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EFFECT OF SODIUM NITROPRUSSIDE AND S-NITROSOGLUTATHIONE ON PIGMENT CONTENT AND ANTIOXIDANT SYSTEM OF TOCOPHEROL-DEFICIENT PLANTS OF *Arabidopsis thaliana*

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Sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO) were used as a source of exogenous nitric oxide (NO) to investigate their effects on biochemical parameters and antioxidant enzyme response in leaves of wild type Columbia and tocopherol-deficient vte4 and vte1 mutant lines of Arabidopsis thaliana plants and possible tocopherol involvement in regulation of antioxidant response under NO-induced stress. SNP enhanced the activity of the enzymes, that scavenge hydrogen peroxide in leaves of all studied lines, and increased glutathione reductase and glutathione-S-transferase activity there. In addition, it decreased the intensity of lipid peroxidation in vte1 mutant line leaves. At the same time, GSNO increased the levels of protein carbonyls and inactivated enzymes ascorbate peroxidase, guaiacol peroxidase and dehydroascorbate reductase in almost all investigated plant lines. In contrast to wild type, GSNO increased superoxide dismutase activity and decreased catalase activity and chlorophyll a/b ratio in the leaves of two mutant lines. It can be assumed that tocopherols in some way are responsible for plant protection against NO-induced stress. However the mechanisms of this protection remain unknown.

K e y w o r d s: sodium nitroprusside; S-nitrosoglutathione; nitric oxide; antioxidant enzymes; Arabidopsis thaliana; protein oxidation; lipid peroxidation; tocopherols.

Nitric oxide (NO) is a free radical with different biological functions in plants – either cytotoxic or cytoprotective [1]. The cytoprotection is partly based on NO ability to regulate the level and toxicity of reactive oxygen species (ROS) under oxidative stress conditions and terminate the propagation of lipid oxidation mediated by free radicals [2–5]. NO-mediated toxicity can be attributed to various derivatives of NO, collectively referred as reactive nitrogen species (RNS). The latter ones comprise not only the NO radical, nitroxyl (NO^-) and nitrosonium (NO^+) ions, but also peroxynitrite ($ONOO^{\bullet-}$), S-nitrosothiols, higher oxides of nitrogen and dinitrosyl-iron complexes [1, 5].

Previous studies demonstrated that the lipophilic antioxidant tocopherol represented by α-, β-, γ-, and δ-forms can scavenge membrane-soluble RNS [6, 7]. Both α-tocopherol and γ-tocopherol interact with nitric oxide, but the products formed during *in vitro* reaction are different. However, experiments *in vivo* showed that nitric oxide species directly interact with γ-tocopherol and that this reaction is more beneficial for the organism than the reaction of α-tocopherol with NO [7].

In most experiments NO donors are used for clarification of the biological NO role in living organisms. This investigation aimed to determine the effects of two NO-donors, SNP and GSNO, on biochemical parameters and antioxidant enzyme response of *Arabidopsis* plants and possible tocopherol involvement in the regulation of antioxidant response under NO-induced stress. We used wild type of *Arabidopsis thaliana* plants and tocopherol deficient *vte4* and *vte1* mutant lines of this plant. Wild type of *Arabidopsis* plants accumulates α-tocopherol only in its leaves. The *vte1* mutant is deficient in tocopherol cyclase activity. It does not synthesize tocopherols, but accumulates the redox-active tocopherol biosynthetic pathway intermediate 2,3-dimethyl-6-phytyl-1,4-benzoquinol (DMPBQ) at the level comparable to α-tocopherol in wild type plants [8]. The *vte4* mutant is defective in γ-tocopherol methyltransferase activity and devoid of α-tocopherol, but accumulates γ-tocopherol in leaves [9].

Materials and Methods

Seeds of wild type (Columbia) and mutant lines *vte4* (SALK_03676) and *vte1* (GABI_11D07) of *Arabidopsis thaliana* plants, defective in *vte4* and *vte1* genes, respectively, were obtained from the Salk Institute [10] and GABI-Kat [11] and selected homozygote plants from the seeds at the

Institute of Botany (Kiel, Germany) were used in present investigation. The plants were grown in hydroponic system at 23 ± 2 °C and daily light/dark regime of 16/8 h under low light intensity (15 μmol photons $m^{-2}s^{-1}$) as described in [12] with some modifications. The Gibeaut nutrient solution [13] was used and changed every two weeks. Leaves of basal rosette from 12-week-old plants were used for experiments. GSNO synthesis was performed as described in [14] and its concentration was determined spectrophotometrically at 335 nm (ε = 586 $M^{-1}cm^{-1}$). Leaves (1 g) were collected from wild type and mutant lines *vte1* and *vte4* of *A. thaliana* and immediately submerged into water (control) or in either of the following NO-donors, SNP (1 mM) and GSNO (1 mM) in the round-bottomed flasks with constant shaking at 150 rpm and temperature 20 °C. The 1 mM GSNO solution was adjusted to pH 5.7. Potassium hexacyanoferrate (II), $K_4[Fe(CN)_6]$, was used as an additional control. The flasks were illuminated by 18 W fluorescent light with intensity 3 W/m^2. The amount of nitric oxide released from 1 mM NO-donor solutions was determined by the Griess reaction, measuring the concentration of nitrite generated at spontaneous conversion of NO to NO_2^- [15]. Arabidopsis leaves were collected after 24 h exposure with the above reagents, briefly rinsed in water and frozen with liquid nitrogen.

