

## INDUCTION OF PLANT CELLS HEAT RESISTANCE BY HYDROGEN SULFIDE DONOR IS MEDIATED BY $H_2O_2$ GENERATION WITH PARTICIPATION OF NADPH OXIDASE AND SUPEROXIDE DISMUTASE

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The participation of enzymatic systems carrying out generation and conversion of reactive oxygen species (ROS), in realization of the stress-protective effect of hydrogen sulfide ( $H_2S$ ) on wheat coleoptile cells was investigated. It has been shown that the treatment of isolated coleoptiles with a 100  $\mu$ M hydrogen sulfide donor sodium hydrosulfide (NaHS) caused a transient enhancement of the generation of superoxide anion radical ( $O_2^{\cdot-}$ ), an increase of hydrogen peroxide content and superoxide dismutase (SOD) activity in them. The increase in ROS generation was eliminated by the inhibitor of NADPH oxidase imidazole, but not by the peroxidase inhibitor sodium azide. Treatment of coleoptiles with SOD inhibitor sodium diethyldithiocarbamate (DDC) enhanced the generation of  $O_2^{\cdot-}$  and neutralized the effect of increasing  $H_2O_2$  content induced by NaHS. One day after treatment with the  $H_2S$  donor, the generation of ROS decreased to a control level, while the activity of antioxidant enzymes increased markedly and the resistance of coleoptiles to damaging heating was increased. These effects of the hydrogen sulfide donor were eliminated by coleoptiles' treatment with inhibitors of NADPH oxidase (imidazole) and SOD (DDC). It was concluded that both NADPH oxidase, generating  $O_2^{\cdot-}$ , and SOD, which turns it into  $H_2O_2$  performing signaling functions, are involved in the formation of a signal that induces protective systems and causes an increase in heat resistance of plant cells.

**Key words:** hydrogen sulfide, signal mediators, reactive oxygen species, NADPH oxidase, superoxide dismutase, plant cells, heat resistance.

At present, it is known that the formation of plant resistance to stress factors occurs with the participation of a number of signal mediators. The role of calcium ions, reactive oxygen species (ROS) and nitric oxide in transduction of stress signals into genetic apparatus of a plant cell and formation of adaptive reactions is most studied [1-3].

In recent years, hydrogen sulfide ( $H_2S$ ) has been considered as another inorganic mediating molecule both in animals [4] and in plant cells [5, 6].

Effects of endogenous hydrogen sulfide content increasing in plants under action of stressors, in particular, drought [7], salinity [8], heavy metals [9] were shown. An increase in content of hydrogen sulfide at treatment of maize seedlings with NO do-

nors or hydrogen peroxide inducing heat resistance was revealed [10, 11]. This indicates a close relationship between hydrogen sulfide, ROS and NO as signaling molecules.

Data have also been obtained that indicate the role of ROS in transduction of  $H_2S$  signal. Increasing resistance of barley plants to UV-B by hydrogen sulfide donor was accompanied by an increase in content of hydrogen peroxide in leaves and this effect was eliminated by the  $H_2O_2$  scavenger dimethylthiourea [12]. We have previously shown that induced by the hydrogen sulfide donor NaHS increase in heat resistance of wheat coleoptiles was leveled by treatment with an antioxidant ionol [13]. At the same time, however, the role of ROS in realization of the effects of hydrogen sulfide as a physiologically active

molecule remains poorly investigated. In particular, enzyme systems involved in the formation of signaling ROS, when hydrogen sulfide donors act on plant objects, are hardly studied.

It can be assumed that an increase in ROS content upon hydrogen sulfide exposure of plant cells may be due to activation of NADPH oxidase and/or apoplastic peroxidases [3], generating a superoxide anion radical ( $O_2^{\cdot-}$ ), which turns into a more stable ROS hydrogen peroxide under the action of SOD [14]. However, in the literature, we were able to find only indirect indications of the role of NADPH oxidase in ROS generation during the treatment of barley leaves with sodium hydrosulfide [12].

The purpose of this work was to elucidate the possible role of NADPH oxidase, extracellular peroxidase and SOD in the formation of ROS involved in transmission of the signal of exogenous hydrogen sulfide, which induces heat resistance of plant cells, to genetic apparatus. Wheat coleoptile segments were used for the studies, which are a convenient model for studying the effect of exogenous compounds on resistance determined by cellular mechanisms, as well as for evaluating the generation of superoxide anion radical by non-destructive testing methods [15, 16].

