason to believe that similar mechanisms may be responsible for the germination enhancement of aged wheat and triticale seeds by hydrogen sulfide donor. Both cereal species showed an increase in amylase activity under the influence of hydrogen sulfide donor priming (Fig. 2, *A*).

Zhang et al. [37] hypothesized that under the influence of exogenous hydrogen sulfide, β-amylase is activated by breaking the S-S bonds that bind this form of amylase to the periphery of starch grains and limit its activity in both wheat and triticale [55]. However, direct evidence for such a mechanism of hydrogen sulfide action on β-amylase activity in germinating grains of cereals has not been obtained. In our work, a slight increase in β-amylase activity was recorded only on the second day from the beginning of seed germination. At the same time, under the influence of hydrogen sulfide donor, a more pronounced increase in total activity was observed in grains of both cereals after 24 h from the beginning of seed germination. These data indicate a possible involvement of α-amylase in hydrogen sulfide donor-stimulated seed germination, at least during the first day. At the same time, β-amylase activity increased on the second day in the hydrogen sulfide donor seed treatment variants (Fig. 2, *B*). It is possible that at different stages of grain germination the contribution of different forms of amylase to the process of

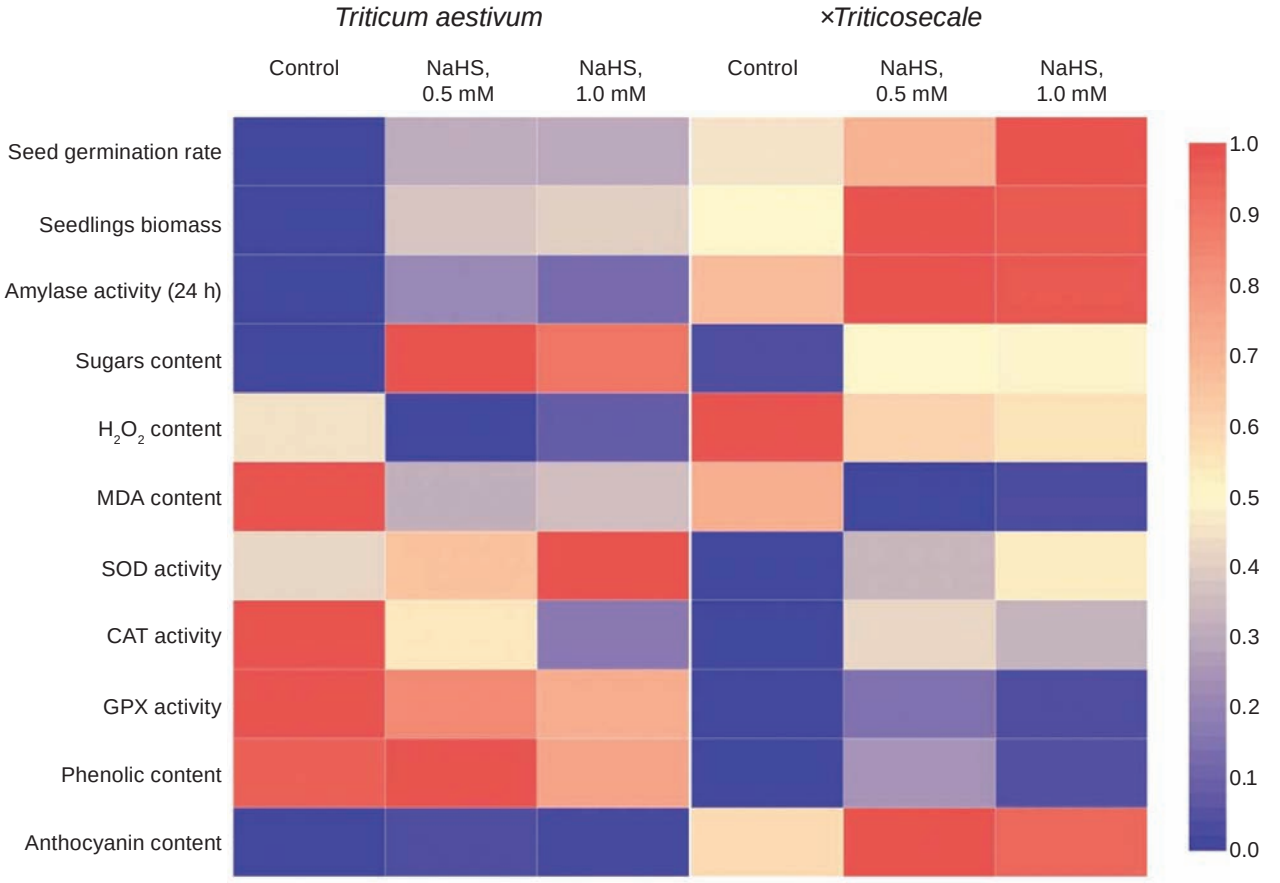

Fig. 7. Heat map of changes in growth and biochemical parameters of wheat and triticale seedlings under the influence of hydrogen sulfide donor. Morphometric indices and soluble carbohydrates content were determined 48 h after the start of seed germination; other biochemical analyses (except for the analysis of amylase activity) were carried out 72 h after the start of seed germination. Each indicator was normalized from 0 to 1 in the construction of the map

reserve starch hydrolysis is different. The magnitude of the activating effect of the hydrogen sulfide donor on amylase may also differ.

Under the conditions of our experiments, it can be assumed that the increase in amylase activity in grains under the influence of hydrogen sulfide donor treatment was the main reason for the increase in sugar content in seedling shoots (Fig. 3). Sugars are known to function as active metabolites and energy sources required for seedling growth [56-59]. At the same time, sugars can also be considered as multifunctional stress metabolites [56]. In particular, the phenomenon of their membrane-stabilizing effect has been known for quite some time [60, 61]. Most soluble carbohydrates also have pronounced antioxidant effects [56]. For example, the disaccharides sucrose, trehalose, maltose, and lactose can effectively neutralize free radicals in vitro [59]. Fructans have an even higher antioxidant activity [66]. They stabilize membranes by scavenging hydroxyl radicals, thereby preventing LPO development. It is assumed that soluble carbohydrates, together with classical antioxidants (ascorbate, glutathione, etc.), form a cytoplasmic antioxidant network [64].

