Journal of Siberian Federal University. Biology 2024 17(2): 177–189
~ ~ ~

EDN: VWZMMZ

УДК 574

Effect of Exogenous Salicylic Acid on the Superoxide Dismutase Activity in Cucumber Seedlings (*Cucumis sativus* L.) Under Chilling

Natalia S. Repkina*, Anna A. Ignatenko and Vera V. Talanova
Institute of Biology, Karelian Research Centre RAS
Petrozavodsk, Russian Federation

Received 25.01.2023, received in revised form 20.06.2024, accepted 24.06.2024

Abstract. Phytohormones play a key role in adaptation to stress factors, including low temperature. There are data indicating a protective effect of salicylic acid (SA), a phenolic compound, on plants under chilling. Previously, it was shown that SA application had a positive effect on antioxidant enzyme activity but less data are available about changes in expression of genes encoding antioxidant enzymes. For this reason, the effects of exogenous SA on the superoxide dismutase (SOD) activity and *Cu/Zn-SOD* and *Mn-SOD* gene expression in cucumber (*Cucumis sativus* L.) seedlings under chilling stress (12 °C and 4 °C) were investigated. An analysis of electrolyte leakage from leaf cells and malondialdehyde (MDA) content showed that cucumber seedlings could survive at the temperature of 12 °C, but the temperature of 4 °C caused a significant damage. SOD activity in leaves increased gradually for 3 days at 12 °C, while at 4 °C, it increased during the first 5 h of exposure with a further decrease. The temperature of 12 °C induced accumulation of *Cu/Zn-SOD* and *Mn-SOD* gene transcripts in leaves, whereas 4 °C did not affect gene expression. Exogenous salicylic acid (SA) (100 μM) application reduced the level of electrolyte leakage and MDA concentration but increased SOD activity and *Cu/Zn-SOD* and *Mn-SOD* gene expression at both 12 °C and 4 °C. It is suggested, that the protective role of SA under the chilling stress is associated with its involvement in the regulation of the antioxidant system of plants, particularly the activity of SOD and expression of genes encoding SOD isoforms.

Keywords: *Cucumis sativus*, antioxidants, low temperature, phytohormone, gene expression.

Acknowledgements. The study was carried out under the state order (Project FMEN-2022–0004).

* Corresponding author E-mail address: nrt9@ya.ru
ORCID: 0000-0002-8555-4701 (Repkina N.)

Citation: Repkina N. S., Ignatenko A. A., Talanova V. V. Effect of exogenous salicylic acid on the superoxide dismutase activity in cucumber seedlings (*Cucumis sativus* L.) under chilling. J. Sib. Fed. Univ. Biol., 2024, 17(2), 177–189. EDN: VWZMMZ

Влияние экзогенной предобработки салициловой кислотой на активность супероксиддисмутазы у проростков огурца (*Cucumis sativus* L.) при холодовом стрессе

Н. С. Репкина, А. А. Игнатенко, В. В. Таланова
Институт биологии Карельского научного центра Российской академии наук (ИБ КарНЦ РАН)
Российская Федерация, Петрозаводск

Аннотация. Фитогормоны играют ключевую роль в адаптации растений к действию разных стресс-факторов, в том числе низких температур. Известно, что салициловая кислота (СК) – соединение фенольной природы, оказывает проекторное действие на растения в условиях гипотермии. Ранее было показано, что обработка СК положительно влияет на активность антиоксидантных ферментов, однако наряду с этим практически нет данных об ее влиянии на экспрессию генов, кодирующих эти ферменты. Учитывая это, цель даной работы заключалась в изучении влияния экзогенной обработки СК на активность супероксиддисмутазы (СОД) и экспрессию генов *Cu/Zn-SOD* и *Mn-SOD* у проростков огурца (*Cucumis sativus* L.) при холодовом стрессе (12 °C and 4 °C). Анализ выхода электролитов из клеток листа и содержания малонового диальдегида (МДА) показал, что проростки огурца адаптируются к действию температуры 12 °C, тогда как температура 4 °C оказывает повреждающее действие. Активность СОД в листьях огурца увеличивалась в течение 3 дней при 12 °C, тогда как при температуре 4 °C повышение активности СОД наблюдалось через 5 часов от начала воздействия, снижаясь при более длительной экспозиции. Кроме того, температура 12 °C приводила к накоплению транскриптов генов *Cu/Zn-SOD* и *Mn-SOD* в листьях огурца, в отличие от этого температура 4 °C не оказывала эффекта на экспрессию исследуемых генов. Экзогенная предобработка СК (100 мкМ) способствовала снижению выхода электролитов, концентрации МДА, а также стимулировала активность СОД и повышение экспрессии генов *Cu/Zn-SOD* и *Mn-SOD* в листьях огурца при действии низких температур 12 °C и 4 °C. Предполагается, что защитная роль СК при холодовом стрессе связана с ее участием в регуляции активности антиоксидантных ферментов, в частности СОД и экспрессии генов, кодирующих изоформы данного фермента.

Ключевые слова: *Cucumis sativus*, антиоксидантные ферменты, низкая температура, фитогормоны, экспрессия генов.

Благодарности. Работа выполнена в рамках государственного задания FMEN-2022–0004.