### Materials and Methods

Seeds of wheat (*Triticum aestivum* L.) variety of forest-steppe ecotype Doskonala (moderate heat- and drought-resistant) were decontaminated by 6% hydrogen peroxide solution for 30 min, carefully washed with sterile distilled water and germinated in the dark for 4 days at 20 °C. The basal parts of the coleoptiles were separated from the seedlings, they were incubated on distilled water for 2 h to remove the “wound stress” effect, and then transferred to Petri dishes with the main incubation medium – a sterilized 2% sucrose solution with penicillin (Na-salt, 100,000 Unit/l). Medium of the respective variants in addition to sucrose and penicillin contained 100  $\mu$ M hydrogen sulfide donor NaHS or inhibitors of NADPH oxidase – imidazole (1  $\mu$ M) [17], peroxidase – sodium azide ( $NaN_3$  – 500  $\mu$ M) [18], Cu/Zn-SOD – sodium diethyldithiocarbamate (DDC – 1 mM) [19, 20] or a combination of each inhibitor with NaHS. The time of incubation of coleoptiles on a medium containing the hydrogen sulfide donor was 24 h. In variants with combined treatment, enzyme inhibitors were added to the coleoptiles incubation medium 2 h before NaHS was added thereto.

Concentrations of enzyme inhibitors and time of their treatment with coleoptiles were selected based on preliminary experiments. The optimal concentration of the hydrogen sulfide donor, causing the maximum increase in the heat resistance of wheat coleoptiles, was established earlier [13]. Previously, it was also shown the removal of the positive effect of NaHS treatment of coleoptiles on their heat resistance by the action of the hydrogen sulfide scavenger hydroxylamine [13], indicating that the physiological effects of NaHS are associated specifically with the formation of hydrogen sulfide.

At the end of incubation time of coleoptiles, they were exposed to a potentially lethal heating in a water ultrathermostate in sterile distilled water for 10 min at  $43.0 \pm 0.1$  °C [13]. The coleoptiles were then placed in Petri dishes with a sterile 2% solution of sucrose with penicillin. After 2 days, they were assessed for their damage by loss of turgor and appearance of a specific shade due to tissue infiltration.

Generation of superoxide anion radicals ( $O_2^{\cdot-}$ ) by segments of coleoptiles was determined from reduction of nitroblue tetrazolium (NBT), as described earlier [16]. The optical density was determined on a spectrophotometer SF-46 (LOMO, Russia) at 530 nm. To check the specificity of generation, in special experiments, SOD (50 U/ml) was added to the samples, that inhibited the reduction of NBT by at least 90%. In this regard, it was believed that the amount of reduced NBT is determined by the amount of superoxide anion radical. The superoxide producing activity was evaluated as a change in the optical density of the reaction mixture ( $\Delta A_{530}$ ) per hour of incubation per one piece of coleoptile. The value of the optical density in the control was taken as 100%.

The hydrogen peroxide content was determined by the ferrothiocyanate method, extracting it from coleoptiles homogenized in the cold by 5% TCA. The samples were centrifuged on a centrifuge MPW 350R (MPW MedInstruments, Poland) at 8000 g for 10 min at a temperature no higher than 4 °C, and the concentration of  $H_2O_2$  was determined in the supernatant [21].

The activity of cytosolic superoxide dismutase (SOD, EC 1.15.1.1) and soluble peroxidase (EC 1.11.1.7) was determined by the methods described earlier [22]. Samples of coleoptiles were homogenized in cold 0.15 M K,Na-phosphate buffer (pH 7.6) with addition of EDTA (0.1 mM) and dithiothreitol (1 mM). For the analysis, the supernatant was used

after centrifuging the homogenate at 8000 g for 10 min at a temperature no higher than 4 °C.

The SOD activity was determined at pH 7.6 of the reaction mixture using a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions formed as a result of the aerobic interaction of NADH and phenazine methosulfate.

The peroxidase activity was analyzed using guaiacol as the hydrogen donor, hydrogen peroxide as the substrate.

The protein content in the samples was determined by Bradford [23] using bovine serum albumin as a standard.

To estimate the activity of extracellular peroxidase, 15 segments of coleoptiles were placed in tubes with 5 ml of 0.06 M K<sub>2</sub>Na-phosphate buffer (pH 6.2) supplemented with 0.1% Triton X-100 shaking on a shaker (120 rpm) for 1 h [16]. 0.15% H<sub>2</sub>O<sub>2</sub> was used as a substrate, and 0.7% guaiacol as a reducing agent. The optical density of the product of its oxidation was measured at 470 nm.

Measurements were carried out in three biological and three analytical replications. The mean values and their standard errors are given. Except the cases noted specifically, the differences significant at  $P \leq 0.05$  are discussed.

## Results and Discussion

Generation of superoxide anion radical in segments of wheat coleoptiles practically did not change during the day of observations (Table 1). At the same time, 2-4 h after the beginning of exposure of wheat coleoptiles to hydrogen sulfide donor NaHS, the formation of O<sub>2</sub><sup>•-</sup> was significantly enhanced, but this effect was transient and, after 24 h of treatment with

sodium hydrosulfide, the generation of superoxide anion radical in the experimental variant was even slightly lower, than in the control (Table 1).

