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EFFECT OF SODIUM CHLORIDE AND NITROPRUSSIDE ON PROTEIN CARBONYL GROUPS CONTENT AND ANTIOXIDANT ENZYME ACTIVITY IN LEAVES OF CORN SEEDLINGS *Zea mays* L.

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The effect of sodium nitroprusside (SNP) and sodium chloride (NaCl) on protein carbonyl group content and activity of antioxidant enzymes was investigated in leaves of maize seedlings. Incubation with NaCl and SNP+NaCl increased the content of carbonyl proteins after 24 h. Treatment with SNP+NaCl during 48 h showed lower and after 72 h higher carbonyl protein content than that in the control. Catalase activity was higher in the leaves of SNP+NaCl-treated than in the leaves of SNP-treated seedlings after 24 h. Ascorbate peroxidase activity increased after incubation with 0.2 mM SNP for 24 h. Significant increment of guaiacol peroxidase activity was obtained in all treated groups in comparison with the control after 72 h. Glutathione-S-transferase activity increased after 48 h seedling treatment with NaCl or SNP and 72 h seedling incubation with NaCl. Under experimental conditions used, glutathione reductase activity was virtually not affected. It is proposed that SNP can be used to prevent salt-induced oxidative stress in maize.

Key words: corn seedlings, salt stress, sodium nitroprusside, oxidative stress, antioxidant enzymes.

Higher plants growing in natural environments experience various abiotic stresses. Soil salinization due to global climate changes and human agricultural activity is an increasing environmental problem affecting crop production worldwide. Up to 20% of the world's arable lands and about 50% of irrigated lands are already adversely affected by salinity. Continuous accumulation of salt in cultivated soils resulting from human activity increases the significance of this stressful factor and calls for development of approaches to solve this problem [1]. Ideally, these approaches should be based on knowledge of plant physiology and exploitation of their natural potential to adapt to high salinity either naturally or artificially.

Nitric oxide (NO) is an important signalling molecule in plants participating in transduction of hormone signals either alone or jointly with reactive oxygen species (ROS) [2]. Besides, NO could significantly enhance antioxidant capacity by increasing the activity of catalase, ascorbate peroxidase and accumulating proline, in wheat seedlings under aluminium stress [3]. More recently, NO was found to reduce aluminium toxicity by preventing development of oxidative stress in *Cassia tora* roots [4]. The pre-treatment of roots of citrus plants with nitric oxide increased the activity of leaf superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase [1]. Sodium nitroprusside (SNP), commonly used NO donor, completely prevented leaf chlorosis under iron deficiency and increased chlorophyll content in the leaves [5].

The effects of salt stress on plants have both osmotic (cell dehydration) and direct toxic (ion accumulation) components at the whole organism and leaf levels [6]. Salinity also reduces the supply of CO_2 to leaves, and further depresses already low CO_2/O_2 in chloroplasts [7]. Although the underlying signalling function of NO has long been masked by its inherent toxic nature [8], the NO-triggering defence response during environmental stress now is widely recognized [9–13]. In addition, there is the substantial evidence of NO involvement in redox-mediated priming, in which transient pre-exposure to NO can increase tolerance against the subsequent more severe stress [14, 15]. Particularly, Gossett et al. [16–18] and Hernandez et al. [19] have demonstrated that the salt stress elicits an oxidative response in plants, and cotton cultivars, and the cell lines with elevated levels of antioxidant enzymes, either constitutive or induced, have been shown to exhibit greater tolerance to NaCl stress. Valderrama et al. [10] showed that salinity may also be accompanied by enhanced production of NO and other reactive nitrogen species leading to nitrosative stress.

Recent data by Zhou et al. [20] indicated that NO serves as a signal in inducing salt tolerance

by increasing the potassium (K^+) to sodium (Na^+) ratio through the increased expression of PM H^+-ATPase activity in two ecotypes of common reed. In cotton calli, the NaCl-induced stress was accompanied by oxidative stress through the increase in superoxide anion production which may serve as an early signal molecule [21] for the upregulation of activity of antioxidant enzymes [16–18]. Nitric oxide suppressed oxidative damage in the roots of wheat seedlings under the salt stress [22].