Contents of chlorophylls, carotenoids and anthocyanins were measured spectrophotometrically in leaves as described in [16]. Tissues were homogenized in a Potter–Elvjeham glass homogenizer with ice-cold 96% ethanol (1:10, w/v) in the presence of $CaCO_3$ (for preventing of pheophytinization). The homogenates were centrifuged at 8000 g during 10 min (4 °C) using centrifuge OPN-8 (USSR), supernatants were collected and the pigments were repeatedly extracted two times from pellets with 1 ml of ice-cold 96% ethanol. All supernatants were collected and the concentrations of pigments were measured spectrophotometrically in the combined resulting extracts using specific absorption coefficients [16]. The content of chlorophylls and carotenoids was calculated as described in [17]. Anthocyanin content was determined after extract acidification with concentrated HCl to its resulting 1% concentration. The anthocyanin concentration was assayed spectrophotometrically at 530 nm wavelength and the absorption coefficient of 30 $mM^{-1}cm^{-1}$ was used [18].

To measure the level of carbonyl proteins, lipid peroxidation and activity of antioxidant enzymes the frozen leaves were powdered in liquid nitrogen with mortal and pestle and mixed (1/5, w/v) with 50 mM potassium-phosphate buffer (pH 7.0) that contained 1 mM ethylenediamine-tetraacetic acid (EDTA) and 1 mM phenylmethylsulfonylfluoride (PMSF). Ascorbic acid (1 mM) was added to potassium-phosphate buffer in the case of ascorbate peroxidase (APX) assay. The homogenates were centrifuged at 13,000 g for 20 min at 4 °C in Eppendorf 5415R (USA) centrifuge. The supernatant obtained from each sample was collected and used for further assay.

Supernatants were mixed with an equal aliquot of 40% (w/v) trichloroacetic acid (TCA) and then centrifuged for 10 min at 5000 g. The pellets were used for carbonyl proteins assay and the supernatants were used for determination of lipid peroxide level. The concentration of protein carbonyls (CP) was evaluated by reaction with 2,4-dinitrophenylhydrazine (DNPH) [19]. The degree of lipid peroxidation was evaluated as the level of thiobarbituric acid reactive substances (TBARS) as described in [20].

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed as a function of its inhibitory action on quercetin oxidation [21]. One unit of SOD activity is defined as the amount of enzyme (per protein milligram) that inhibits quercetin oxidation reaction by 50% of the maximum value, which was calculated using 'KINETICS' program for non-linear inhibition [22].

Catalase (EC 1.11.1.6) activity was measured spectrophotometrically at 240 nm [23]. The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was monitored following the decrease of absorbance at 290 nm (ε = 2.8 $mM^{-1}cm^{-1}$) due the oxidation of ascorbic acid to dehydroascorbate [24]. Guaiacol peroxidase (GuPx; EC 1.11.1.7) activity was assayed spectrophotometrically following the increase in absorbance at 470 nm due to guaiacol oxidation (ε = 26.6 $mM^{-1}cm^{-1}$) [25]. Dehydroascorbate reductase (DHAR; EC 1.8.5.1) activity was determined by measuring the increase in absorbance at 265 nm due to the formation of ascorbic acid (ε = 14 $mM^{-1}cm^{-1}$) [26]. Glutathione-S-transferase (GST; EC 2.5.1.18) activity was measured by monitoring the formation of adduct between GSH and 1-chloro-2,4-dinithrobenzene (CDNB) at 340 nm (ε = 9.6 $mM^{-1}cm^{-1}$) [21]. Glutathione reductase (GR; EC 1.6.4.2) activity was determined as the decrease in absorbance at 340 nm (ε = 6.22 $mM^{-1}cm^{-1}$) due to the oxidation of reduced NADPH [21].

One unit of CAT, APX, GuPx, DHAR, GR and GST activity is defined as the amount of the enzyme consuming 1 μmol of substrate or generating 1 μmol of product per minute; activities were expressed as international units (or milliunits) per milligram of protein.

Protein concentration was determined with Coomassie brilliant blue G-250 according to Brad-

ford's method [27] with bovine serum albumin as a standard.

All values were expressed as means ± S.E.M. of three independent experiments. For statistical analysis, one-way 'ANOVA' was used as appropriate. The Dunnett's test was used to compare values at all conditions with their corresponding control values. The Student's *t*-test was used to compare *vte4* and *vte1* mutant lines with the wild type.

Results and Discussion

Nitric oxide is highly reactive free radical which can modify different cellular components. In plants it also acts as an important inter- and intracellular signaling molecule involved in many plant physiological processes [1]. Therefore, for plants the mechanisms of NO detoxification as well as reactivity and signal functions of this molecule are required. Tocopherols (α-, β-, γ- and δ-tocopherols) are lipophylic antioxidants synthesized by photosynthetic organisms only [28]. It is known that α-tocopherol is the most widespread form in plant leaves and it possesses the highest antioxidant activity among natural tocopherols described to date. However, γ-tocopherol is a better nucleophile than α-tocopherol and may scavenge electrophiles with higher affinity compared to α-tocopherol [28]. Previous *in vitro* investigations revealed that γ-tocopherol has the capability to scavenge NO via nitration leading to the formation of 5-nitro-γ-tocopherol and this reaction could be crucial for NO detoxication. Desel and coauthors [7] showed the presence of 5-nitro-γ-tocopherol in the leaves of *Arabidopsis vte4* mutant line, confirming that nitration of γ-tocopherol occurs *in vivo*. Accordingly, the level of NO_x in the leaves of *vte4* was significantly lower than in the leaves of wild type and tocopherol-deficient *vte1* mutant [7].