The dynamics of the hydrogen peroxide content in the coleoptiles of wheat was similar to that of the superoxide. Thus, in the control, the H<sub>2</sub>O<sub>2</sub> content did not change during the experiment, but in the variant with sodium hydrosulfide it slightly increased after 2 h and reached a maximum 3-4 h after the beginning of the treatment (Table 1). After 24 h of exposure to the hydrogen sulfide donor, the amount of H<sub>2</sub>O<sub>2</sub> in the coleoptiles corresponded to the control values.

To elucidate the possible contribution of NADPH oxidase and apoplastic peroxidase to the generation of superoxide anion radical in wheat coleoptiles, the effect of imidazole and sodium azide on this process was investigated. The treatment of coleoptiles with imidazole by itself caused a small but significant decrease in the formation of O<sub>2</sub><sup>•-</sup> at  $P \leq 0.05$  (Fig. 1). At the same time, this inhibitor almost completely suppressed the effect of amplification of the superoxide anion radical generation on cell surface, which was caused by the hydrogen sulfide donor. At the same time, the inhibitor of apoplastic peroxidase by itself only slightly reduced the generation of O<sub>2</sub><sup>•-</sup> and completely did not affect the hydrogen sulfide-induced increase in the formation of superoxide anion radical by wheat coleoptiles (Fig. 1). In this connection, it can be assumed that the hydrogen sulfide-induced enhancement of O<sub>2</sub><sup>•-</sup> generation on cell surface is associated with an increase in the activity of NADPH oxidase, rather than the extracellular forms of peroxidase. In favor of such a conclusion, the absence of an increase in the activity of apoplastic peroxidase of coleoptiles

Table 1. Dynamics of generation of superoxide anion radical and H<sub>2</sub>O<sub>2</sub> content in wheat coleoptiles at treatment with a hydrogen sulfide donor NaHS

Variant	Time of exposure, h			
	2	3	4	24
O <sub>2</sub> <sup>•-</sup> generation (% of value at the first point of control variant)				
Control	100.0 ± 2.2	101.0 ± 2.6	102.0 ± 2.3	102.0 ± 2.3
NaHS (100 μM)	136.0 ± 3.0*	142.0 ± 2.5*	140.0 ± 2.5*	92.5 ± 2.6
H <sub>2</sub> O <sub>2</sub> content (nmol/g f. w.)				
Control	114.0 ± 3.1	117.0 ± 2.9	119.0 ± 3.6	117.0 ± 4.3
NaHS (100 μM)	148.0 ± 3.9*	212.0 ± 5.2*	208.0 ± 4.0*	116.0 ± 4.5

\* Differences are significant at  $P \leq 0.05$  relative to control

during the NaHS treatment is also evidence (Fig. 2, A). Moreover, the donor of hydrogen sulfide not only did not activate, but even inhibited this form of peroxidase. Note, that treatment with sodium azide also reduced the activity of the enzyme, and at combined action of the hydrogen sulfide donor and  $\text{NaN}_3$  on coleoptiles, there was a significant inhibition of extracellular peroxidase activity (Fig. 2, A), which, however, did not affect the generation of the superoxide anion radical (Fig. 1).

It is known that sodium azide is an inhibitor not only of peroxidase, but of cytochrome oxidase too [24] and thus can influence the ROS formation in mitochondria. However, as noted above, in our experiments sodium azide had no significant effect on the ROS formation stimulated by the hydrogen sulfide donor in wheat coleoptiles, but at the same time inhibited extracellular peroxidase. In this regard, we can talk about a rather specific action of it as an inhibitor of the apoplasmic form of this enzyme.

An increase in the hydrogen peroxide content occurring in wheat coleoptiles under the influence of a hydrogen sulfide donor may be due to the conversion of a superoxide anion radical to  $\text{H}_2\text{O}_2$ , which occurs spontaneously or with the participation of SOD [20, 25]. As our results showed, 3 h after the start of coleoptiles' treatment with NaHS, when the maximum content of hydrogen peroxide was observed, an increase in the activity of SOD was observed (Fig. 2, B). The SOD inhibitor DDC markedly reduced the activity of the enzyme in the coleoptiles and completely eliminated the effect of its increase caused by the hydrogen sulfide donor.

