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ORIGINAL PAPER

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# ANTIOXIDANT POTENTIAL OF TOMATO (*Solanum lycopersicum* L.) SEEDLINGS AS AFFECTED BY THE EXOGENOUS APPLICATION OF ORGANOIODINE COMPOUNDS

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## ABSTRACT

Salicylic acid is one of the regulatory compounds involved in numerous processes in plants. Previous studies indicated that also its halogen derivatives may exhibit similar roles. The aim of the work was to evaluate the influence of iododerivatives of salicylic acid such as: 5-iodosalicylc acid (5I-SA) and 3,5-diiodosalicylic acid (3,5diI-SA) on selected aspects of antioxidant capacity of tomato seedlings. The efficiency of improving iodine accumulation in tomato seedlings was also studied. No tested organoiodine compound had a negative effect on the growth and development of tomato seedlings. The presence of iodosalicylic acids in the nutrient solution led to a decrease of the content of salicylic acid, ascorbic acid and phenolic compounds in tomato seedlings. A modifying effect of tested organoiodine compounds on the antioxidant activity of tomato seedling extracts varied with respect to analyzed enzyme and applied assays. It has been confirmed that higher plants can take up and accumulate iodine from organoiodine compounds in levels not causing any symptoms of toxicity.

**Key words:** ascorbic acid, antioxidant enzymes, iodosalicylic acids, phenolic compounds, biofortification

**Abbreviations:** SA – salicylic acid, 5I-SA – 5-iodosalicylic acid, 3,5diI-SA – 3,5-diiodosalicylic acid, CAT – catalase, POX – guaiacol peroxidase, APX – ascorbate peroxidase, DPPH – 2,2-diphenyl-1-picryl-hydrazyl, CUPRAC – cupric ion reducing antioxidant capacity, FRAP – ferric reducing antioxidant power

## INTRODUCTION

Salicylic acid (SA) is included into the group of endogenous regulators of plant growth and development. It takes part, among others, in the control of seed germination, uptake and transport of various compounds, transpiration, stomatal conductance and photosynthesis [Hayat et al. 2010]. Salicylic acid stimulates plant reaction to various stress factors mainly through the activation of antioxidant system which is crucial for the neutralization of reactive oxygen species (ROS) generated in plants. Numerous studies have revealed that not only endo-, but also exogenous SA improves the efficiency of plant antioxidant system, particularly in stress conditions [Hayat et al. 2008]. The positive effect of foliar or root application of SA has been revealed with respect to the activity of such antioxidant enzymes as: ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) or guaiacol peroxidase (POX) in plants subjected to heavy metals [Panda and Patra 2007], water stress [Hayat et al. 2008], salinity [Yusuf et al. 2008] or low temperature

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[Kang et al. 2003]. Also, in non-stressed plants exogenous salicylic acid may improve the efficiency of ROS scavenging [Agarwal et al. 2005]. Salicylic acid is also one of the factors regulating the activity of PAL enzyme (phenylalanine ammonia lyase) responsible for the deamination of L-phenylalanine to transcinnamic acid, the latter being the precursor of the most phenols in plants [Boudet 2007].

SA derivatives, including halogen ones, may exhibit similar action with respect to the stimulation of resistance response or the improvement of phenolic biosynthesis in plant cells [Wang et al. 2012, Safari et al. 2013]. Among them, chlorosalicylic acid is described as particularly active. Its stimulative action on phenol biosynthesis and the improvement of the resistance of horticultural plants to selected pathogens has already been documented [Siegrist et al. 1994]. However, little information is available on the modifying effect of other halogen SA derivatives, including iodosalicylates, on plants.

Recognition of such influence, regarding organoiodine compounds, may be of substantial importance with respect to plant biofortification purposes [Sady et al. 2014]. Crop plants containing increased iodine levels (biofortified crops) may become an important source of that micronutrient in a daily diet for human and animal populations. It is worth to notice that low intake of iodine does not concern only populations from developing countries as is the case of other micronutrient deficiencies [Zimmermann and Boelaert 2015]. So far, studies on the improvement of crops with iodine, including the efficiency of such process as well as possible implications regarding plant physiology, have been mainly focused on the application of inorganic compounds such iodides and iodates [Dai et al. 2004, Blasco et al. 2008, Caffagni et al. 2011, Gonzali et al. 2017]. Recently, however, it has been revealed that higher plants can relatively easily absorb and accumulate iodine from organoiodine compounds [Sady et al. 2014, Smoleń et al. 2017]. The effect of the application of organoiodine compounds on plant growth, physiology and metabolism is however very poorly documented. Prior to the implementation of iodosalicylates into agricultural practice, detailed studies need to be conducted towards plant response to such compounds as well as the safety of such obtained iodine-enriched crops for the consumers.