This article aimed to investigate the capability of tocopherol composition to modulate physiological and antioxidant Arabidopsis response, induced by NO donors. Two different NO-donors SNP and GSNO were used. They release nitrosonium cation (NO^+) and NO radical, respectively [29]. In the present investigation, kinetics of NO release from SNP and GSNO in solutions was evaluated indirectly by measuring the amount of nitrite using the Griess method [15]. The NO-donors differed in the amount of NO_2^- released during the experiment (Fig. 1). Production of NO_2^- from SNP and GSNO increased within the time, and maximum NO_2^- concentrations were observed after 18 h of incubation under continuous illumination conditions in both NO-donor solutions. However, the concentration of NO_2^- produced at 1 mM GSNO decomposition was ~2-fold higher than that in

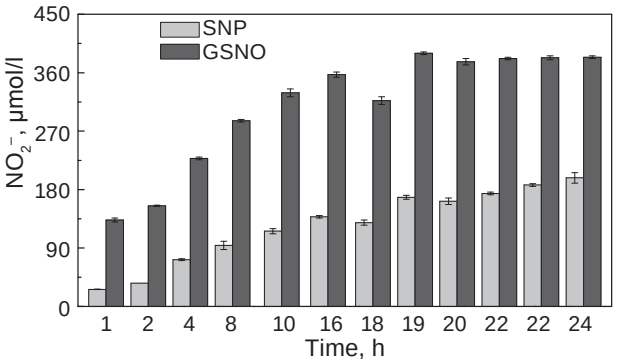

Fig. 1. The amount of NO_2^- released in 1 mM SNP and 1 mM GSNO solutions during 24 h (n = 3)

SNP solution (Fig. 1). Previous research showed that under comparable experimental conditions and donor concentrations, the rate of NO generation by these donors varied for tested compounds and was higher in GSNO solution [29, 30].

Chlorophyll (Chl) content and the Chl *a/b* ratio are fundamental parameters for determination of photosynthetic activity and these parameters are often used as indicators of stress in plants [16]. Table shows the concentration of total Chl in the leaves of wild type and tocopherol-deficient lines *vte4* and *vte1* of *A. thaliana* plants. Incubation of plant leaves with 1 mM SNP resulted in a 1.3-fold increase of total Chl concentration in the leaves of wild type and *vte4* mutant line plants, while in the leaves of *vte1* plants they did not change. SNP was found to enhance Chl content in different plant species [31, 32]. During decomposition, SNP may release other biologically active compounds together with NO. To evaluate the responsibility of NO for the increase of Chl concentrations in the leaves of plant lines, control experiments were carried out using potassium hexacyanoferrate (II), $K_4[Fe(CN)_6]$, since it has a chemical structure similar to SNP but lacks the ability to produce NO [33]. The concentrations of total Chl, as well as Chl *a/b* ratio did not change in the leaves of plants incubated for 24 h with 1 mM $K_4[Fe(CN)_6]$ compared to the control values (Table). Other NO-donor tested, GSNO, induced an increase of total Chl concentrations in the leaves of *vte4* mutant line plants. The Chl *a/b* ratio decreased 1.2-times in the leaves of both mutant lines incubated with GSNO (Table). Similarly, an increase of total Chl level and decrease of Chl *a/b* ratio were observed in *Brassica napus* leaves after SNP treatment [31]. The increase in Chl content might be related with activation of chlorophyll biosynthesis or/and its slow degradation [34] or involvement of NO in the iron metabolism of plants [32]. Concentrations of total carotenoids and anthocyanins are also given

Effect of NO-donors on pigment content (μmol/gww) in leaves of wild type, vte4, vte1 plants of A. thaliana

	Total chlorophyll	Chlorophyll *a/b*	Carotenoids	Anthocyanins
		Wild type		
H_2O	0.55 ± 0.05	1.65 ± 0.10	0.12 ± 0.01	0.27 ± 0.02
$K_4[Fe(CN)_6]$	0.61 ± 0.05	1.60 ± 0.08	0.12 ± 0.01	0.27 ± 0.02
SNP	0.74 ± 0.05*	1.63 ± 0.10	0.14 ± 0.01	0.30 ± 0.02
GSNO	0.67 ± 0.06	1.44 ± 0.10	0.10 ± 0.01	0.32 ± 0.03
		vte4		
H_2O	0.60 ± 0.05	1.85 ± 0.09	0.12 ± 0.01	0.25 ± 0.02
$K_4[Fe(CN)_6]$	0.67 ± 0.05	1.89 ± 0.08	0.14 ± 0.01	0.27 ± 0.02
SNP	0.80 ± 0.06*	2.20 ± 0.13*	0.19 ± 0.02*	0.33 ± 0.03*
GSNO	0.87 ± 0.08**	1.54 ± 0.09*	0.13 ± 0.02	0.38 ± 0.03**
		vte1		
H_2O	0.73 ± 0.03^{wt,vte4}	1.79 ± 0.08	0.16 ± 0.01^{wt,vte4}	0.31 ± 0.01^{wt,vte4}
$K_4[Fe(CN)_6]$	0.84 ± 0.07	1.63 ± 0.11	0.16 ± 0.02	0.38 ± 0.02
SNP	0.72 ± 0.08	1.71 ± 0.10	0.15 ± 0.02	0.31 ± 0.03
GSNO	0.70 ± 0.06	1.49 ± 0.06**	0.14 ± 0.01	0.34 ± 0.03

*Significantly different from the respective control group (H_2O) with $P < 0.05$, **$P < 0.01$. ^{wt}Significantly different from respective group of wild type plants, ^{vte4} *vte4* mutant line ($P < 0.05$)

in Table. SNP treatment resulted in the 1.6-fold increase of carotenoid concentration only in the leaves of *vte4* mutant line. Incubation with two NO-donors led to an increase of anthocyanin content only in the leaves of *vte4* mutant line plants.