The NADPH oxidase inhibitor imidazole itself did not significantly affect the content of hydrogen peroxide in the wheat coleoptiles, but almost completely removed the increase in its amount caused by the action of the hydrogen sulfide donor (Fig. 3). Under the influence of the SOD inhibitor DDC, a decrease in the hydrogen peroxide content in wheat coleoptiles was noted, and also the treatment of DDC completely eliminated the effect of increasing the content of hydrogen peroxide in tissues caused by the action of NaHS (Fig. 3). It is noteworthy that the treatment of coleoptiles with DDC in itself caused an increase in the amount of detected  $\text{O}_2^{\cdot-}$  and enhanced the manifestation of the influence of the hydrogen sulfide donor on the superoxide anion radical generation (Fig. 1). The totality of these results indicates a significant contribution of SOD in the conversion of

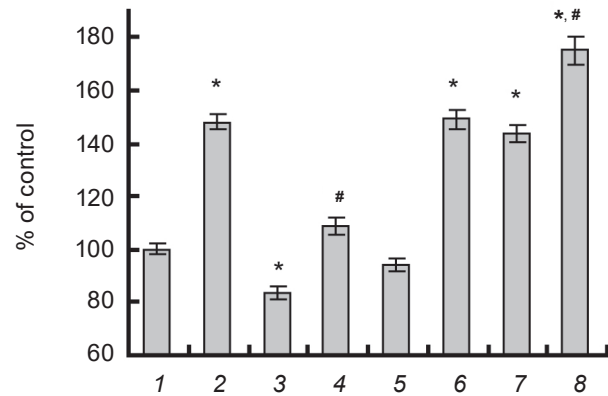


Fig. 1.  $\text{O}_2^{\cdot-}$  generation by wheat coleoptiles at the action of NaHS, imidazole, DDC and  $\text{NaN}_3$ : 1 – control; 2 – NaHS (100  $\mu\text{M}$ ); 3 – imidazole (1  $\mu\text{M}$ ); 4 – NaHS (100  $\mu\text{M}$ ) + imidazole (1  $\mu\text{M}$ ); 5 –  $\text{NaN}_3$  (500  $\mu\text{M}$ ); 6 – NaHS (100  $\mu\text{M}$ ) +  $\text{NaN}_3$  (500  $\mu\text{M}$ ); 7 – DDC (1 mM); 8 – NaHS (100  $\mu\text{M}$ ) + DDC (1 mM). Note. Here and in Fig. 2, 3: coleoptiles NaHS treatment was carried out for 3 h, enzyme inhibitors were added to the incubation medium of coleoptiles 2 h before adding NaHS. \*Differences are significant at  $P \leq 0.05$  relative to control; # differences are significant at  $P \leq 0.05$  relative to NaHS

$\text{O}_2^{\cdot-}$  to hydrogen peroxide at action of the  $\text{H}_2\text{S}$  donor on wheat coleoptiles.

Consequently, under the influence of exogenous hydrogen sulfide, the pool of signal hydrogen peroxide in wheat coleoptile cells appears to be formed by increasing the activity of NADPH oxidase and SOD. It can be assumed that an increase in the ROS generation may lead to an increase in the activity of antioxidant enzymes. And indeed, 24 h after the beginning of treatment of coleoptiles with the hydrogen sulfide donor, the activity of SOD was higher than the values observed after 3 h after the beginning of the treatment (Fig. 2, B, Table 2). At the same time, the increase in SOD activity induced by the NaHS treatment of coleoptiles was completely leveled by the action of the NADPH oxidase inhibitor imidazole.

There was observed increased activity of the soluble form of peroxidase 24 h after the beginning of the NaHS treatment of coleoptiles (Table 2), which, in contrast to the apoplast forms, performs mainly antioxidant functions [26]. Inhibitors of NADPH oxidase imidazole and SOD DDC, by themselves not causing changes in peroxidase activity,

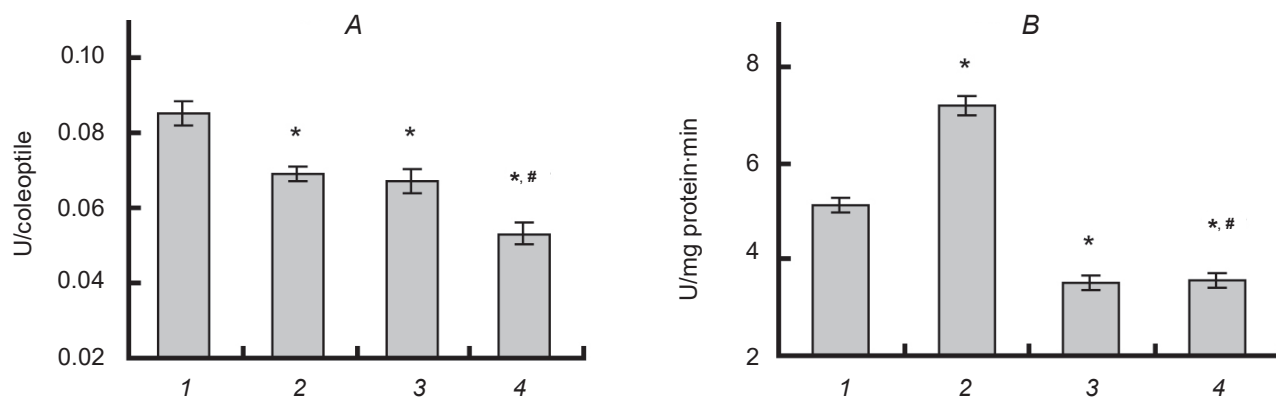


Fig. 2. Activity of extracellular peroxidase (A) and SOD (B) in wheat coleoptiles. A: 1 – control; 2 – NaHS (100  $\mu$ M); 3 –  $\text{NaN}_3$  (500  $\mu$ M); 4 – NaHS (100  $\mu$ M) +  $\text{NaN}_3$  (500  $\mu$ M); B: 1 – control; 2 – NaHS (100  $\mu$ M); 3 – DDC (1 mM); 4 – NaHS (100  $\mu$ M) + DDC (1 mM)