Therefore, the aim of the work was to confirm the ability to take up iodine from iododerivatives of salicylic acid (namely: 5-iodosalicylic and 3,5-diiodosalicylic acid) by tomato seedlings along with determining its influence on selected parameters of antioxidant activity of tomato seedlings at an early stage of growth. It was aimed to analyze the accumulation of selected compounds engaged in the antioxidant system in tomato plants subjected to the presence of iodosalicylic acids in the nutrient solution.

## MATERIAL AND METHODS

**Plant cultivation.** Seedlings of tomato (*Solanum lycopersicum* L. cv. 'Kmicic') were cultivated in a pot experiment in the spring season of 2016. Tomato seeds were sown into peat substrate and watered with the Hoagland solution. Seedlings at the two-leaf stage were transferred into the pots filled with perlite. From that time, each pot was watered daily with app. 40 ml of the nutrient solution containing macro- and micronutrients in the amounts recommended by Wysocka-Owczarek [2011] (Tab. 1). Nutrient solution was also supplemented with salicylic acid, 5-iodosalicylic acid (5I-SA) or 3,5-diiodosalicylic acid (3,5diI-SA) in the concentration of 10 or 50 μM depending on the tested combination. Doses of tested compounds were chosen on the basis of previous studies conducted by Sady et al. [2014]. Each combination consisted of four pots with five plants per pot. Seedling harvest followed by the leaf sampling was conducted at the four-leaf stage (30-day-old seedlings). Leaf, shoot and root biomass of tomato seedling was measured during harvest.

**Determination of iodine content.** Fresh leaves were dried at 50°C in a laboratory dryer with forced air circulation and ground in a small laboratory mill. A 0.2 g sample of dried leaves was incubated for 3 h at 70°C with 10 ml of demineralized water and 1 ml of 25% TMAH solution (tetramethylammonium hydroxide). After incubation, samples were cooled down to a room temperature, filled up with demineralized water to the volume of 30 ml and centrifuged for 15 min at 4500 rpm, room temperature. The measurements were conducted in the supernatant [PN-EN 15111 2008, Smoleń et al. 2016] using ICP-OES Prodigy spectrometer (Teledyne Leeman Labs, USA) and iodine content expressed as mg $I \cdot g^{-1}$ f.w.

**Determination of antioxidant properties of tomato seedlings.** The content of phenolic compounds as well as antioxidant and antiradical capacity of tomato leaves were measured in 96% ethanol extracts of fresh plant material and the obtained results were expressed on the fresh weight basis. The content of phenolic compounds was measured after the reaction with Folin-Ciocalteau reagent according to the method described by Swain and Hillis [1959]. The assessment of antioxidant capacity of tomato leaves was conducted after the reaction with free stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [Brand-Williams et al. 1995] as well as with the use of: CUPRAC (cupric ion reducing antioxidant capacity) [Apak et al. 2010] and FRAP methods (ferric reducing antioxidant power) [Benzie and Strain 1996].

In order to analyze the activity of selected antioxidant enzymes, extraction of fresh leaf samples with 100 mM phosphate buffer (pH 7.5) containing 1mM EDTA (ethylenediaminetetraacetic acid) and 1% PVP (polyvinylpyrrolidone) was conducted [Lin and Wang 2002 with further modifications]. Samples were homogenized and centrifuged for 15 min (4500 rpm, 5°C). The collected supernatant was further centrifuged (20 min, 10 000 rpm, 2°C) and stored at –80°C until analyses. In such obtained extracts the activity of catalase (CAT), guaiacole peroxidase (POX) and ascorbate peroxidase (APX) was determined along with the content of salicylic acid. In order to analyze APX activity, extraction buffer was supplemented with 1 mM ascorbic acid.