In this work we hypothesized that NO-donor sodium nitroprusside might improve plant adaptation under normal physiological and stress conditions. For this purposes we investigated the effects of SNP in the control conditions and under salt stress induced by sodium chloride in seedlings of maize *Zea mays* L. The latter one was used in concentration of 100 mM which is rather high for this salt-sensitive plant, but we expected this approach might disclose SNP potential to prevent deleterious salt effects. Since it is known that the salt stress induces secondary oxidative stress, we carried out the investigation of some parameters of this stress as well as the activity of antioxidant and associated enzymes.

Materials and Methods

Plants and reagents. Seeds of maize hybrid Kharkivskiy 195 MB (*Zea mays* L.) were used for the experiments. The chemicals were obtained from Sigma (USA), Fluka (Germany) and Reakhim (Russia). Other chemicals were of the highest purity available.

Plant material and growth conditions. Maize seeds were allowed to soak for 24 h in tap water at 25 °C and then germinated under a wet cloth for 3 days. The germinated seeds were transferred in Hoagland solution [23] and grown for 5 days at 6700 lux of light intensity, 16/8 h day/night regime and temperature 26 °C.

Treatment with sodium nitroprusside and sodium chloride. Ten-day-old maize seedlings were treated with 0.2 mM sodium nitroprusside ($Na_2[Fe(CN)_5NO]$), 100 mM sodium chloride (NaCl) or their combination in Hoagland solution. Sodium chloride in concentration of 100 mM was used as described previously by Kurylenko and Palladina [24]. Solutions were changed every 24 h. The plants were incubated for 24, 48 and 72 h.

Enzyme activity assay. Leaves of maize seedlings were ground in liquid nitrogen and stored in liquid nitrogen until use. Leaves powder was supplemented 1 : 10 (w/v) with 50 mM potassium-phosphate (KPi) buffer (pH 7.0) containing 0.5 mM EDTA and 1.0 mM phenylmethylsulfonylfluoride. Ascorbic acid (1 mM) was added to KPi buffer in the case of ascorbate peroxidase (APX) assay. The homogenates were centrifuged at 13,200 g for 15 min at 4 °C. The resulted supernatants were used for the experiments. The activity of catalase (1.11.1.6) was measured spectrophotometrically at 240 nm [25]. Guaiacol peroxidase (GuPX, 1.11.1.7) activity was assayed spectrophotometrically following the increase in absorbance at 470 nm wavelength due to guaiacol oxidation (ε = 26,600 $M^{-1}cm^{-1}$) [26]. Ascorbate peroxidase (APX; 1.11.1.11) activity was measured spectrophotometrically following the decrease of absorbance at 290 nm (ε = 2.80 $M^{-1}cm^{-1}$) [26]. Glutathione reductase (GR; 1.6.4.2) activity was assayed spectrophotometrically following the decrease in absorbance at 340 nm (ε = 6.22 $mM^{-1}cm^{-1}$) due to oxidation of NADPH, according to Halliwell and Foyer [27]. Glutathione-S-transferase (GST, 2.5.1.18) activity was measured by monitoring the formation of adduct between glutathione (GSH) and 1-chloro-2,4-dinitrobenzene at 340 nm (ε = 9.6 $mM^{-1}cm^{-1}$) [28].

Measurement of protein carbonyl level. The content of protein carbonyl groups (CP) was evaluated with 2,4-dinitrophenylhydrazine according to the method described previously [29] and modified by Lushchak et al. [30].

Protein measurements and statistics. The protein content was determined according to Bradford method with Coomassie Brilliant Blue R-250 [31] with bovine serum albumin as a standard. Experimental data are expressed as mean ± SEM, and statistical testing used the Student *t*-test for comparison of two means or ANOVA followed by post-hoc Dunnet's test to compare several experimental groups against a single control.