Table 2. Activity of antioxidant enzymes in wheat coleoptiles under the influence of NaHS, imidazole and DDC and their survival after damaging heating (43  $^{\circ}$ C, 10 min)

Variant	SOD activity (U/mg protein·min)	Soluble guaiacol peroxidase activity ( $\mu$ mol guaiacol/mg protein·min)	Survival of coleoptiles, %
Control	6.20 $\pm$ 0.14	397 $\pm$ 14	43.0 $\pm$ 2.0
NaHS (100 $\mu$ M)	10.26 $\pm$ 0.20*	589 $\pm$ 17*	68.4 $\pm$ 2.5*
Imidazole (1 $\mu$ M)	5.98 $\pm$ 0.16	401 $\pm$ 11	41.5 $\pm$ 1.8
NaHS (100 $\mu$ M) + imidazole (1 $\mu$ M)	7.27 $\pm$ 0.17*#	429 $\pm$ 13#	42.5 $\pm$ 1.5#
DDC (2 mM)	–	366 $\pm$ 16	40.0 $\pm$ 2.0
NaHS (100 $\mu$ M) + DDC (2 mM)	–	419 $\pm$ 14#	37.5 $\pm$ 2.4#

\* Differences are significant at  $P \leq 0.05$  relative to control; # differences are significant at  $P \leq 0.05$  relative to NaHS

eliminated its increase induced by the action of the  $\text{H}_2\text{S}$  donor (Table 2).

Thus, inducing of peroxidase by hydrogen sulfide in wheat coleoptiles depends on the increase in ROS content, which occurs with the participation of NADPH oxidase and SOD already in the first hours after the action of the  $\text{H}_2\text{S}$  donor. These data are consistent with earlier findings on the role of hydrogen peroxide, formed with the participation of SOD, in controlling the expression of the ascorbate peroxidase gene in plant cells [19]. It should also be noted the dependence of SOD inducing on the formation of a superoxide radical associated with an increase in the activity of NADPH oxidase (Table 2). This conclusion is supported by the removal of the  $\text{H}_2\text{S}$ -induced increase in SOD activity by the inhibitor of NADPH oxidase imidazole (Table 2). It can be assumed that antioxidant enzymes are components

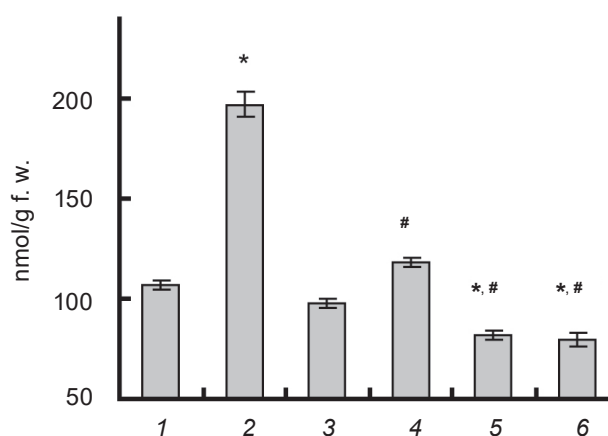


Fig. 3. Content of hydrogen peroxide in wheat coleoptiles under the action of NaHS, imidazole and DDC. 1 – control; 2 – NaHS (100  $\mu$ M); 3 – imidazole (1  $\mu$ M); 4 – NaHS (100  $\mu$ M) + imidazole (1  $\mu$ M); 5 – DDC (1 mM); 6 – NaHS (100  $\mu$ M) + DDC (1 mM)

of the protective systems of coleoptiles cells induced by exogenous hydrogen sulfide. Activation of anti-oxidant enzymes and, probably, of other protective systems causes an increase in the heat resistance of wheat coleoptiles when hydrogen sulfide donor acts on them (Table 2). It is noteworthy that the positive effect of NaHS on the heat resistance of coleoptiles was suppressed both by inhibitors of NADPH oxidase (imidazole) and SOD (DDC). At the same time the inhibitors themselves at the used concentrations did not have a significant effect on the survival of coleoptiles after heat stress.