Nitric oxide reacts readily with the superoxide anion, that resulting in peroxynitrite ion formation. Peroxinitrite caused a variety of protein modifications, including cysteine and tryptophan oxidation, tyrosine nitration and formation of protein carbonyls [35]. After exposure of the plants with SNP a 30% decrease of CP concentration was observed only in the leaves of wild type plants (Fig. 2, *A*). However, virtually the same result was observed in the leaves incubated with potassium hexacyanoferrate (II), suggesting that this effect could be caused by other compounds, besides NO. In response to GSNO treatment, the level of CP in the leaves of wild type, *vte4* and *vte1* mutant lines of *A. thaliana* increased by 39, 43 and 180%, respectively (Fig. 2, *A*). The highest, 3-fold increase of CP concentration was observed in the leaves of *vte4* mutant line plants, lacking in α-tocopherol synthesis.

Along with protein oxidation we measured the intensity of lipid peroxidation in the leaves of *Arabidopsis* plants. The decomposition of lipid hydroperoxides produces low-molecular mass products, including malondialdehyde (MDA). In this work the product of MDA condensation with thiobarbituric acid (TBA) was measured as thiobarbituric acid reactive substance (TBARS) [36]. SNP treatment did not change TBARS concentration in the wild type and *vte4* mutant plants (Fig. 2, *B*). Incubation of leaves with 1 mM GSNO decreased the level of TBARS by 24 and 53% in the leaves of wild type and *vte*4 mutant line plants, respectively. Both NO-donors caused 15% decrease of TBARS content in the leaves of *vte1* plants. In contrast to enhanced protein oxidation, the level of TBARS content decreased in the leaves of all plant lines treated with GSNO. A protective role of NO against lipid peroxidation was previously reported by many researcher [2–5]. Nitric oxide can affect lipid peroxidation due to interaction with lipid alcoxyl ($LO^{\cdot}$) and peroxyl ($LOO^{\cdot}$) radicals [1]. It is possible, that the increased of protein carbonyl content could be associated with the action of NO-derived species, such as $ONOO^{\cdot-}$, whereas a decrease of TBARS content could result from NO action. In the leaves of *vte1* mutant line both, SNP and GSNO, suppressed the level of TBARS content, but to smaller extent than GSNO in the plants of the other line and wild type plants. Tocopherols are key antioxidants, that protect the polyunsaturated fatty acids from lipid peroxidation [28]. The *vte1* mutant plants do not synthesize tocopherols which probably resulted in

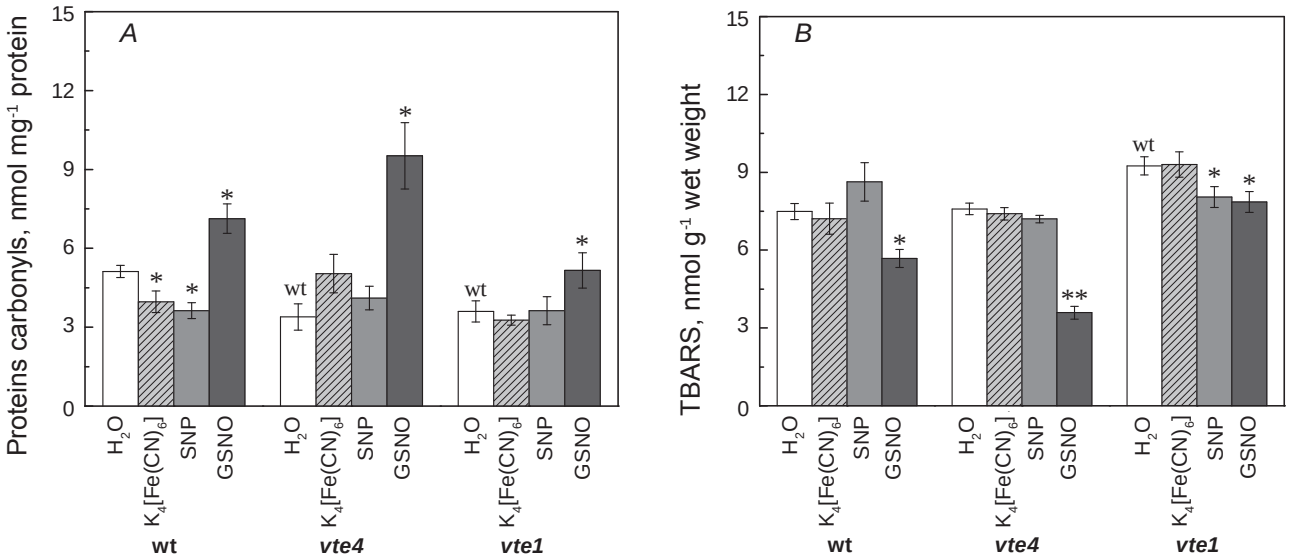

*Fig. 2. Protein carbonyl (A) and thiobarbituric acid reactive substances content (B) in leaves of wild type, vte4 and vte1 plants of A. thaliana after 24 h treatment with 1 mM SNP or 1 mM GSNO *Significantly different from respective control group (H_2O) with $P < 0.01$, **$P < 0.05$. ^{wt}Significantly different from respective group of wild type plants ($P < 0.05$)*

the highest TBARS content under control conditions. A significant decrease in TBARS concentration was observed in *vte1* mutant after SNP and GSNO treatment. These results suggest possible ROS scavenging by NO in the studied plants. Under conditions of ROS-related toxicity, NO can play the role in a cell as a limiting factor of the chain reaction of lipid peroxidation and thus limit oxidative damage. Previously it was reported that α-tocopherol and NO can act cooperatively to inhibit the processes of lipid peroxidation [37]. This can explain only 15% of decrease of TBARS content in the leaves of mutant plants of *vte1* line, which lacks all tocopherols.