Results and Discussion

Levels of protein carbonyl groups. The formation of additional protein carbonyl groups (CP), resulting from oxidative modification of arginine, cysteine, proline, lysine, histidine and other amino acid residues, is widely used and is a very popular marker of oxidative stress [32, 33]. Under maize seedling exposure for 24 h to 100 mM NaCl, 58% increase in leaf CP content was found (Fig. 1). Similar results were obtained with citrus plants after 16 h treatment with 150 mM NaCl [1]. However, in our work at this time 0.2 mM SNP virtually did not affect CP content, but when we applied jointly SNP and NaCl, the CP level was higher than that in the control plants. At the first stage of incubation, i.e. at 24 h, SNP application partially protected maize seedlings against salt-induced stress.

No significant changes in CP leaf content were found in seedlings incubated for 48 and 72 h

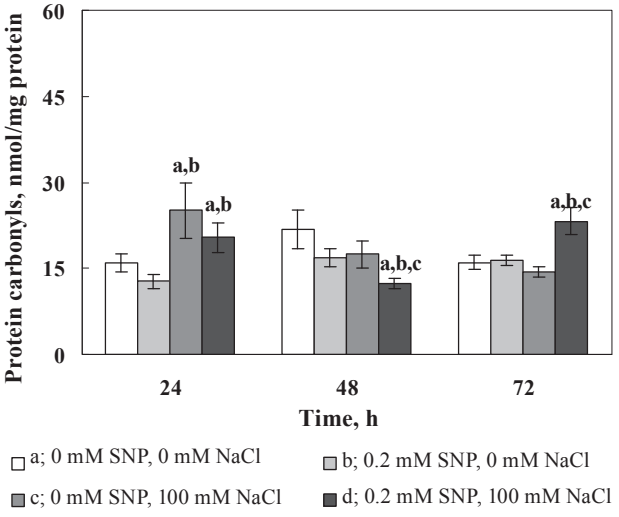

Fig. 1. Effects of 0.2 mM SNP and 100 mM NaCl and their combination on the content of carbonyl proteins in maize leaves for 24, 48 and 72 h. Data are means ± S.E.M (n = 3). [a,b,c] Significantly different as compared to a, b and c groups respectively (P < 0.05)

with 0.2 mM SNP and 100 mM NaCl alone or in combination (Fig. 1). But when the plants were affected by the combination of these two chemicals, a 43% decrease at 48 h exposure and 44% increase at 72 h one were found in the leaf CP content. That seems somewhat strange that at these two exposures the effects were opposite and at the moment we cannot provide any reasonable explanation of this phenomenon. It is clear that on the first day of maize seedling treatment, at the level of leaf CP content, SNP showed protective effect against NaCl-induced ROS-promoted oxidation of proteins. At further exposure the picture looks uncertain and would probably need additional investigation.

Hydrogen peroxide-detoxifying enzymes. Hydrogen peroxide-detoxifying enzymes form the first line of high molecular mass antioxidants. Catalases and peroxidases such as ascorbate peroxidase and guaiacol peroxidase are supposed to be primary H_2O_2-scavenging enzymes in plants [34].

In our experiments, after 24 h exposure of maize seedlings we observed only 43% higher catalase activity in the leaves of SNP+NaCl-treated ones than in those of SNP-treated maize seedlings (Fig. 2, *A*). Earlier Vital et al. [35] showed that the addition of 0.2 mM SNP to 150 mM NaCl incubation media increased significantly the catalase activity compared to 150 mM NaCl in cotton calli after 2 h exposure. The longer treatment of maize seedlings for 48 and 72 h did not result in leaf catalase activity changes. Our results do