The increase in antioxidant activity and, as a consequence, the resistance of plants to stressors under the influence of hydrogen sulfide donors was also registered in a number of other studies. So, an increase in the activity of catalase and ascorbate peroxidase in wheat seedlings under osmotic stress [27] was shown. In the same species, an increase in activity of antioxidant enzymes was revealed when treated by the hydrogen sulfide donor under the subsequent action of toxic doses of copper [28]. In grape plants under conditions of hypothermia (4 °C), an increase in the SOD activity was observed at their treatment with NaHS [29]. In pelargonium plants, subjected to hydrogen sulfide donor treatment, an increase in the content of ascorbate and reduced glutathione was revealed during cold stress [30].

As was noted, in only one work, using inhibitor of NADPH oxidase diphenyliodonium, the possible participation of this enzyme as an ROS generator was shown when inducing the resistance of barley plants to the action of UV-B [12] by the exogenous hydrogen sulfide. In our work using the inhibitory method, the causal relationship between hydrogen sulfide-stimulated and NADPH oxidase-dependent superoxide anion radical generation, induction of antioxidant enzymes, and development of heat resistance of wheat coleoptiles was first demonstrated. For the first time, using the SOD inhibitor DDC, the value of this enzyme in the formation of hydrogen peroxide signal pool, necessary for activating antioxidant enzymes and improving the heat resistance of coleoptiles, has been shown. In the future, it seems relevant to clarify the question of the functional interaction of hydrogen sulfide with other signal mediators, primarily calcium ions and nitric oxide, which have close functional links with ROS [6, 31, 32].

It is quite natural that the antioxidant system is not the only protector system induced by hydrogen

sulfide. Thus, to date, data have been obtained on the increase in content of polyfunctional low-molecular-weight protectors – sucrose, trehalose, betaine, in maize plants under the influence of exogenous hydrogen sulfide [33, 34]. The strawberry has been shown to induce the synthesis of various groups of heat shock proteins and aquaporins at the action of exogenous H<sub>2</sub>S [30]. The study of the “spectrum” of hydrogen sulfide-induced protective reactions of plants, as well as the mechanisms of their activation, will create prerequisites for their use as part of stress-protective preparations for plant growing.

### ІНДУКУВАННЯ ТЕПЛОСТІЙКОСТІ РОСЛИННИХ КЛІТИН ДОНОРОМ ГІДРОГЕН СУЛЬФІДУ ОПОСЕРЕДКОВАНО ГЕНЕРАЦІЮ Н<sub>2</sub>О<sub>2</sub> З УЧАСТЮ NADPH-ОКСИДАЗИ І СУПЕРОКСИДДИСМУТАЗИ

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Досліджували участь ензиматичних систем, що забезпечують генерацію і перетворення активних форм кисню (АФК), в реалізації стрес-протекторного ефекту гідроген сульфід (H<sub>2</sub>S) на клітини колеоптилів пшениці. Показано, що обробка ізольованих колеоптилів 100 мкМ розчином донора гідроген сульфід – гідросульфід натрію (NaHS) – спричиняла в них транзиторне посилення генерації супероксидного аніон-радикала (O<sub>2</sub><sup>-</sup>), підвищення вмісту пероксиду водню та активності супероксиддисмутази (СОД). Посилення генерації АФК усувалося інгібітором NADPH-оксидази – імідазолом, але не інгібітором пероксидази – азидом натрію. Обробка колеоптилів інгібітором СОД – діетилдитіокарбаматом натрію (ДДК) – посилювала генерацію O<sub>2</sub><sup>-</sup> і нівелювала ефект підвищення вмісту Н<sub>2</sub>О<sub>2</sub>, індукований дією NaHS. Через добу після обробки донором Н<sub>2</sub>S генерація АФК знижувалася до рівня контролю, при цьому помітно збільшувалася активність антиоксидантних ензимів і підвищувалася стійкість колеоптилів до ушкоджуючого нагрівання. Ці ефекти донора гідроген сульфід

усувалися обробкою колеоптилів інгібіторами NADPH-оксидази (імідазолом) і СОД (ДДК). Дійшли висновку, що у формуванні сигналу, який індукуює протекторні системи і зумовлює підвищення теплостійкості рослинних клітин, задіяні NADPH-оксидаза, що генерує  $O_2^{\cdot-}$ , і СОД, яка перетворює його на  $H_2O_2$ , що виконує сигнальні функції.

**Ключові слова:** гідроген сульфід, сигнальні посередники, активні форми кисню, NADPH-оксидаза, супероксиддисмутаза, рослинні клітини, теплостійкість.