Цитирование: Репкина Н. С. Влияние экзогенной предобработки салициловой кислотой на активность супероксиддисмутазы у проростков огурца (*Cucumis sativus* L.) при холодовом стрессе / Н. С. Репкина, А. А. Игнатенко, В. В. Таланова // Журн. Сиб. федер. ун-та. Биология, 2024. 17(2). С. 177–189. EDN: VWZMMZ

Introduction

Cucumber (*Cucumis sativus* L.) is an important fruit vegetable belonging to the family Cucurbitaceae (Jeffrey, 1980). Cucumber is sensitive to low temperatures and suffers injuries at temperatures above 0 °C that affect the yield and quality of fruits (Lu, Lu, 2020). Low temperatures as well as other stresses lead to over production of reactive oxygen species (ROS) in plants that damage macromolecules (proteins, lipids, nucleic acids), which ultimately results in oxidative stress (Pal et al., 2013; Dumanović et al., 2021). Plants possess very efficient enzymatic (superoxide dismutase, catalase, glutathione reductase, etc.) and non--enzymatic (ascorbic acid, glutathione, phenolic compounds, etc.) antioxidant defense systems which work in concert to deal with the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Carmody et al., 2016). Superoxide dismutases (SODs) are the key enzymes catalyzing dismutation of the superoxide radical into hydrogen peroxide and oxygen (Perry et al., 2010; Dinakar et al., 2012; Miller, 2012; Dumanović et al., 2021). Based on the metal co-factor used by the enzyme, SODs are classified into three groups: iron SOD (Fe-SOD), manganese SOD (Mn-SOD) and copper-zinc SOD (Cu/Zn-SOD) (Bowler et al., 1994). Mn-SOD is present in mitochondria and peroxisomes; Cu/Zn-SOD is mainly a cytosolic, mitochondrial and plastidic enzyme; Fe-SOD has been found in the chloroplasts, cytosol, mitochondria and peroxisomes (Mittler et al., 2004; Szőllősi, 2014). One of the features of plant SOD isozymes is the multiplicity of isoforms, the number of which is species-specific. For instance, leaves of cucumber have four isoforms of Mn-SOD and two isoforms of Cu/Zn-SOD, Fe-SOD isoforms were not observed in this plant (Lee, Lee, 2000).

SOD plays an important role in plant adaptation to different stressors by protecting cells from ROS accumulation. Previously, it was shown that SOD activity in plants increased under various stresses, such as water deficit (Zhang et al., 2007; Sánchez-Rodríguez et al., 2016), salt stress (Mandhania et al., 2006; Yan et al., 2016), UV radiation (Tang et al., 2010; Inostroza-Blancheteau et al., 2016), heavy metals (Goswami, Das, 2016), high (Asthir et al., 2012; Chen et al., 2014) and low temperatures (Fortunato et al., 2010) and others. On the other hand, there are data indicating a decrease in activity of SOD under stress: heavy metals (Dandan et al., 2011), low temperatures (Lado et al., 2016), salt stress (Oufdou et al., 2014) and so on. This demonstrates the ambiguity of the antioxidant system reactions to stress; these reactions may vary depending on the duration and intensity of stress.

It is well known that phytohormones including salicylic acid (SA) play an essential role in plant stress tolerance (Hayat et al., 2010; Mir et al., 2021). Quite a large number of studies suggest the involvement of this important signal molecule in plant resistance to biotic stresses, whereas the information about its role in the tolerance to abiotic stress is scarce (Janda, Ruelland, 2015). It has been hypothesized that one of the mechanisms for building up resistance to abiotic stress is based on the SA effect on the antioxidant enzyme activity which prevents the development of oxidative stress and protects cells against ROS-induced damage (Pál et al., 2013; Yan et al., 2018). However, the effect of SA application on the antioxidant enzyme activity, particularly SOD, under the stress conditions is ambiguous. For instance, both positive and negative

effects of exogenous treatments with SA on SOD activity in plants were observed under cadmium stress (Agami, Mohamed, 2013; Saidi et al., 2013; Lopez-Orenes et al., 2014) and UV radiation (Choudhary, Agrawal, 2014).

The aim of this study was to investigate the effects of exogenous salicylic acid on the activity of superoxide dismutase and its encoding genes (*Cu/Zn-SOD* and *Mn-SOD*) in cucumber seedlings under low temperatures.

Material and methods

Plant materials and treatments

Cucumber (*Cucumis sativus* L. cv. Zozulya) plants were grown on Hoagland nutrient solution (pH 6.2–6.4) in the growth chamber for 7 d at the air temperature of 22 °C, air relative humidity of 60–70 %, photosynthetic photon flux density (PPFD) of 180 μmol $m^{-2}s^{-1}$and 14-h photoperiod. At the age of 1 week, seedlings were placed on the SA water solution (100 μM) for 24 hours, then placed back on Hoagland solution and exposed to low temperatures (12 °C or 4 °C) for 72 h. Seedlings without the SA pre-treatment served as the control.

Dry weight (DW) was evaluated after drying the leaves to a constant weight at 80 °C.

Analysis of relative electrolyte leakage

Relative electrolyte leakage (REL) was measured by the electrical conductivity method as described by Luo et al. (2005) with slight modifications. Fresh leaves were cut into pieces of 0.5 cm^2, rinsed with deionised water and put into 30 mL of deionised water in a test tube. After 10-min infiltration of leaf samples using a vacuum pump, the tubes were stored at room temperature for 4 h. Initial electrolyte leakage (EL0) was measured using a conductometer (HANNA, Italy). The tubes were subsequently boiled at 100 °C for 30 min to release all the electrolytes into the solution, cooled to 25 °C and final electrolytic leakage (EL1) was measured. REL was calculated according to the formula: (EL0/EL1) × 100 and expressed as percentage of total conductivity.

Determination of lipid peroxidation

The level of lipid peroxidation was assessed by malondialdehyde (MDA) content determined by the thiobarbituric acid reaction using the Stewart and Bewley method (1980). Cucumber leaves (0.1 g) were homogenized with 2 cm^3 5 % thiobarbituric acid in 20 % trichloroacetic acid and centrifuged at 10,000×g for 15 min at 4 °C. The mixture was heated at 95 °C in the water bath for 30 min, then cooled quickly in the icebath and centrifuged at 10,000×g for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The MDA content was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW (fresh weight).