Catalase (hydrogen-peroxide : hydrogen-peroxide oxidoreductase; EC 1.11.1.6; CAT) activity was measured according to Beers and Sizer [1952] with modifications. Reaction mixture of 3 ml contained 100 mM phosphate buffer (pH 7.5) 200 mM $H_2O_2$ and enzyme extract. Absorbance of the mixture was measured at 240 nm as the change of $H_2O_2$ concentration ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$) occurring 45 and 60 s after the addition of enzyme extract. Specific activity of CAT was expressed as µmol of oxidized $H_2O_2$ · mg $protein^{-1}$ · $min^{-1}$ (U · mg $protein^{-1}$).

Peroxidase (guaiacol : hydrogen-peroxide oxidoreductase; EC 1.11.1.7; POX) activity was analyzed according to Reuveni et al. [1992] with further modifications. A total volume of 2 ml of reaction mixture contained 15 mM phosphate buffer (pH 6.5), 10 mM guaiacol, enzyme extract and 1 mM $H_2O_2$ as an initiating factor. The increase of absorbance of oxidized guaiacol ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$) was measured at 470 nm. Enzymatic activity of POX was expressed as µmol of oxidized guaiacol · mg $protein^{-1}$ · $min^{-1}$ (U · mg $protein^{-1}$).

Ascorbate peroxidase (L-ascorbate : hydrogen-peroxide oxidoreductase; EC 1.11.1.11; APX) activity was measured according to Nakano and Asada [1981]. Reaction mixture of the total volume of 2 ml contained 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, 5 mM ascorbate, enzyme extract and 1 mM $H_2O_2$ as an initiating factor. A decrease of sample absorbance at 290 nm was measured within three minutes as a result of ascorbate ($\varepsilon$ = 2.8 $mM^{-1}$ $cm^{-1}$) oxidation. Specific enzyme activity of APX was expressed as the amount of µmol of oxidized ascorbate · mg $protein^{-1}$ · $min^{-1}$ (U · mg $protein^{-1}$). Protein content in extracts was determined by the Lowry method with the use of bovine albumin as a protein standard [Waterborg 2009].

The level of salicylic acid in extracts of fresh leaves was analyzed with the use of capillary electrophoresis Capel 105 M system in 10 mM Tris buffer adjusted to pH 2.78 with formic acid [Coolen et al. 1998]. The content of L-ascorbic (AA) and L-dehydroascorbic acid (DHA) in tomato leaves was assayed after homogenization of fresh plant material in 2% oxalate acid. Homogenates were centrifuged for 15 min at 4500 rpm, 5°C and supernatant was collected for further analysis. Directly before measurements samples were again centrifuged for 10 min at 10 000 rpm (room temperature) and analyzed with the use of capillary electrophoresis with photodiode array detector (DAD) system (Beckman PA 800 Plus). Running buffer containing: 30 mM $NaH_2PO_4$, 15 mM $Na_2B_4O_7$ and 0.2 mM CTAB (pH 8.80) was prepared according to the method by Zhao et al. [2011]. The content of L-dehydroascorbic acid (DHA) was determined after the reduction of DHA to AA with the use of 50 mM dithiothreitol (DTT) as proposed by Dresler and Maksymiec [2013]. The content of DHA in plant samples was calculated by subtracting the initial content of AA from the total AA content after DHA reduction.

The obtained results were statistically verified with the use of ANOVA module of STATISTICA 12 PL software. Significance of differences between means was estimated using Tukey's test, $p < 0.05$. All measurements were performed in triplicates (n = 3).

## RESULTS AND DISCUSSION

**Biomass of tomato seedlings.** No negative influence of salicylic acid and its iododerivatives (5-iodosalicylic acid, 3,5-diiodosalicylic acid) was noted with respect to the growth and development of 30-day old tomato seedlings. Introduction of 10 μM 5I-SA into the nutrient solution increased the biomass of tomato seedlings as compared to the control (Fig. 1). No symptoms of unfavorable effect of tested compounds, such as chlorosis and necrosis, were noted on plants (Fig. 2). The obtained observations are in the agreement with literature data confirming that exogenous application of low doses of SA improves seed germination and seedling growth [Choudhury and Panda 2004, Hayat et al. 2010]. What is more, it was demonstrated that the applied doses of tested iododerivatives were not toxic for plants.