### **ИНДУЦИРОВАНИЕ ТЕПЛОУСТОЙЧИВОСТИ РАСТИТЕЛЬНЫХ КЛЕТОК ДОНОРОМ СЕРОВОДОРОДА ОПОСРЕДОВАНО ГЕНЕРАЦИЕЙ $H_2O_2$ С УЧАСТИЕМ NADPH-ОКСИДАЗЫ И СУПЕРОКСИДДИСМУТАЗЫ**

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Исследовали участие энзиматических систем, обеспечивающих генерацию и превращение активных форм кислорода (АФК), в реализации стресс-протекторного эффекта сероводорода ( $H_2S$ ) на клетки колеоптилей пшеницы. Показано, что обработка изолированных колеоптилей 100 мкМ раствором донора сероводорода – гидросульфида натрия ( $NaHS$ ) – вызвала у них транзиторное усиление генерации супероксидного анион-радикала ( $O_2^{\cdot-}$ ), повышение содержания пероксида водорода и активности супероксиддисмутазы (СОД). Усиление генерации АФК устранялось ингибитором NADPH-оксидазы – имидазолом, но не ингибитором пероксидазы – азидом натрия. Обработка колеоптилей ингибитором СОД – диэтилдитиокарбаматом натрия (ДДК) – усиливала генерацию  $O_2^{\cdot-}$  и нивелировала эффект повышения содержания  $H_2O_2$ , индуцируемый действием  $NaHS$ . Через сутки после обработки донором  $H_2S$  генерация АФК снижалась до уровня контроля, при

этом заметно увеличивалась активность антиоксидантных энзимов и повышалась устойчивость колеоптилей к повреждающему нагреву. Эти эффекты донора сероводорода устранялись обработкой колеоптилей ингибиторами NADPH-оксидазы (имидазолом) и СОД (ДДК). Сделано заключение, что в формировании сигнала, индуцирующего протекторные системы и обуславливающего повышение теплоустойчивости растительных клеток, задействованы NADPH-оксидаза, генерирующая  $O_2^{\cdot-}$ , и СОД, превращающая его в  $H_2O_2$ , выполняющий сигнальные функции.

**Ключевые слова:** сероводород, сигнальные посредники, активные формы кислорода, NADPH-оксидаза, супероксиддисмутаза, растительные клетки, теплоустойчивость.

### **References**

1. Mori IC, Schroeder JI. Reactive oxygen species activation of plant  $Ca^{2+}$  channels. A signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction. *Plant Physiol.* 2004; 135(2): 702-708.
2. Jeandroz S, Lamotte O, Astier J, Rasul S, Trapet P, Besson-Bard A, Bourque S, Nicolas-Francès V, Ma W, Berkowitz GA, Wendehenne D. There's more to the picture than meets the eye: nitric oxide cross talk with  $Ca^{2+}$  signaling. *Plant Physiol.* 2013; 163(2): 459-470.
3. Kolupaev YuE, Karpets YuV. Reactive oxygen species and stress signaling in plants. *Ukr Biochem J.* 2014; 86(4): 18-35. (In Russian).
4. Zaichko NV, Melnik AV, Yoltukhivskyy MM, Olhovskiy AS, Palamarchuk IV. Hydrogen sulfide: metabolism, biological and medical role. *Ukr Biochem J.* 2014; 86(5): 5-25.
5. Lisjak M, Teklic T, Wilson ID, Whiteman M, Hancock JT. Hydrogen sulfide: environmental factor or signalling molecule? *Plant Cell Environ.* 2013; 36(9): 1607-1616.
6. Hancock JT. Harnessing evolutionary toxins for signaling: reactive oxygen species, nitric oxide and hydrogen sulfide in plant cell regulation. *Front Plant Sci.* 2017; 8: 189.
7. Jin Z, Shen J, Qiao Z, Yang G, Wang R, Pei Y. Hydrogen sulfide improves drought resistance in *Arabidopsis thaliana*. *Biochem Biophys Res Commun.* 2011; 414(3): 481-486.