Analysis of SOD activity

Leaves of cucumber plants were separated from the stem, rapidly weighed (0.3 g fresh weight) and crushed with a pestle in an ice-cold mortar with 3 ml 0.1 M phosphate buffer (pH 7.8). The homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C. SOD (EC 1.15.1.1) activity was determined according to Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50 % of photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture (2 ml) contained 0.1 M K, Na-phosphate buffer (pH 7.8), 9.3 mM methionine, 152.3 μM NBT, 1.1 μM EDTA, 2.4 % Triton X-100, 111.3 μM riboflavin, and 100 μl of enzyme extract. Riboflavin was added last. The tubes were shaken and illuminated at 180 μmol m^{-2} s^{-1} for 30 min. The tubes were then immediately covered with black cloth and the absorbance was measured spectrophotometrically at

560 nm. Total leaf soluble proteins were measured according to Bradford (1976).

Analysis of genes expression

Frozen leaf tissues were homogenised with liquid nitrogen using a mortar and pestle. Total RNA was extracted using the TRizol reagent (Evrogen, Russia) as instructed by the manufacturer. Total RNA was treated with RNase-free DNase (Syntol, Russia) to remove genomic DNA. The purity and concentrations of RNA samples were determined spectrophotometrically (NanoPhotometer C 40, IMPLEN, Germany), and the resultant A260/A280 ratios were within 1.8–2.0. One μg of total RNA was reverse-transcribed using the MMLV RT kit (Evrogen, Russia) following the supplier's recommendations. A quantitative real-time PCR was performed using the CFX96 Real-time System with the C 1000 Touch Thermal Cycler (Bio-Rad, USA). PCRs were performed using the SYBR Green PCR kit (Evrogen, Russia). The PCR procedure consisted of denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 40 s and extension at 72 °C for 45 s. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using the software with the iCycler iQ Real-time PCR Detection System. To minimize sample variations, mRNA expression of the target gene was normalized relative to the expression of the housekeeping gene encoding actin. The mRNA levels of target genes (*Cu/Zn-SOD* and *MnSOD*) were quantified in comparison to the control by $2^{-\Delta\Delta Ct}$ (Livak, Schmittgen, 2001). PCR primers of *Cu/Zn-SOD*, *MnSOD* and *actin* genes are listed below:
Cu/Zn-SOD (EF121763), forward (fw) – 5′-GACTGGGCCACATTTCAACC-3′ and reverse (rv) – 5′-GCCTTGCCATCTTCACCAA-3′;
MnSOD (EF203086), fw – 5′-CAATGGCGGAGGTCACATTA-3′ and rv – 5′-AGAGCAAGCCACACCCATC-3′;
actin (AB 010922), fw – 5′-GGTCGTGACCTTACTGATGC-3′ and rv – 5′-CAATAGAGGAACTGCTCTTTG-3′.

Statistical analyses

The experiment had three replicates. The data were subjected to the analysis of variance (ANOVA). Data were processed using Excel 2007 (Microsoft Corp., Redmond, WA, USA) and analyzed using the Statgraphics Plus 5.0 (Statgraphics Technologies, Inc., The Plains, VA, USA) statistical software. Data are presented as mean values ± standard error (SE). The Fisher's least significant difference (LSD) test was used to compare the treatment means. Differences at $p \leq 0.05$ were considered statistically significant. The research was carried out using the equipment of the Core Facility Sharing of KarRC of RAS.

Results

Dry weight

At the temperature of 12 °C leaf DW of cucumber seedlings increased on day 3, whereas at 4 °C DW remained at the same level throughout the experiment (Table). SA pre-treatment did not affect cucumber leaf DW accumulation under the low temperatures.

Values include mean ± SE (n=15). Different letters indicate significant differences between treatments at $p < 0.05$ (*ANOVA*).

Relative electrolyte leakage

The exposure to the temperature of 12 °C resulted in a slight increase in electrolyte leakage (EL) from leaves of cucumber seedlings after one day of treatment, followed by a decrease on the 3rd day, and reached the initial values (Table). After one day at 4 °C, electrolyte leakage was 4-fold higher than the initial level and remained

Table. Effect of SA on leaf DW, electrolyte leakage and MDA content in cucumber seedlings under low temperatures

Exposure, h	Temperature, °C			
	12		4	
	SA concentration, μM		SA concentration, μM	
	0	100	0	100
	Leaves DW, mg			
0	16.9 ± 0.2^{b}	17.3 ± 0.4^{b}	16.9 ± 0.2^{b}	17.3 ± 0.4^{b}
24	17.9 ± 0.5^{ab}	18.1 ± 0.6^{ab}	17.4 ± 0.3^{b}	17.8 ± 1.0^{ab}
72	19.4 ± 0.7^{a}	20.1 ± 0.8^{a}	17.2 ± 0.4^{b}	17.2 ± 0.5^{b}
	Electrolyte leakage, %			
0	15.12 ± 0.41^{f}	14.11 ± 0.27^{g}	15.12 ± 0.41^{f}	14.11 ± 0.27^{g}
24	18.37 ± 0.67^{e}	15.30 ± 0.70^{f}	54.73 ± 1.94^{c}	45.16 ± 1.69^{d}
72	13.93 ± 0.44^{h}	12.27 ± 0.51^{i}	86.17 ± 1.26^{a}	74.02 ± 1.40^{b}
	MDA content, nmol g^{-1} fresh mass			
0	8.8 ± 0.5^{f}	10.1 ± 0.4^{e}	8.8 ± 0.5^{f}	10.1 ± 0.4^{e}
24	12.1 ± 0.3^{d}	11.0 ± 0.3^{e}	15.9 ± 0.7^{c}	12.8 ± 1.1^{d}
72	16.1 ± 0.6^{c}	12.8 ± 0.5^{d}	41.7 ± 1.6^{a}	35.1 ± 1.5^{b}

increased during 3 days. It is important to note, that SA pretreatment reduced electrolyte leakage from leaf cells at optimal growth condition prior to exposure to low temperature. After one day of chilling SA pre-treatment repressed the increase of electrolyte leakage from leaf cells in comparison to untreated plants at both 12 °C and 4 °C (Table).