**Iodine accumulation in the leaves of tomato seedlings.** The application of organoiodine compounds caused a significant increase in the iodine content in leaves of tomato seedlings when compared to the control and the combination with salicylic acid (Fig. 3). For both tested iododerivatives of SA the increase in the applied dose heightened the level of iodine accumulation in the leaves. It is worth mentioning that the iodine content in leaves grown in the presence of 10 μM 3,5diI-SA was lower than in the combination with the same dose of 5I-SA. However, when the applied dose increased, the greater accumulation of this element was noted for 3,5diI-SA and the plants from that combination were characterized by the highest I level (Fig. 3). The obtained results confirm the ability of higher plants to absorb and accumulate organoiodine compounds [Smoleń et al. 2017].

**Accumulation of antioxidant compounds and antioxidant potential of leaf extracts.** Most studies conducted so far have described a stimulation effect of exogenous SA on its accumulation in plant tissues [e.g. Dat et al. 2000]. What is more, salicylic acid is relatively easily transported within a plant both as a free compound and glycoside derivatives [Rocher et al. 2006]. Taking above into consideration, the results obtained in the study are difficult to interpret as plants grown in the nutrient solution containing SA or its iododerivatives were characterized by a significant decrease of SA accumulation in leaves (Fig. 4). The only exception was the leaves of plants grown in the presence of 10 μM 3,5diI-SA that contained SA in the level comparable to the control values. The lowest concentration of SA was noted in plants cultivated in the nutrient solution containing 5I-SA in both tested doses. Only in the case of 3,5diI-SA, a statistically significant effect of its dose on SA accumulation in tomato leaves was noted – higher concentration of 3,5diI-SA in the nutrient solution decreased SA level in leaves. A decrease of SA accumulation after the application of its iododerivatives (5I-SA, 3,5diI-SA) may have resulted from the course and direction of the distribution and metabolism processes of absorbed compounds in plants. However, mechanisms of such processes are yet to be described.

Ascorbic acid and phenolics are the abundant compounds that play a substantial antioxidant role in plant cells. Ascorbic acid is one of the most important low molecular agents participating in numerous processes that neutralize free radicals. In plant cell it is present in two forms: reduced (L-ascorbic acid, AA) and oxidized (L-dehydroascorbic acid, DHA). It is estimated that in physiological conditions a reduced form represents approximately 90% of total ascorbic acid in plant cells [Blokhina et al. 2003]. Phenolic compounds are a group of secondary metabolites exhibiting a wide range of action on plants. Due to its high reactivity and the ability to chelate transition metal ions they represent a crucial element of plant antioxidant system [Rice-Evans et al. 1997].

Results of the conducted studies revealed a decrease of ascorbic acid content in tomato seedlings from all combinations with the application of SA and its iododerivatives (Tab. 2). The lowest level of AA was noted in plants cultivated in the nutrient solution containing the lower dose of 5I-SA (10 μM). There was also a tendency of increasing AA accumulation after the application of 50 μM of tested compounds. Higher level of DHA, exceeding the control value, was found only in the plants grown in the nutrient solution containing 50 μM SA. In the studies conducted so far, no clear effect of exogenous application of SA on the content of ascorbic acid in plants has been determined. Chen et al. [2016] presented that root application of SA decreased DHA accumulation in wheat seedlings, while its effect on AA level depended on SA dose. It is worth underlining that SA doses applied in the