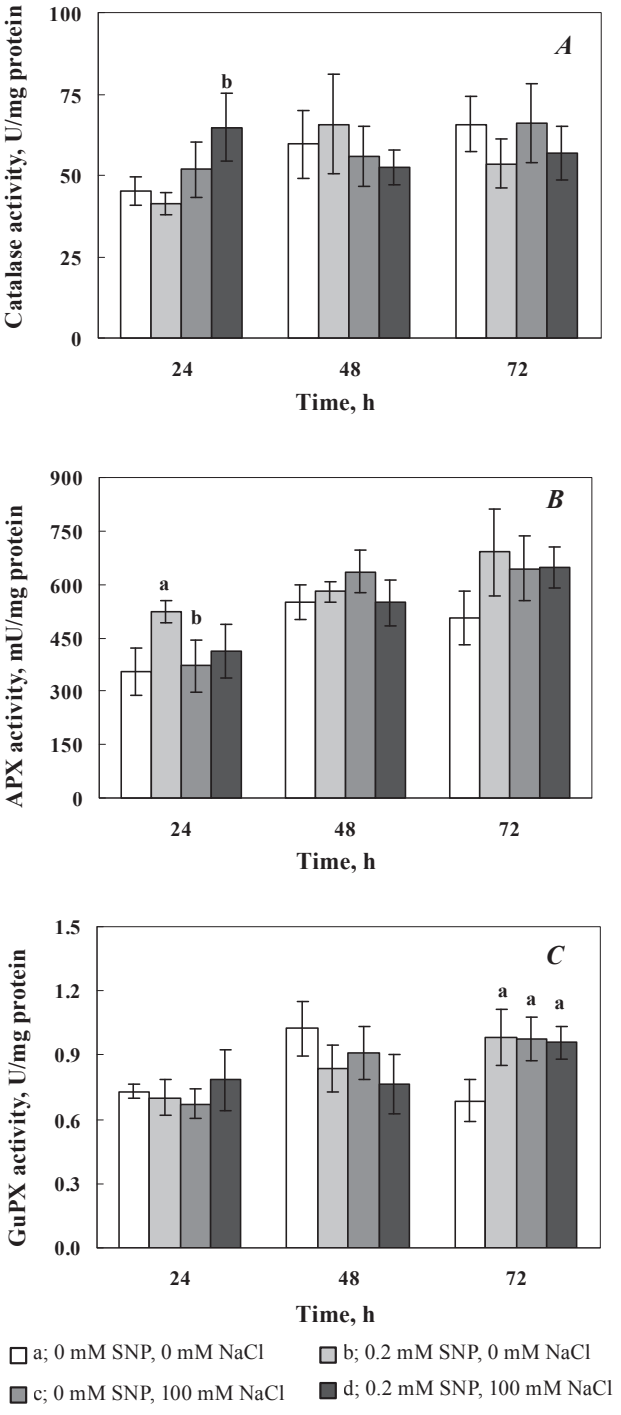

Fig. 2. Effects of 0.2 mM SNP and 100 mM NaCl on the activity of catalase (A), ascorbate peroxidase, APX (B) and guaiacol peroxidase, GuPx (C) in maize leaves for 24, 48 and 72 h. Data are means ± S.E.M (n = 3). [a,b] Significantly different as compared to a and b groups respectively (P < 0.05)

not correspond to ones received by Tanou et al. [1] in citrus plants. They showed that treatment with 100 mM SNP increased the catalase activi-

ty, treatment with 150 mM NaCl decreased the catalase activity and their combination decreased the enzyme activity as compared to control after 16-days exposure. Kurylenko and Palladina [36] showed that 100 mM NaCl decreased the catalase activity after 3-days exposure. Interestingly, maize seedling incubation with 0.2 mM SNP for 24 h increased the leaf ascorbate peroxidase (APX) activity by 43%, but neither NaCl-treated groups, nor those incubated jointly with NaCl and SNP were different from the control one (Fig. 2, *B*). Further seedling incubation with the chemicals alone or in combination did not show any significant differences. Tian and Lei [37] showed that wheat seedling treatment with 0.2 mM SNP during 7-days exposure activated antioxidant enzymes under drought, UV-B radiation, and combined stresses.

Peroxidases, particularly, guaiacol peroxidase are very important players in the antioxidant system. In our hands, maize seedling exposure for 24 and 48 h to either 0.2 mM SNP or 100 mM NaCl as well as their combination did not affect leaf GuPX activity (Fig. 2, *C*). However, the extension of exposure time to 72 h increased significantly the leaf GuPX activity in all treated groups in comparison with the control, but there was no significant difference between them. Results of Vital et al. [35] showed that in cotton calli an addition of 0.2 mM SNP to 150 mM NaCl significantly increased GuPX activity as compared to 150 mM NaCl after 2 h exposure.