Lipid peroxidation rate

MDA content of leaves increased after one day of low temperature exposure (12 °C and 4 °C) and remained increased on the 3rd day of the experiment (Table). At 4 °C, the MDA content increased more significantly compared to the influence of the 12 °C exposure. SA application resulted in an increase in MDA at 12 °C on the 3rd day but its amount was lower compared to untreated plants. The same pattern was observed at 4 °C (Table).

SOD activity

It was found that the temperatures of 12 °C and 4 °C influenced SOD activity in cucumber leaves differently. The temperature of 12 °C promoted SOD activity during 3 days of the experiment (Fig. 1A), while the temperature of 4 °C increased the activity of SOD in the initial period (5 hours), but after one day it decreased (Fig. 1B). SA treatment of cucumber seedlings had a positive effect on SOD activity. Particularly, SA-pretreatment lead to an increase in enzyme activity under 12 °C with further increase during 3 days (Fig. 1A). SA application also enhanced SOD activity at 4 °C, but on the 3rd day the enzyme activity decreased to the initial level (Fig. 1B).

Relative gene expression

The exposure of cucumber seedlings to 12 °C caused accumulation of transcripts of *Cu/Zn-SOD* and *Mn-SOD* genes in leaves (Fig. 2A, B). SA application resulted in an increase in the mRNAcontent of both *Cu/Zn-SOD* and *Mn-SOD* genes. With SA, *Cu/Zn-SOD* gene expression increased prior to chilling treatments as well as when exposed to 12 °C during 3 days. On the

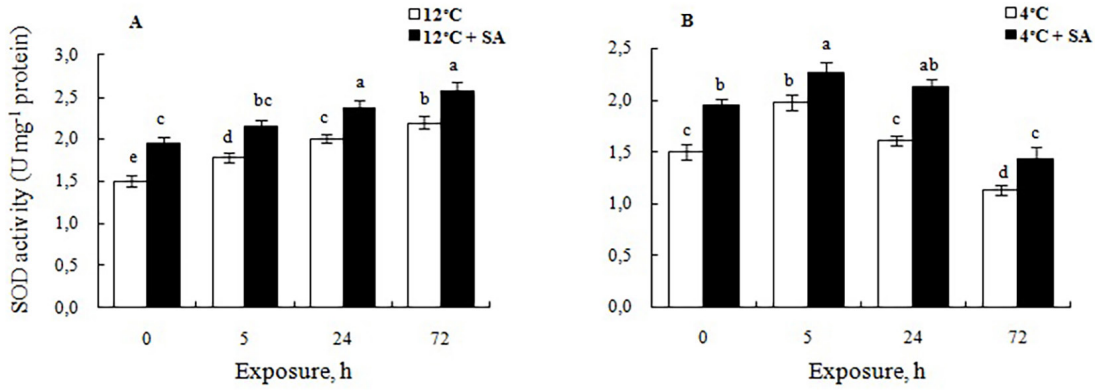

Fig. 1. Effect of low temperatures 12 °C (A) and 4 °C (B) with and without SA pre-treatment on SOD activity in the leaves of cucumber. Means ± SEs, (n = 12). Different letters indicate significant differences between means at $p < 0.05$ (*ANOVA*)