Under the conditions of our experiments, priming of aged wheat and triticale seeds with a hydrogen sulfide donor, together with an increase in the sugar content of the seedlings, caused a decrease in the content of LPO product MDA (Fig. 4, *B*). Certainly, this effect may be related not only to the probable antioxidant effect of the accumulated sugars, but also to other changes in the pro/antioxidant system. In particular, there is evidence for a decrease in the activity of lipoxygenase involved in LPO processes in wheat under the action of hydrogen sulfide donor [54]. In addition, the decrease in LPO levels may be the result of an increase in the activity of antioxidant enzymes when

seeds are treated with hydrogen sulfide donor. Thus, in our experiments, an increase in the activity of one of the key antioxidant enzymes, SOD, was observed in the shoots of wheat and triticale seedlings obtained from seeds primed with H_2S donor (Fig. 5, *A*). It is known to be the only enzyme that neutralizes the superoxide anion radical [65]. At the same time, the effect of priming with hydrogen sulfide donor on the activity of other antioxidant enzymes in shoots of seedlings of two cereal species was not so unambiguous. In particular, in the exogenous H_2S treatments, an increase in catalase activity was observed in triticale, while in wheat, the activity of this enzyme slightly decreased (Fig. 5, *B*). It should be noted that a decrease in catalase activity under the influence of hydrogen sulfide donor was also observed in maize seedlings [36]. It has been reported that persulfidation has a direct inhibitory effect on catalase [66]. However, specific studies are needed to conclude such an effect of H_2S donor on catalase in wheat seedlings.

The effect of treatment of seeds of two cereal species with H_2S donor on guaiacol peroxidase activity was also different. The enzyme activity increased in triticale under NaHS action, but at the same time decreased in wheat (Fig. 5, *C*). It is possible that hydrogen sulfide exerts a direct inhibitory effect on heme-containing antioxidant enzymes [63], but contributes to the formation of redox signals that activate gene expression of antioxidant system enzymes [67, 68]. It is also possible that modulations of antioxidant enzyme activity in seedlings derived from seeds primed with hydrogen sulfide donor are the consequence of different processes, including substrate regulation. It is noteworthy that in our experiments, a decrease in hydrogen peroxide content was observed in the shoots of both studied cereals under seed treatment with hydrogen sulfide donor. This could be related both to the improvement of the antioxidant system and to the reduction of ROS generation. In particular, there is information in the literature on the reduction of ROS generation in plant cell mitochondria under the action of hydrogen sulfide [15, 69]. However, the involvement of such a mechanism in the alteration of mitochondrial function in aging seeds requires specific experimental studies.