It was found that NO promoted alleviation of oxidative stress which was associated with induction of activity of various ROS-scavenging enzymes [38]. Superoxide dismutase, an important primary antioxidant enzyme, catalyzing superoxide radical dismutation to H_2O_2, was found in different compartments of plant cells [39]. In our experiments the incubation of leaves with SNP, as well as potassium hexacyanoferrate (II), did not affect the SOD activity in leaves of all investigated plants (Fig. 3, *A*). However, GSNO treatment increased this enzyme activity by 50% in the leaves of *vte4* and *vte1* mutants lines of *Arabidopsis* plants, whereas in the wild type leaves the only tendency of the increase of SOD activity was found. In several previous investigations it was suggested to regard the nitric oxide as an inductor of high SOD activity in plants [2, 3, 40, 41]. The induction of SOD activity in the leaves of both mutant plants of Arabidopsis in response to GSNO treatment may reflect its important role in the defense mechanisms of these plants.

Catalase, APX and GuPx are important H_2O_2 scavenging enzymes in plants [39]. The exposure to SNP led to increase of catalase activity in the leaves of wild type, *vte4* and *vte1* mutant lines of *A. thaliana* plants by 55, 39 and 41%, respectively (Fig. 3, *B*). No changes in catalase activity were observed in leaves of plants of all lines incubated with potassium hexacyanoferrate (II). At the same time, GSNO suppressed catalase activity by 30% in leaves of *vte4* and *vte1* mutants. Similar tendencies were observed in APX activity. It increased by 80, 25 and 33% in the leaves of wild type, *vte4* and *vte1* plants, exposed to SNP action (Fig. 3, *C*). However, $K_4[Fe(CN)_6]$ enhanced APX activity in the leaves of wild type plants also, whereas this effect was not observed in leaves of mutant plants. GSNO treatment induced the decrease of APX activity by 46, 77 and 40% in the leaves of wild type, *vte4* and *vte1* plants, respectively (Fig. 3, *C*). Under the same treatment, GuPx activity increased by 97 and 33% in the leaves of wild type plants and *vte1* mutants, respectively, whereas in *vte4* mutant it decreased by 27% (Fig. 3, *D*). However, GuPx activity was enhanced in the wild type leaves after potassium hexacyanoferrate (II) treatment as compared to control values. Incubation with GSNO resulted in 45, 75 and 51% decrease of GuPx activity in leaves of wild type, *vte4* and *vte1*