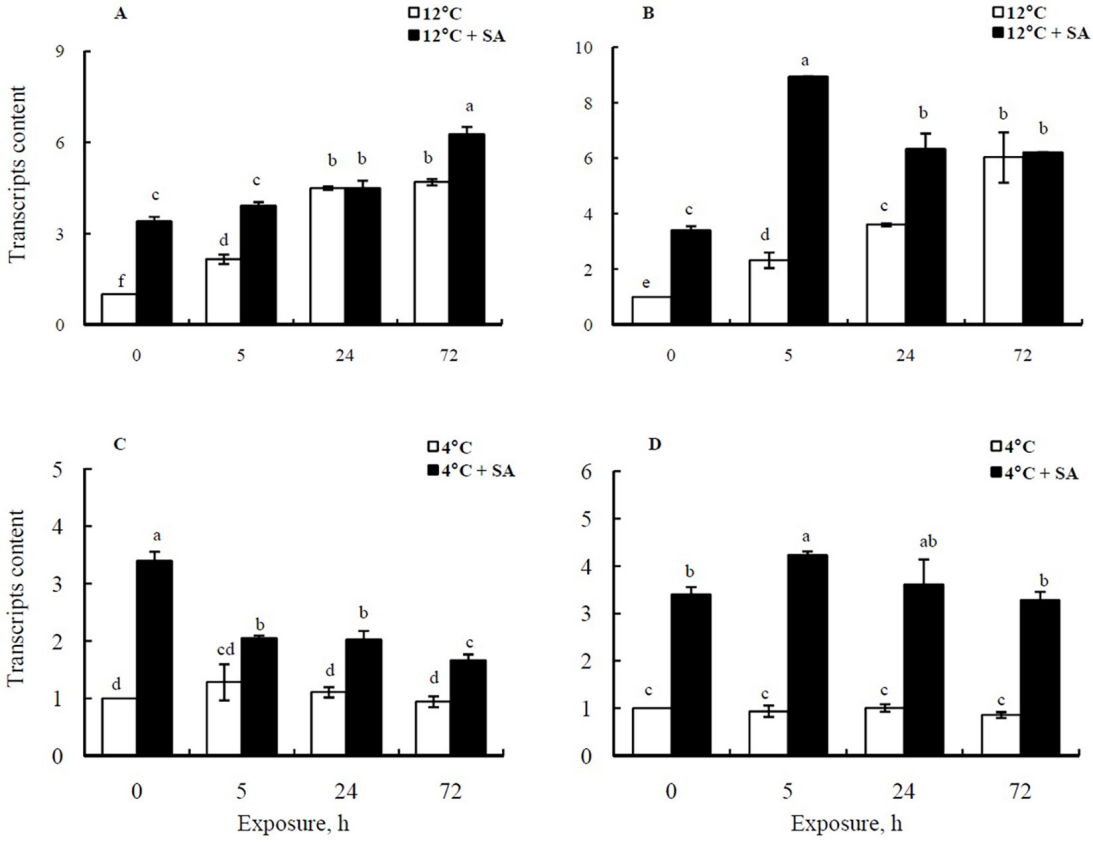

Fig. 2. Effect of low temperatures 12 °C (A, B) and 4 °C (C, D) with and without SA pre-treatment on *Cu/Zn-SOD* (A, C) and *MnSOD* (B, D) genes expression in the leaves of cucumber. Means ± SEs, (n = 12). Different letters indicate significant differences between treatments at $p < 0.05$ (*ANOVA*)

3rd day the mRNA of *Cu/Zn-SOD* content was higher in SA-treated seedlings compared to untreated plants. In contrast, the *Mn-SOD* gene expression was higher in SA-treated compared to untreated plants on the 1st day, but on the 3rd day no difference was observed (Fig. 2A, B).

The temperature of 4 °C did not affect the expression of *Cu/Zn-SOD* and *Mn-SOD* genes during 3 days of the experiment (Fig. 2C, D). SA application caused an increase in *Cu/Zn-SOD* and *Mn-SOD* genes expression. Moreover, mRNA content of these both genes was higher even on the 3rd day of the experiment, compared to untreated plants (Fig. 2C, D).

Discussion

It is generally accepted that environmental stresses are detrimental to plants by reducing their growth and biomass production. Nevertheless, inhibition of growth in response to low positive temperatures is one of the prerequisites for the adaptation to cold (Klimov, 2009). In the present study leaf DW increased on the 3rd day of 12 °C exposure, i.e. cucumber was capable of growing under this temperature, whereas at 4 °C DW accumulation did not occur. The decrease in DW accumulation under low temperatures was also observed previously (Cao et al., 2014). However, in our experiments SA-application did not increase DW in cucumber. A similar effect was described by Miao et al. (2020). This effect can be associated with the dose of SA; the dose-dependent effect on growth parameters was also shown by Barba-Espín et al. (2011).

It is known that one of non-specific reactions of plants to low temperatures is disturbance of membrane permeability and, consequently, an increase in electrolyte leakage from cells. In our experiments, a 24 h exposure to the temperature of 4 °C already caused an increase in electrolyte leakage from the cells of cucumber leaves, indicating a damaging effect. This effect was also supported by the data on MDA content in leaves. In contrast, even 3-day exposure to 12 °C did not negatively affect membrane permeability in leaves. According to these data, it can be assumed that cucumber plants are able to adapt to the temperature of 12 °C. It was noted, that low temperatures can have a negative effect, but over time plants can adapt to them (hardening temperatures), while low temperatures closer to 0 °C have an extremely negative effect, which over time will lead to the death of plants (damaging temperatures). Our data are consistent with the results of other authors. For example, electrolyte leakage from the cells of cucumber plants was intensified by exposure to the temperatures of 8 °C and 2 °C (Hu et al., 2006; Qian et al., 2013), while at 12 °C the increase of electrolyte leakage was not detected (Erez et al., 2002). The MDA level also increased in cucumber plants subjected to a low temperature (Hu et al., 2006; Liu et al., 2020).

Our results indicated the protective effect of SA application on cucumber seedlings under low temperatures influence including 4 °C, when there was a considerable reduction in electrolyte leakage from the leaf cells and MDA concentration. There are data about a protective effect of SA on cell membranes of cucumber subjected to 10 °C (Lei et al., 2010), 4 °C (Sayyari, 2012) and cucumber fruits under 2 °C (Zhang et al., 2015). The dose-dependent effect of SA application on MDA content in cucumber was also described (Nie et al., 2018). These data suggest that SA pre-treatment can neutralize the negative effect of chilling on cucumber.

Low temperatures directly or indirectly intensify generation of ROS that can damage macromolecules and disrupt physiological and biochemical processes in plants. An important role in plant cell protection from ROS is performed by antioxidant enzymes that are capable of scavenging them (Pal et al., 2013; Dumanović et al., 2021). Particularly, SOD is a key enzyme which converts superoxide radicals into molecular oxygen and hydrogen peroxide (Perry et al., 2010; Zeinali et al., 2015). In our study, it was shown that SOD activity depends on the intensity and duration of exposure to low temperatures. The

12 °C treatment resulted in an increase in SOD activity in cucumber leaves, which indicates its involvement in plant adaptation to chilling. In contrast, an increase in SOD activity under the damaging temperature (4 °C) was only short-term, and a more prolonged exposure negatively affected the activity of the enzyme. Previously, it was shown that at low temperatures SOD activity increased in cucumber (Gao et al., 2009; Yang et al., 2011; Chen et al., 2015), barley (Mutlu et al., 2013), chickpea (Kazemi-Shahandashti et al., 2014), wheat (Li et al., 2014; Scebba et al., 1999). However, the response of SOD activity to chilling was ambiguous. For instance, SOD activity in cucumber plants exposed to 15 °C rose on the 2nd day of the treatment but declined on the 4th day (Gao et al., 2009; Cao et al., 2014).