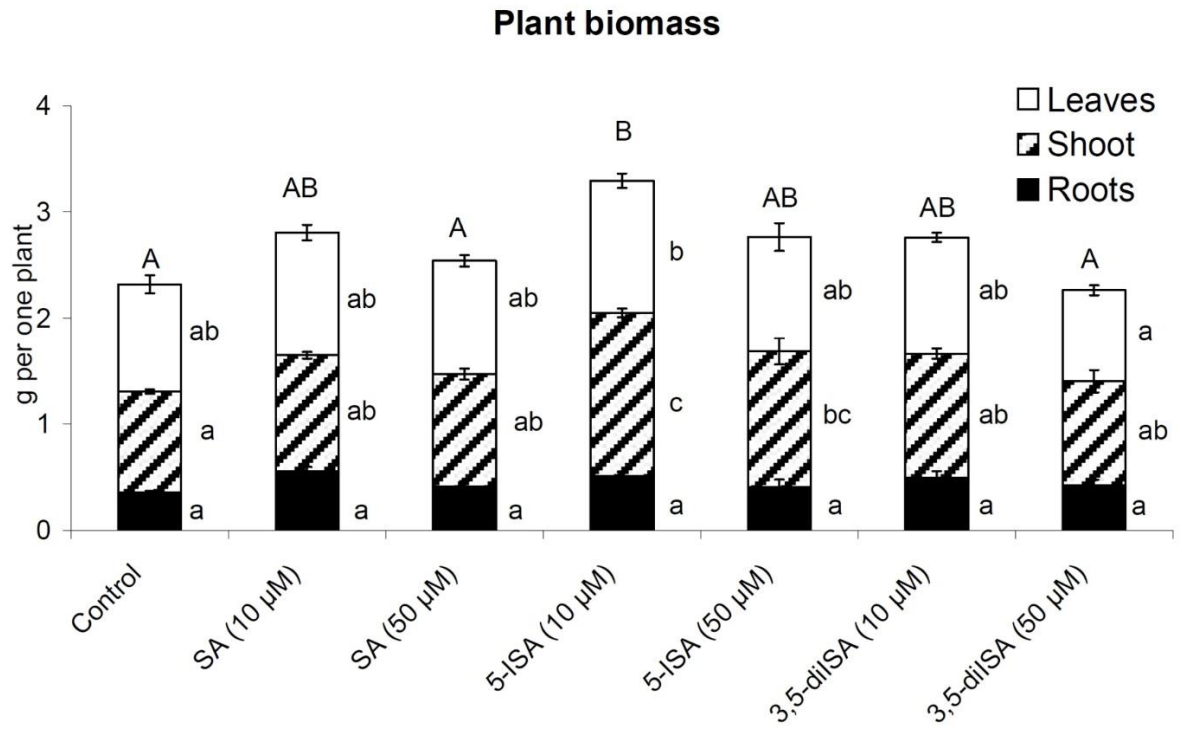


**Fig. 1.** Biomass of tomato seedlings grown in the nutrient solution containing salicylic acid or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 16)