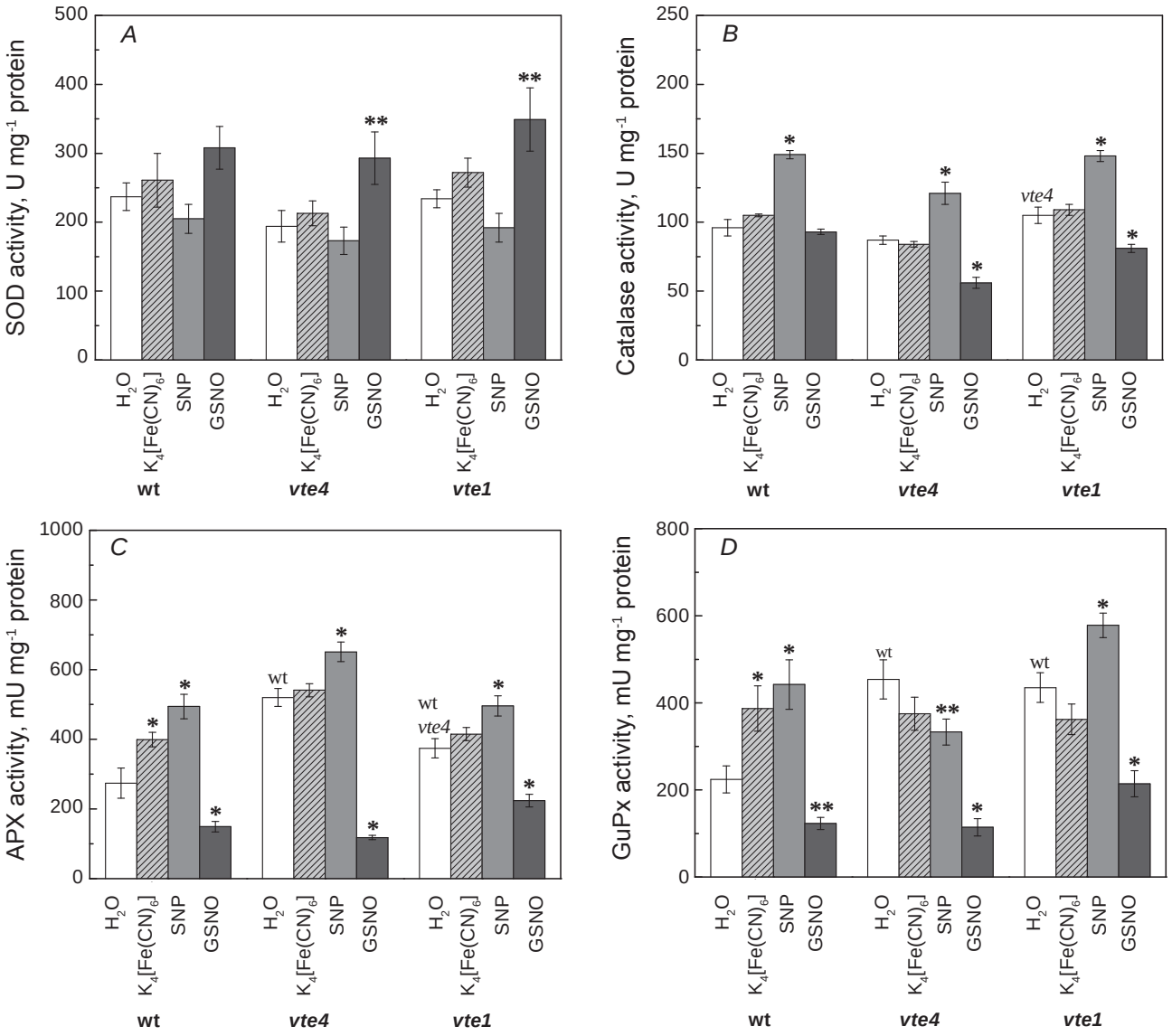

*Fig. 3. Activity of superoxide dismutase (A), catalase (B), ascorbate peroxidase (C) and guaiacole peroxidase (D) in leaves of wild type, vte4 and vte1 plants of A. thaliana after 24 h treatment with 1 mM SNP or 1 mM GSNO. *Significantly different from respective control group (H_2O) with $P < 0.01$, **$P<0.05$. ^{wt}Significantly different from respective group of wild type plants, ^{vte4} vte4 mutant line ($P < 0.05$)*

plants, respectively. Some authors supposed that NO could increase the activity of antioxidant enzymes by stimulation of H_2O_2 producing system(s) [42]. Similar increase in catalase, APX and peroxidase activity was observed in SNP-treated leaves of *Stylosanthes guianensis* [41] and *Zea mays* [4] plants as well as in adventitious roots of *Panax ginseng* plants [2, 3]. The opposite effect was observed in the activity of these enzymes in the leaves of plants treated with GSNO. This NO-donor drastically decreased the activity of catalase, APX and GuPx in all investigated plants, excepting catalase activity in the wild type plants. It can be assumed that such effects were mediated by different concentrations of NO released by SNP and GSNO (Fig. 1). For example, under lower concentrations of NO peroxidase activity increased in the leaves of Brassica plants, whereas under higher concentrations of NO this activity was decreased [43]. In addition, pure NO (55 μM) inhibited peroxidase in the xylem of *Zinnia elegans* plants [44], as well as GSNO inhibited catalase and APX in *Nicotiana tabacum* plants [45]. The inhibition could result from NO binding to prosthetic heme group of peroxidases and heme nitrosylation which, in turn, prevented H_2O_2 interaction with active centre [45]. Clarck and colleagues [45] suggested that the inhibition of APX by GSNO was mediated by peroxynitrite