In the present study, it was shown that SA application stimulated an increase in SOD activity under 12 °C and 4 °C. The positive effect of SA on SOD activity was demonstrated previously in barley under low temperatures (Mutlu et al., 2013), soybean (Ardebili et al., 2014) and radish (Bukhat et al., 2020) at high salinity, as well as in wheat and bean plants under cadmium stress (Agami, Mohamed, 2013; Saidi et al., 2013). These data suggest that regulation of the antioxidant enzyme activity by SA under stress conditions, including low temperatures, is associated with generation of hydrogen peroxide (Hayat et al., 2010). It is possible, that SA enhances tolerance of cucumber seedlings under chilling due to the increase in SOD activity and consequently, generation of H_2O_2 as a key signaling molecule involved in the cascade of reactions resulting in activation of plant defense mechanisms.

In our experiments, the temperature of 12 °C increased SOD activity in parallel with the rise in expression of *Cu/Zn-SOD* and *Mn-SOD* genes encoding SOD isoforms. Similar results were obtained in plants of cucumber at the temperature of 15 °C: accumulation of transcripts of *Cu/Zn-SOD* gene was observed on the 2nd day of the exposure, but on the 4th day, their content decreased, whereas an increase in the level of *Mn-SOD* transcripts did not occur (Gao et al., 2009). In the present study, the temperature of 4 °C did not affect gene expression. Overall, these data indicate that there is no direct relation between the activity of SOD and the content of transcripts of SOD encoding genes. It could mean that regulation of SOD activity can be performed at the transcriptional and post-transcriptional levels. This conclusion is also supported by another set of data on the low activity of SOD along with up-regulation of *Cu/Zn-SOD* gene expression in cucumber under a low temperature (Cao et al., 2014). In contrast, the content of transcripts of *Cu/Zn-SOD* and *Mn-SOD* genes decreased in cucumber at dehydration, but at the same time SOD activity increased (Huang et al., 2013). In our experiments, SA application led to an increase in accumulation of *Mn-SOD* and *Cu/Zn-SOD* gene transcripts in the leaves of cucumber at hardening (12 °C) and damaging (4 °C) temperatures. It should be noted that the data on the effect of SA on the expression of the SOD encoding gene are fragmentary. According to our data, it is possible to propose, that SA can regulate SOD activity at the transcriptional level. However, the mechanisms of regulation of the expression of SOD encoding genes by SA at chilling needs further research.

Conclusions

In conclusion, it is supposed that the enzyme activity of SOD in the leaves of cucumber seedlings depends on the intensity and duration of low temperature exposure, and changes differently at hardening and damaging temperatures. Considering the fact that SA pre-treatment improved tolerance to chilling stress and led to an increase in SOD activity and accumulation of transcripts of *Cu/Zn-SOD* and

Mn-SOD genes in the leaves, we can assume that SA plays an important role in regulation of SOD activity contributing to greater plant resistance to cold stress. Thus, activation of SOD may be one of the key processes in adaptation to low temperatures induced by SA in cucumber plants.

References

Agami R. A., Mohamed G. F. (2013) Exogenous treatment with indole-3-acetic acid and salicylic acid alleviates cadmium toxicity in wheat seedlings. *Ecotoxicology and Environmental Safety*, 94: 164–171

Ardebili N. O., Saadatmand S., Niknam V., Khavari-Nejad R. A. (2014) The alleviating effects of selenium and salicylic acid in salinity exposed soybean. *Acta Physiologiae Plantarum*, 36(12): 3199–3205

Asthir B., Koundal A., Bains N. S. (2012) Putrescine modulates antioxidant defense response in wheat under high temperature stress. *Biologia Plantarum*, 56(4): 757–761

Barba-Espín G., Clemente-Moreno M. J., Álvarez S., García-Legaz M. F., Hernández J. A., Díaz-Vivancos P. (2011) Salicylic acid negatively affects the response to salt stress in pea plants. *Plant Biology*, 13(6): 909–917

Beauchamp C., Fridovich I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1): 276–287

Bowler C., Van Camp W., Van Montagu M., Inzé D., Asada K. (1994) Superoxide dismutase in plants. *Critical Reviews in Plant Sciences*, 13(3): 199–218

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2): 248–254

Bukhat S., Manzoor H., Athar H.-U.-R., Zafar Z. U., Azeem F., Rasul S. (2020) Salicylic acid induced photosynthetic adaptability of *Raphanus sativus* to salt stress is associated with antioxidant capacity. *Journal of Plant Growth Regulation*, 39(2): 809–822

Cao Y.-Y., Yang M.-T., Li X., Zhou Z.-Q., Wang X.-J., Bai J.-G. (2014) Exogenous sucrose increases chilling tolerance in cucumber seedlings by modulating antioxidant enzyme activity and regulating proline and soluble sugar contents. *Scientia Horticulturae*, 179: 67–77

Carmody M., Waszczak C., Idänheimo N., Saarinen T., Kangasjärvi J. (2016) ROS signaling in a destabilized world: A molecular understanding of climate change. *Journal of Plant Physiology*, 203: 69–83

Chen J., Zhao Y., Chen X., Peng Y., Hurr B. M., Mao L. (2014) The role of ethylene and calcium in programmed cell death of cold-stored cucumber fruit. *Journal of Food Biochemistry*, 38(3): 337–344

Chen A., Yang Z., Zhang N., Zhao S., Chen M. (2015) Effects of cold shock intensity on physiological activity of harvested cucumbers during storage. *Scientia Horticulturae*, 197: 420–427