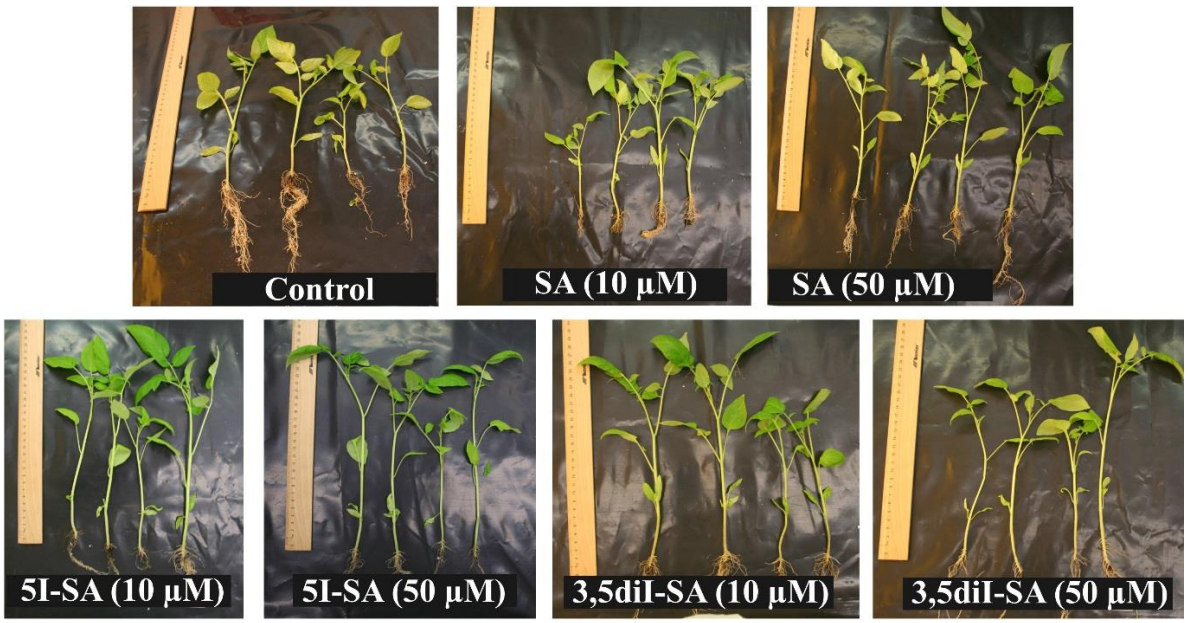


**Fig. 2.** Phenotype of 30-day-old tomato seedlings at harvest

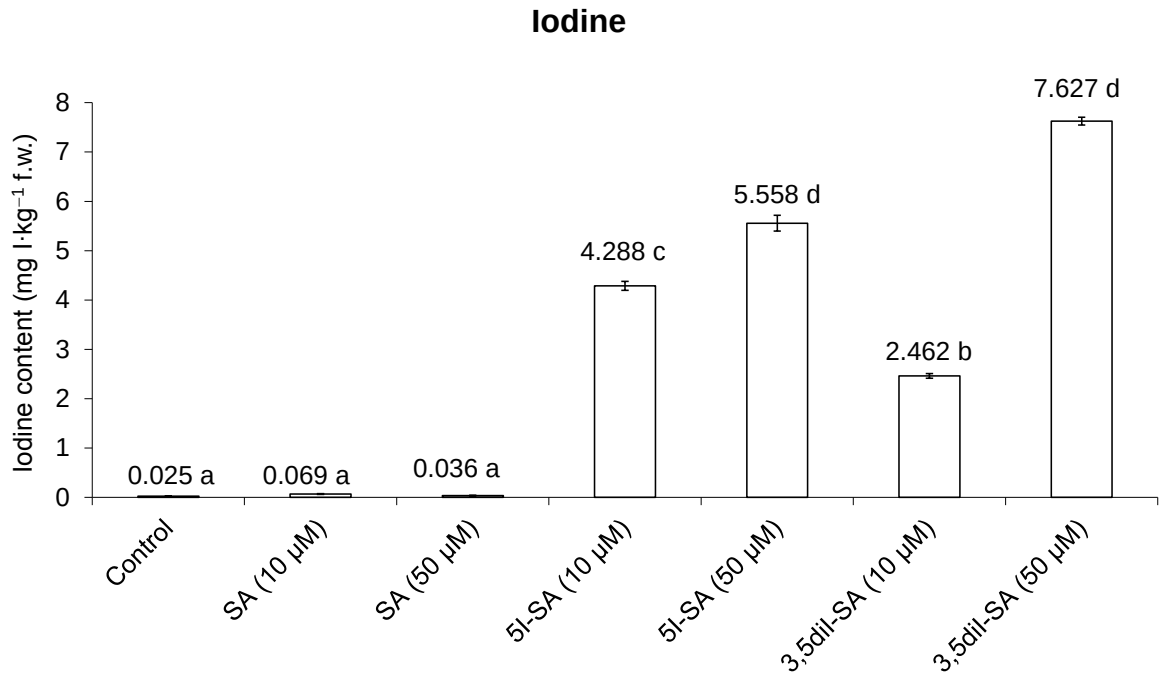


**Fig. 3.** Iodine content in the leaves of tomato seedlings grown in the nutrient solution containing SA or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