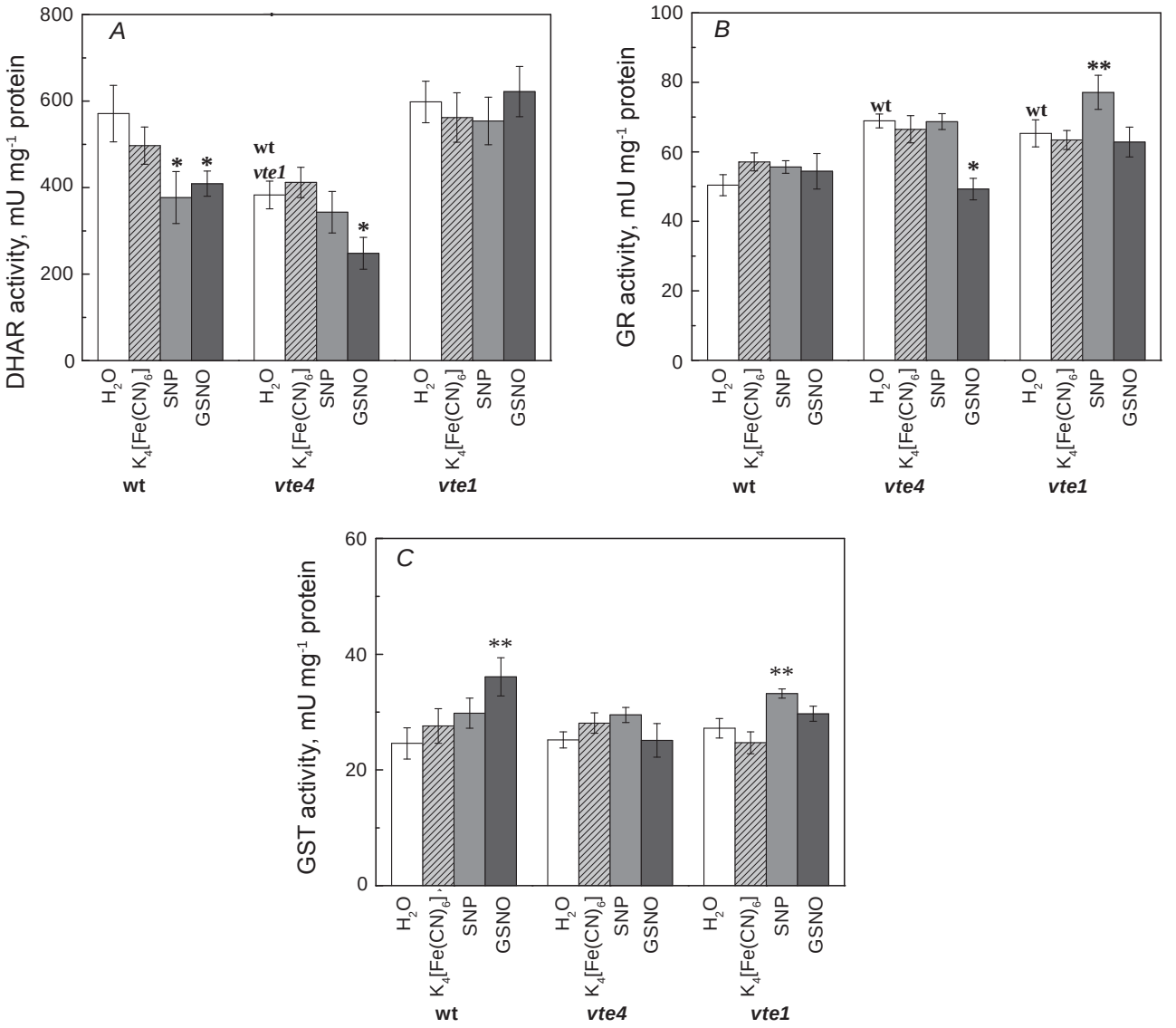

*Fig. 4. Activity of dehydroascorbate reductase (A), glutathione reductase (B) and glutathione-S-transferase (C) in leaves of wild type, vte4 and vte1 plants of A. thaliana after 24 h treatment with 1 mM SNP or 1 mM GSNO. *Significantly different from respective control group (H_2O) with $P < 0.01$, **$P<0.05$. ^{wt}Significantly different from respective group of wild type plants, ^{vte1} vte1 mutant line ($P < 0.05$)*

rather than by NO. We did not observe any specific response of peroxidases to NO-donors in tocopherol deficient *vte1* and *vte4* lines, except for GuPx activity in the leaves of *vte4* mutants. The activity of this enzyme decreased in the leaves of *vte4* mutant after treatment of plants by NO-donors.

An ascorbate-glutathione cycle is the most important H_2O_2-detoxifying system in plant chloroplasts [39], which operates also in cytosol, peroxisomes, and mitochondria. The enzymes of the ascorbate-glutathione cycle DHAR and GR play an essential role in plant tolerance to the action of various biotic and abiotic stresses by sustaining of reduced status of ascorbate and glutathione, respectively [39]. Both NO-donors used in this work led to ~30% decrease in DHAR activity and did not change GR activity in the leaves of wild type plants (Fig. 4, *A*, *B*). In leaves of *vte4* mutant plants we observed the decrease of DHAR activity by 35% and decrease of GR activity by 28% after GSNO treatment. These two enzymes are thiol-containing and could be inactivated by oxidants via the oxidation of their thiol groups [46, 47]. Moreover, it is considered, that sulfhydryl oxidation may be a major mechanism of NO action [47]. In *vte1* plants the GR activity increased by 14% after SNP treatment as compared to respective controls (Fig. 4, *B*). Similarly, an increase in GR activity was ob-

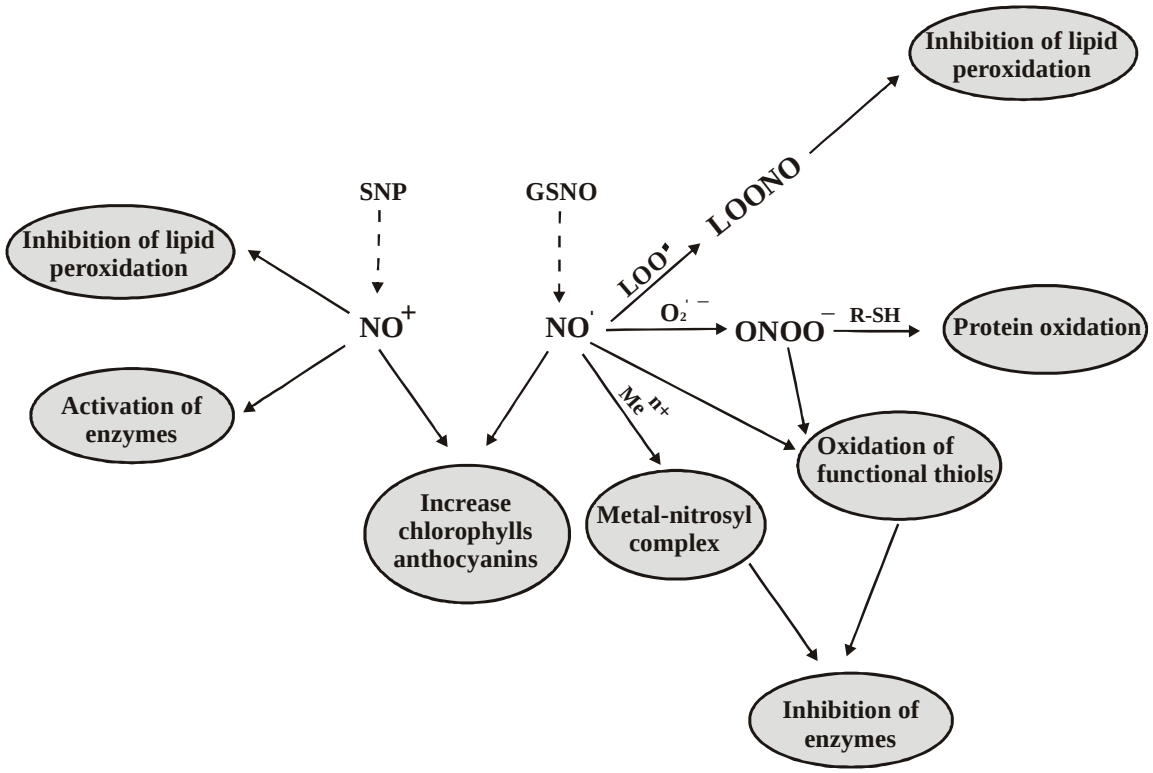

Fig. 5. Possible action of NO-donors in plants

served also in the adventitious roots of mountain ginseng [2, 3] and in the leaves of citrus plants [48] and in the leaves of pelargonium [5] under SNP treatment. Potassium hexacyanoferrate (II) did not change GR activity in *vte1* plants. In the leaves of *vte1* mutant both NO-donors did not cause any changes in DHAR activity (Fig. 4, *A*).