Choudhary K. K., Agrawal S. B. (2014) Cultivar specificity of tropical mung bean (*Vigna radiata* L.) to elevated ultraviolet-B: Changes in antioxidative defense system, nitrogen metabolism and accumulation of jasmonic and salicylic acids. *Environmental and Experimental Botany*, 99: 122–132

Dandan L., Dongmei Z., Peng W., Nanyan W., Xiangdong Z. (2011) Subcellular Cd distribution and its correlation with antioxidant enzymatic activities in wheat (*Triticum aestivum*) roots. *Ecotoxicology and Environmental Safety*, 74(4): 874–881

Dinakar C., Djilianov D., Bartels D. (2012) Photosynthesis in desiccation tolerant plants: Energy metabolism and antioxidative stress defense. *Plant Science*, 182: 29–41

Dumanović J., Nepovimova E., Natić M., Kuča K., Jaćević V. (2021) The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Frontiers in Plant Science*, 11: 552969

Erez A., Cohen E., Frenkel C. (2002) Oxygen-mediated cold-acclimation in cucumber (*Cucumis sativus*) seedlings. *Physiologia Plantarum*, 115(4): 541–549

Fortunato A. S., Lidon F. C., Batista-Santos P., Leitão A. E., Pais I. P., Ribeiro A. I., Ramalho J. C. (2010) Biochemical and molecular characterization of the antioxidative system of *Coffea* sp. under cold conditions in genotypes with contrasting tolerance. *Journal of Plant Physiology*, 167(5): 333–342

Gao J.-J., Li T., Yu X.-C. (2009) Gene expression and activities of SOD in cucumber seedlings were related with concentrations of Mn^{2+}, Cu^{2+}, or Zn^{2+} under low temperature stress. *Agricultural Sciences in China*, 8(6): 678–684

Goswami S., Das S. (2016) Copper phytoremediation potential of *Calandula officinalis* L. and the role of antioxidant enzymes in metal tolerance. *Ecotoxicology and Environmental Safety*, 126: 211–218

Hayat Q., Hayat S., Irfan M., Ahmad A. (2010) Effect of exogenous salicylic acid under changing environment: A review. *Environmental and Experimental Botany*, 68(1): 14–25

Hu W. H., Shi K., Song X. S., Xia X. J., Zhou Y. H., Yu J. Q. (2006) Different effects of chilling on respiration in leaves and roots of cucumber (*Cucumis sativus*). *Plant Physiology and Biochemistry*, 44(11–12): 837–843

Huang Y.-W., Nie Y.-X., Wan Y.-Y., Chen S.-Y., Sun Y., Wang X.-J., Bai J.-G. (2013) Exogenous glucose regulates activities of antioxidant enzyme, soluble acid invertase and neutral invertase and alleviates dehydration stress of cucumber seedlings. *Scientia Horticulturae*, 162: 20–30

Inostroza-Blancheteau C., Acevedo P., Loyola R., Arce-Johnson P., Alberdi M., Reyes-Díaz M. (2016) Short-term UV-B radiation affects photosynthetic performance and antioxidant gene expression in highbush blueberry leaves. *Plant Physiology and Biochemistry*, 107: 301–309

Janda M., Ruelland E. (2015) Magical mystery tour: Salicylic acid signaling. *Environmental and Experimental Botany*, 114: 117–128

Jeffrey C. (1980) A review of the Cucurbitaceae. *Botanical Journal of the Linnean Society*, 81(3): 233–247

Kazemi-Shahandashti S.-S., Maali-Amiri R., Zeinali H., Khazaei M., Talei A., Ramezanpour S.-S. (2014) Effect of short-term cold stress on oxidative damage and transcript accumulation of defense-related genes in chickpea seedlings. *Journal of Plant Physiology*, 171(13): 1106–1116

Klimov S. V. (2009) Freezing tolerance of winter wheat plants depends on adaptation of photosynthesis and respiration in different time intervals. *Biology Bulletin*, 36(3): 259–266

Lado J., Rodrigo M. J., López-Climent M., Gómez-Cadenas A., Zacarías L. (2016) Implication of the antioxidant system in chilling injury tolerance in the red peel of grapefruit. *Postharvest Biology and Technology*, 111: 214–223

Lee D. H., Lee C. B. (2000) Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Science*, 159(1): 75–85

Lei T., Feng H., Sun X., Dai Q.-L., Zhang F., Liang H.-G., Lin H.-H. (2010) The alternative pathway in cucumber seedlings under low temperature stress was enhanced by salicylic acid. *Plant Growth Regulation*, 60(1): 35–42

Li X., Cai J., Liu F., Dai T., Cao W., Jiang D. (2014) Cold priming drives the sub-cellular antioxidant systems to protect photosynthetic electron transport against subsequent low temperature stress in winter wheat. *Plant Physiology and Biochemistry*, 82: 34–43

Liu F., Zhang X., Cai B., Pan D., Fu X., Bi H., Ai X. (2020) Physiological response and transcription profiling analysis reveal the role of glutathione in H_2S-induced chilling stress tolerance of cucumber seedlings. *Plant Science*, 291: 110363

Livak K.J., Schmittgen T.D. (2001) Analysis of relative gene expression data using real-time quantative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25(4): 402–408

López-Orenes A., Martínez-Pérez A., Calderón A.A., Ferrer M.A. (2014) Pb-induced responses in *Zygophyllum fabago* plants are organ-dependent and modulated by salicylic acid. *Plant Physiology and Biochemistry*, 84: 57–66

Lu Y., Lu R. (2020) Enhancing chlorophyll fluorescence imaging under structured illumination with automatic vignetting correction for detection of chilling injury in cucumbers. *Computers and Electronics in Agriculture*, 168: 105145