8. Lai D, Mao Y, Zhou H, Li F, Wu M, Zhang J, He Z, Cui W, Xie Y. Endogenous hydrogen sulfide enhances salt tolerance by coupling the reestablishment of redox homeostasis and preventing salt-induced K<sup>+</sup> loss in seedlings of *Medicago sativa*. *Plant Sci*. 2014; 225: 117-129.
9. Shi H, Ye T, Chan Z. Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (*Cynodon dactylon* (L.) Pers.). *Plant Physiol Biochem*. 2014; 74: 99-107.
10. Li ZG, Yang SZ, Long WB, Yang GX, Shen ZZ. Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant Cell Environ*. 2013; 36(8): 1564-1572.
11. Li ZG, Luo LJ, Sun YF. Signal crosstalk between nitric oxide and hydrogen sulfide may be involved in hydrogen peroxide induced thermotolerance in maize seedlings. *Rus J Plant Physiol*. 2015; 62(4): 507-514.
12. Li Q, Wang Z, Zhao Y, Zhang X, Zhang S, Bo L, Wang Y, Ding Y, An L. Putrescine protects hullless barley from damage due to UV-B stress via H<sub>2</sub>S- and H<sub>2</sub>O<sub>2</sub>-mediated signaling pathways. *Plant Cell Rep*. 2016; 35(5): 1155-1168.
13. Kolupaev YuE, Firsova EN, Yastreba TO, Shvidenko NV. Induction of antioxidant system and heat resistance of wheat coleoptiles by hydrogen sulfide donor: connection with reactive oxygen species formation. *Bul Kharkiv Nat Agrar Univ. Series Biology*. 2017; 1(40): 61-68. (In Russian).
14. Alscher RG, Erturk N, Heath LS. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot*. 2002; 53: 1331-1341.
15. Shorning BY, Smirnova EG, Yaguzhinsky LS, Vanyushin BF. Necessity of superoxide production for development of etiolated wheat seedlings. *Biochemistry (Mosc)*. 2000; 65(12): 1357-1361.
16. Kolupaev YuE, Yastreba TO, Shvidenko NV, Karpets YuV. Induction of heat resistance of wheat coleoptiles by salicylic and succinic acids: connection of the effect with the generation and neutralization of reactive oxygen species. *Appl Biochem Microbiol*. 2012; 48(5): 500-505.
17. Hung KT, Hsu YT, Kao CH. Hydrogen peroxide is involved in methyl jasmonate induced senescence of rice leaves. *Physiol Plant*. 2006; 127(2): 293-303.
18. Minibayeva FV, Gordon LK, Kolesnikov OP, Chasov AV. Role of extracellular peroxidase in the superoxide production by wheat root cells. *Protoplasma*. 2001; 217(1-3): 125-128.
19. Morita S, Kaminaka H, Masumura T, Tanaka K. Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress: the involvement of hydrogen peroxide in oxidative stress signalling. *Plant Cell Physiol*. 1999; 40(4): 417-422.
20. Kolupaev YuE, Oboznyi OI. Participation of the active oxygen forms in the induction of ascorbate peroxidase and guaiacol peroxidase under heat hardening of wheat seedlings. *Ukr Biokhim Zhurn*. 2012; 84(6): 131-138. (In Russian).
21. Sagisaka S. The Occurrence of Peroxide in a Perennial Plant, *Populus gelrica*. *Plant Physiol*. 1976; 57(2): 308-309.
22. Karpets YuV, Kolupaev YuE, Lugovaya AA, Oboznyi AI. Effect of jasmonic acid on the pro-/antioxidant system of wheat coleoptiles as related to hyperthermia tolerance. *Russ J Plant Physiol*. 2014; 61(3): 339-346.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72: 248-254.
24. Schwarzländer M, Fricker MD, Sweetlove LJ. Monitoring the in vivo redox state of plant mitochondria: effect of respiratory inhibitors, abiotic stress and assessment of recovery from oxidative challenge. *Biochim Biophys Acta*. 2009; 1787(5): 468-475.
25. Sagi M, Fluhr R. Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiol*. 2006; 141(2): 336-340.
26. Ivanov S, Konstantinova T, Parvanova D, Todorova D, Djilianov D, Alexieva V. Effect of high temperatures on the growth, free proline content and some antioxidants in tobacco plants. *Rep Bulg Acad Sci*. 2001; 54(7): 71-74.
27. Zhang H, Wang MJ, Hu LY, Wang SH, Hu KD, Bao LJ, Luo JP. Hydrogen sulfide promotes wheat seed germination under osmotic stress. *Russ J Plant Physiol*. 2010; 57(4): 532-539.
28. Dai H, Xu Y, Zhao L, Shan C. Alleviation of copper toxicity on chloroplast antioxidant capacity and photosystem II photochemistry of wheat by hydrogen sulfide. *Braz J Bot*. 2016; 39(2): 787-793.
29. Fu PN, Wang WJ, Hou LX, Liu X. Hydrogen sulfide is involved in the chilling stress response



- in *Vitis vinifera* L. *Acta Soc Bot Pol.* 2013; 82(4): 295-302.
30. Christou A, Filippou P, Manganaris GA, Fotopoulos V. Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. *BMC Plant Biol.* 2014; 14: 42.
  31. Karpets YuV, Kolupaev YuE, Yastreb TO, Dmitriev OP. Possible pathways of heat resistance induction in plant cells by exogenous nitrogen oxide. *Cytol Genet.* 2012; 46(6): 354-359.
  32. Singh VP, Singh S, Kumar J, Prasad SM. Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate-glutathione cycle: Possible involvement of nitric oxide. *J Plant Physiol.* 2015; 181: 20-29.
  33. Li ZG, Zhu LP. Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. *Braz J Bot.* 2015; 38(1): 31-38.
  34. Li ZG, Long WB, Yang SZ, Wang YC, Tang JH, Wen L, Zhu BYu, Min X. Endogenous hydrogen sulfide regulated by calcium is involved in thermotolerance in tobacco *Nicotiana tabacum* L. suspension cell cultures. *Acta Physiol Plant.* 2015; 37(10): 219.

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