Glutathione-related enzymes. Glutathione and glutathione-related enzymes are actively involved in maintaining the redox status in plants [38]. At 24 h maize seedling exposure to SNP, NaCl or their combination did not affect the activity of glutathione-S-transferase in maize leaves (Fig. 3, *A*). However, a significant increase (53%) of the activity was found after 48 h seedling treatment with 100 mM NaCl and 0.2 mM SNP, whereas both these components alone did not affect the activity. At 72 h seedling incubation with 100 mM NaCl resulted in 59% higher GST leaf activity than that in the control group, which was cancelled by simultaneous incubation with NaCl and SNP. Previous studies demonstrated that nitric oxide stimulated GST gene expression in soybean plants exposed to pathogens [39].

Under experimental conditions used, glutathione reductase activity of maize leaf seedlings was almost unaffected (Fig. 3, *B*). The exceptions were only 28% difference at 24 h exposure between leaf GR activity in SNP and SNP+NaCl-treated seedlings while in the latter case it was higher, and at 72 h exposure the salt-treated seedlings demonstrated 38% higher activity than those of the control. The latter effects were reversed by SNP

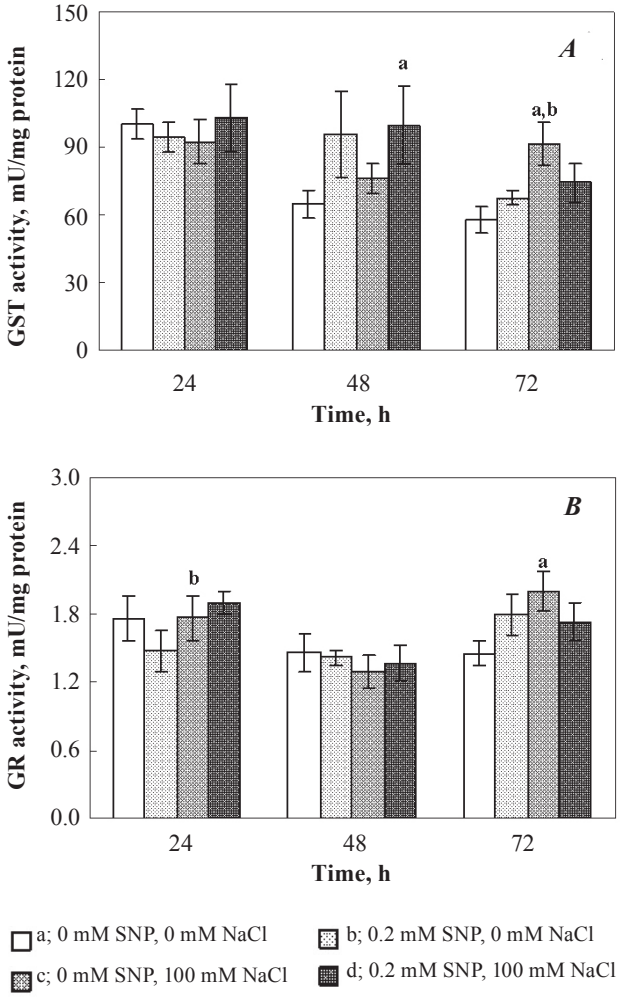

Fig. 3. Effects of 0.2 mM SNP and 100 mM NaCl and their combination on the activity of glutathione-S-transferase, GST (A) and glutathione reductase, GR (B) in maize leaves for 24, 48 and 72 h. Data are means ± S.E.M (n = 3). [a,b] Significantly different as compared to a and b groups respectively (P < 0.05)

addition to NaCl. Similar results were obtained by Vital et al. [35]. They demonstrated that addition of 0.2 mM SNP to 150 mM NaCl significantly decreased GR activity in comparison with treatment by NaCl only during 48 h in cotton calli. Tanou et al. [1] showed that both 100 μM SNP and 150 mM NaCl increased GR activity in the leaves of citrus plants after 48 h of exposure in comparison with the control.