Luo Q., Yu B., Liu Y. (2005) Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress. *Journal of Plant Physiology*, 162(9): 1003–1012

Mandhania S., Madan S., Sawhney V. (2006) Antioxidant defense mechanism under salt stress in wheat seedlings. *Biologia Plantarum*, 50(2): 227–231

Miao Y., Luo X., Gao X., Wang W., Li B., Hou L. (2020) Exogenous salicylic acid alleviates salt stress by improving leaf photosynthesis and root system architecture in cucumber seedlings. *Scientia Horiculturae*, 272: 109577

Miller A.-F. (2012) Superoxide dismutases: Ancient enzymes and new insights. *FEBS Letters*, 586(5): 585–595

Mir R.A., Aryendu, Somasundaram R. (2021) Salicylic acid and salt stress tolerance in plants: a review. *Journal of Stress Physiology & Biochemistry*, 17(3): 32–50

Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. (2004) Reactive oxygen gene network of plants. *Trends in Plant Science*, 9(10): 490–498

Mutlu S., Karadağoğlu Ö., Atici Ö., Taşğin E., Nalbantoğlu B. (2013) Time-dependent effect of salicylic acid on alleviating cold damage in two barley cultivars differing in cold tolerance. *Turkish Journal of Botany*, 37(2): 343–349

Nie W., Gong B., Chen Y., Wang J., Wei M., Shi Q. (2018) Photosynthetic capacity, ion homeostasis and reactive oxygen metabolism were involved in exogenous salicylic acid increasing cucumber seedlings tolerance to alkaline stress. *Scientia Horticulturae*, 235: 413–423

Oufdou K., Benidire L., Lyubenova L., Daoui K., El Abidine Fatemi Z., Schröder P. (2014) Enzymes of the glutathione–ascorbate cycle in leaves and roots of rhizobia-inoculated faba bean plants (*Vicia faba* L.) under salinity stress. *European Journal of Soil Biology*, 60: 98–103

Pál M., Szalai G., Kovács V., Gondor O.K., Janda T. (2013) Salicylic acid-mediated abiotic stress tolerance. *Salicylic acid*. Hayat S., Ahmad A., Alyemeni M. (Eds.) Springer, Dordrecht, p. 183–247

Pal R.S., Agrawal P.K., Bhatt J.C. (2013) Molecular approach towards the understanding of defensive systems against oxidative stress in plant: A critical review. *International Journal of Pharmaceutical Sciences Review and Research*, 22(2): 131–138

Perry J. J. P., Shin D. S., Getzoff E. D., Tainer J. A. (2010) The structural biochemistry of the superoxide dismutases. *Biochimica et Biophysica Acta – Proteins and Proteomics*, 1804(2): 245–262

Qian C.-L., Mi H.-B., Zhao Y.-Y., He Z.-P., Mao L.-C. (2013) Effect of maturity stage on the gene expression of antioxidative enzymes in cucumber (*Cucumis sativus* L.) fruits under chilling stress. *Journal of Integrative Agriculture*, 12(8): 1495–1500

Saidi I., Ayouni M., Dhieb A., Chtourou Y., Chaïbi W., Djebali W. (2013) Oxidative damages induced by short-term exposure to cadmium in bean plants: Protective role of salicylic acid. *South African Journal of Botany*, 85: 32–38

Sánchez-Rodríguez E., Romero L., Ruiz J. M. (2016) Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. *Journal of Plant Physiology*, 190: 72–78

Sayyari M. (2012) Improving chilling resistance of cucumber seedlings by salicylic acid. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 12(2): 204–209

Scebba F., Sebastiani L., Vitagliano C. (1999) Protective enzymes against activated oxygen species in wheat (*Triticum aestivum* L.) seedlings: Responses to cold acclimation. *Journal of Plant Physiology*, 155(6): 762–768

Stewart R. R. C., Bewley J. D. (1980) Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiology*, 65(2): 245–248

Szőllősi R. (2014) Superoxide dismutase (SOD) and abiotic stress tolerance in plants: An overview. *Oxidative damage to plants*. Ahmad P. (Ed.) The Netherlands, Elsevier, p. 89–129

Tang K., Zhan J.-C., Yang H.-R., Huang W.-D. (2010) Changes of resveratrol and antioxidant enzymes during UV-induced plant defense response in peanut seedlings. *Journal of Plant Physiology*, 167(2): 95–102

Yan H., Li Q., Park S.-C., Wang X., Liu Y.-J., Zhang Y.-G., Tang W., Kou M., Ma D.-F. (2016) Overexpression of *CuZnSOD* and *APX* enhance salt stress tolerance in sweet potato. *Plant Physiology and Biochemistry*, 109: 20–27

Yan Y., Pan C., Du Y., Li D., Liu W. (2018) Exogenous salicylic acid regulates reactive oxygen species metabolism and ascorbate–glutathione cycle in *Nitraria tangutorum* Bobr. under salinity stress. *Physiology and Molecular Biology of Plants*, 24(4): 577–589

Yang H., Wu F., Cheng J. (2011) Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. *Food Chemistry*, 127(3): 1237–1242

Zeinali F., Homaei A., Kamrani E. (2015) Sources of marine superoxide dismutases: Characteristics and applications. *International Journal of Biological Macromolecules*, 79: 627–637

Zhang M., Duan L., Tian X., He Z., Li J., Wang B., Li Z. (2007) Uniconazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. *Journal of Plant Physiology*, 164(6): 709–717

Zhang Y., Zhang M., Yang H. (2015) Postharvest chitosan-g-salicylic acid application alleviates chilling injury and preserves cucumber fruit quality during cold storage. *Food Chemistry*, 174: 558–563