Plant glutathione-S-transferases are antioxidant enzymes of the second line of defense and they have the potential capability to remove cytotoxic/genotoxic compounds, which can damage the DNA, RNA and proteins [39]. In response to SNP exposure, GST activity increased only in the leaves of *vte1* mutant plants by 22%, whereas no change in GST activity was observed under $K_4[Fe(CN)_6]$ treatment (Fig. 4, *C*). In wild type plants GST activity increased by 47% under conditions of GSNO incubation. In contrast, Arasimowicz-Jelonek and colleagues [5] found that both SNP and GSNO caused inhibition of GST.

It can be concluded, that the action of two NO-donors caused different or even opposite effects on physiological and antioxidant parameters of investigated plants that can reflect the dual role of NO (Fig. 5). This dual role of NO may depend on its concentration- and time-course release by the used NO-donors and/or components released at their decomposition. SNP released less NO than GSNO and induced H_2O_2-scavenging enzymes in plants of all studied lines, as well as increased GR and GST activity and decreased TBARS content in the leaves of *vte1* mutant. At the same time, GSNO induced the increase of protein carbonyl content and inactivated APX, GuPx and DHAR enzymes in almost all Arabidopsis lines in our experiments. In contrast to wild type, in the leaves of plants of both mutant lines GSNO increased SOD activity and decreased catalase activity. Finally, GSNO decreased Chl *a/b* ratio in the leaves of plants of both mutant lines. We can suggest that tocopherols in some way are involved in plant protection against NO-induced stress, but the molecular mechanisms of above mentioned effects need to be elucidated.

ВПЛИВ НІТРОПРУСИДУ НАТРІЮ ТА S-НІТРОЗОГЛУТАТІОНУ НА ВМІСТ ПІГМЕНТІВ І АНТИОКСИДАНТНУ СИСТЕМУ ДЕФЕКТНИХ ЗА ТОКОФЕРОЛОМ РОСЛИН *Arabidopsis thaliana*

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Нітропрусид натрію та S-нітрозоглутатіон були використані як джерела екзогенного оксиду азоту (NO) для вивчення його впливу на біохімічні параметри та активність антиоксидантних ензимів у листках рослин *Arabidopsis thaliana* дикого типу та дефектних за біосинтезом токоферолу ліній *vte4* та *vte1*, а також можливу участь токоферолу в регуляції антиоксидантної відповіді за стресу, зумовленому NO. Обробка рослин нітропрусидом натрію підвищує активність ензимів, які знешкоджують пероксид водню в усіх досліджуваних ліній, збільшує активність глутатіонредуктази та глутатіон-S-трансферази та зменшує інтенсивність пероксидного окислення ліпідів у мутантної лінії *vte1.* Обробка рослин S-нітрозоглутатіоном призводить до зростання вмісту карбонільних груп протеїнів та інактивації аскорбатпероксидази, гваяколпероксидази та дегідроаскорбатредуктази в листках рослин усіх досліджуваних ліній. На відміну від рослин дикого типу, у мутантних ліній S-нітрозоглутатіон підвищує активність супероксиддисмутази та знижує – каталази та співвідношення хлорофілу *a/b.* Останнє може свідчити про те, що токоферол якимось чином задіяний у захисті рослин від стресу, індукованого NO, але механізм цього процесу ще належить з'ясувати.

Ключові слова: нітропрусид натрію, S-нітрозоглутатіон, оксид азоту,антиоксидантні ензими, *Arabidopsis thaliana*, окислення протеїнів, пероксидне окислення ліпідів, токофероли.

ВЛИЯНИЕ НИТРОПРУССИДА НАТРИЯ И S-НИТРОЗОГЛУТАТИОНА НА СОДЕРЖАНИЕ ПИГМЕНТОВ И АНТИОКСИДАНТНУЮ СИСТЕМУ ДЕФЕКТНЫХ ПО ТОКОФЕРОЛУ РАСТЕНИЙ *Arabidopsis thaliana*

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Нитропруссид натрия и S-нитрозоглутатион были использованы в качестве источников экзогенного оксида азота (NO) для изучения его влияния на биохимические параметры и активность антиоксидантных энзимов у растений *Arabidopsis thaliana* дикого типа и дефектных по биосинтезу токоферола линий *vte4* и *vte1*, а также возможное включение токоферола в регуляцию антиоксидантного ответа растений при стрессе, обусловленном NO. Обработка растений нитропруссидом натрия повышает активность энзимов, которые обезвреживают пероксид водорода в растениях всех исследуемых линий, увеличивает активность глутатионредуктазы и глутатион-S-трансферазы и уменьшает интенсивность пероксидного окисления липидов в растениях мутантной линии *vte1.* Обработка растений S-нитрозоглутатионом приводит к повышению содержания карбонильных групп протеинов и инактивации аскорбатпероксидазы, гваяколпероксидазы и дегидроаскорбатредуктазы во всех исследуемых линиях растений. В отличие от растений дикого типа, у мутантных линий S-нитрозоглутатион повышает активность супероксиддисмутазы и снижает – каталазы и соотношение хлорофилла *a/b* в листьях. Этот факт может свидетельствовать о том, что токоферол каким-то образом задействован в защите растений от NO-индуцированного стресса, но механизм этого процесса еще предстоит выяснить.

Ключевые слова: нитропруссид натрия, S-нитрозоглутатион, оксид азота, антиокислительные энзимы, *Arabidopsis thaliana,* окисление протеинов, пероксидное окисление липидов, токоферолы.

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