The results obtained in this work let us to propose that either sodium nitroprusside or some of products of its decomposition increased the antioxidant capacity of *Zea mays* L. seedlings under salt-induced stress. That can enhance total plant resistance to this and may be to other stresses because the oxidative stress commonly accompanies

any substantial stress directly or secondarily. Since SNP is a commonly used donor of nitric oxide, the latter could be responsible for the described effects.

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ВПЛИВ НІТРОПРУСИДУ ТА ХЛОРИДУ НАТРІЮ НА ВМІСТ КАРБОНІЛЬНИХ ГРУП ПРОТЕЇНІВ ТА АКТИВНІСТЬ АНТИОКСИДАНТНИХ ЕНЗИМІВ У ЛИСТКАХ ПРОРОСТКІВ КУКУРУДЗИ *Zea mays* L.

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Досліджено вплив нітропрусиду (SNP) та хлориду натрію на вміст карбонільних груп протеїнів та активність антиоксидантних ензимів у листках проростків кукурудзи. Обробка проростків 100 мМ NaCl підвищувала концентрацію карбонільних груп протеїнів через 24 год. Інкубація проростків з 100 мМ NaCl та SNP+NaCl після 48 год знижувала, а після 72 год – підвищувала концентрацію карбонільних груп протеїнів порівняно з контролем. Активність каталази була вищою в листках, інкубованих з SNP+NaCl порівняно з листками проростків, обробленими SNP протягом 24 год. Активність аскорбатпероксидази була вищою після обробки проростків 0,2 мМ SNP протягом 24 год. Вірогідне зростання активності гваяколпероксидази спостерігалося в усіх дослідних групах через 72 год. Активність глутатіон-S-трансферази була вищою після 48 год у проростках, оброблених SNP, та після 72 год – у проростках, які інкубували на середовищі з NaCl. За досліджуваних умов активність глутатіонредуктази в листках проростків кукурудзи, практично не змінювалась. Припускається, що SNP може бути використаний для попередження розвитку оксидативного стресу кукурудзи, спричиненого сольовим стресом.

Ключові слова: антиоксидантні ензими, нітропрусид натрію, оксидативний стрес, проростки кукурудзи, сольовий стрес.

ВЛИЯНИЕ НИТРОПРУССИДА И ХЛОРИДА НАТРИЯ НА СОДЕРЖАНИЕ КАРБОНИЛЬНЫХ ГРУП ПРОТЕИНОВ И АКТИВНОСТЬ АНТИОКСИДАНТНЫХ ЭНЗИМОВ В ЛИСТЬЯХ ПРОРОСТКОВ КУКУРУЗЫ *Zea mays* L.

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Исследовали влияние нитропруссида (SNP) и хлорида натрия на содержание карбонильных групп протеинов и активность антиоксидантных энзимов в листьях проростков кукурузы. Обработка проростков 100 мМ NaCl увеличивала концентрацию карбонильных групп протеинов через 24 ч. Инкубация проростков с 100 мМ NaCl и SNP+NaCl после 48 ч снижала, а после 72 ч – увеличивала концентрацию карбонильных групп по сравнению с контролем. Активность каталазы была выше в листьях проростков, инкубированных с SNP+NaCl в течение 24 ч. Активность аскорбатпероксидазы была выше после обработки 0,2 мМ SNP в течение 24 ч. Достоверное увеличение активности гваяколпероксидазы наблюдалось во всех исследованных группах через 72 ч. Активность глутатион-S-трансферазы была выше после 48 ч инкубации проростков с SNP, и ниже – после 72 ч в проростках, которые инкубировали на среде с NaCl. В исследуемых условиях активность глутатионредуктазы в листьях проростов кукурузы практически не изменялась. Предполагается, что SNP может быть использован для предупреждения окислительного стресса, вызванного солевым стрессом.

Ключевые слова: антиоксидантные энзимы, нитропруссид натрия, окислительный стресс, проростки кукурузы, солевой стресс.

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