In addition to basic antioxidant enzymes, many low-molecular-weight antioxidants may be involved in the realization of the stimulatory effect of hydrogen sulfide on aged cereal seeds. Thus, an increase in the content of classical antioxidants such as ascorbic acid and reduced glutathione in seeds under the influence of hydrogen sulfide donor priming has been reported [36, 70, 71]. In our experiments, triticale but not wheat seedlings showed a significant increase in anthocyanins under the influence of seed priming with a hydrogen sulfide donor. This is probably a species characteristic of triticale. In particular, the content of anthocyanins increased more in triticale than in various wheat species in response to heat stress [72]. However, the index of total phenolic compounds content was not changed in either wheat or triticale under the influence of priming with hydrogen sulfide donor (Fig. 6, *A*). It should be noted that the total phenolic compound content does not always correlate with the ability of plants to effectively maintain the pro/antioxidant balance [48].

Conclusions. In general, priming of aged wheat and triticale seeds with hydrogen sulfide donor promoted their germination and improved seedling growth. These effects in both species seem to be associated with an increase in amylase activity in the grains and an increased supply of soluble carbohydrates to the seedling (Fig. 7). It should be noted that the increase in sugar content in shoots under the influence of seed treatment with hydrogen sulfide donor was more pronounced in wheat than in triticale. At the same time, some components of the antioxidant system were activated in triticale under the influence of hydrogen sulfide donor, the indices of which did not change or even decreased in wheat. Thus, only in triticale, the activity of catalase and peroxidase increased, as well as the content of anthocyanins. Nevertheless, treatment of aged seeds of both cereal species with hydrogen sulfide donor decreased the values of oxidative stress markers, hydrogen peroxide and MDA content (Fig. 7). Thus, modulation of antioxidant system components in aged seeds of cereals by hydrogen sulfide depended on their species peculiarities, although for both wheat and triticale an improvement of integral indices characterizing the maintenance of redox homeostasis and growth processes was observed. In this regard, the treatment of aged cereal seeds with hydrogen sulfide donors can be considered an effective tool to increase their germination and improve seedling growth.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОКАЗНИКИ ВУГЛЕВОДНОГО ОБМІНУ ТА СТАНУ АНТИОКСИДАНТНОЇ СИСТЕМИ ЗА ПРОРОСТАННЯ СТАРОГО НАСІННЯ ПШЕНИЦІ ТА ТРИТИКАЛЕ, ОБРОБЛЕНОГО ДОНОРОМ H_2S

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Гідроген сульфід (H_2S) є молекулою-газотрансмітером, яка бере участь у реалізації багатьох функцій рослинного організму, включно з проростанням насіння. Показано, що старіння насіння супроводжується окислювальним стресом і зниженням схожості. Вплив екзогенного H_2S на проростання старого насіння злаків не досліджувався. Мета роботи полягала у дослідженні впливу праймування NaHS як донора H_2S на насіння пшениці та тритикале, які попередньо зазнали природного старіння. Насіння озимої пшениці (*Triticum aestivum*) та озимого ×*Triticosecale* зберігалося в приміщенні протягом 4 років за змінних температури та вологості. Постаріле насіння обробляли 0,2-5 мМ розчином NaHS протягом 3 годин і пророщували в чашках Петрі протягом 3 днів. Як контроль використовували обробку водою (гідропраймінг). Визначали активність амілази в зерні, біомасу пагонів і коренів, вміст загальних цукрів, H_2O_2, продуктів пероксидного окислення ліпідів та антоціанів, а також активність антиоксидантних ензимів у проростках. Показано, що після обробки донором H_2S активність каталази і гваяколпероксидази, а також вміст антоціанів зростали лише в проростках тритикале. Водночас обробка насіння обох видів злаків супроводжувалася посиленням росту пагонів і коренів, підвищенням активності амілази та супероксиддисмутази, зниженням вмісту H_2O_2 і МДА, а також підвищеним накопиченням цукрів у пагонах. Зроблено висновок, що підвищення схожості старого насіння злаків під впливом донора H_2S зумовлене посиленням мобілізації резервних вуглеводів та модуляцією активності антиоксидантної системи. Таку обробку можна розглядати як ефективний інструмент для покращення росту проростків.

Ключові слова: старіння насіння, *Triticum aestivum*, *Triticosecale*, гідроген судьфід, загальний цукор, амілаза, окислювальний стрес, антиоксидантна система.

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