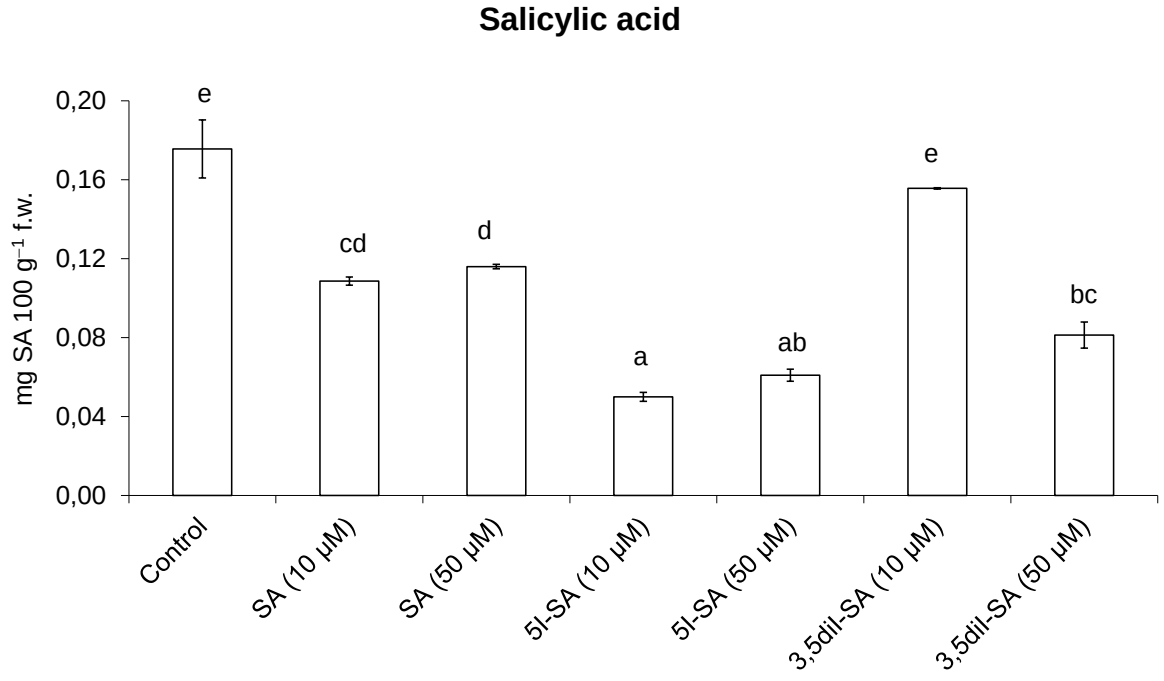


**Fig. 4.** SA content in the leaves of tomato seedlings grown in the nutrient solution containing SA or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

**Table 1.** Composition of the nutrient solution applied during tomato cultivation from the two-leaf stage of growth

| (mg · dm$^{-3}$) | | | | | | | | | | | | | | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $N_{-NO_3}$ | $N_{-NH_4}$ | P | K | Mg | Ca | S | Cl | Fe | Mn | Zn | B | Cu | Mo | |
| 175 | 0 | 30 | 200 | 50 | 170 | 60 | 30 | 2 | 0.55 | 0.33 | 0.33 | 0.15 | 0.05 | 5.7 |

**Table 2.** The content of ascorbic acid in leaves of 30-day-old tomato seedlings grown in the nutrients solution containing SA and its iododerivatives. Means followed by the same letter are not significantly different at $p < 0.05$ (n = 3)

| Combination | mg · 100 g$^{-1}$ f.w. | |
|---|---|---|
| | L-ascorbic acid (AA) | L-dehydroascorbic acid (DHA) |
| Control | 26.13 c | 2.20 a |
| SA (10 µM) | 10.71 ab | 1.90 a |
| SA (50 µM) | 16.96 abc | 4.51 b |
| 5I-SA (10 µM) | 9.33 a | 1.78 a |
| 5I-SA (50 µM) | 14.22 ab | 2.07 a |
| 3,5diI-SA (10 µM) | 11.45 ab | 2.14 a |
| 3,5diI-SA (50 µM) | 20.26 bc | 3.02 a |

cited work (0.25 or 0.5 mM SA) substantially exceeded those tested in the present study. In the case of tobacco plants grown for four weeks in the presence of 100 µM SA, an increase of the content of both AA and DHA was noted as compared to the control [Dat et al. 2000]. On the other hand, soaking of seeds in the same solution reduced the accumulation of ascorbic acid in rice seedlings [Choudhury and Panda 2004]. Studies conducted by Smoleń et al. [2017] revealed that only after the introduction of 40 µM 5I-SA into the nutrient solution the level of AA in lettuce increased or remained at the level comparable to the control. Application of lower doses of 5I-SA reduced the content of ascorbic acid in leaves.

As in the case of ascorbic acid, exogenous application of both salicylic acid and its iododerivatives lowered the level of phenolic compounds in the leaves of 30-day-old tomato seedlings. Introduction of 5I-SA and 3,5diI-SA into the nutrient solution decreased the accumulation of phenolics in leaves also when compared to the plants grown in the presence of SA (Fig. 5). Exceptionally low content of phenolic compounds was noted in both combinations with 3,5diI-SA application. Increase of SA and 5I-SA concentration in the nutrient solution resulted in the growth of phenolic accumulation in tomato leaves but without exceeding the control value. Obtained results are also difficult to interpret, particularly with respect to the combination with salicylic acid application. One of the physiological roles of salicylic acid is the stimulation of the synthesis of phenolic compounds [Hayat et al. 2010] so an increase of biosynthesis and accumulation of phenolic in plant tissues should have been expected. It may be suggested that the applied doses of tested compounds were too low to exhibit any stimulative action on phenolics synthesis. Also, in the studies with lettuce cultivation conducted by Smoleń et al. [2017] significant changes in the content of total phenolic compounds were noted only for the relatively higher dose of 5I-SA (40 µM). It may be of some importance that the observed lack of increase of phenolic accumulation may indirectly confirm that iodosalicylic acids were not stress factors for plants.

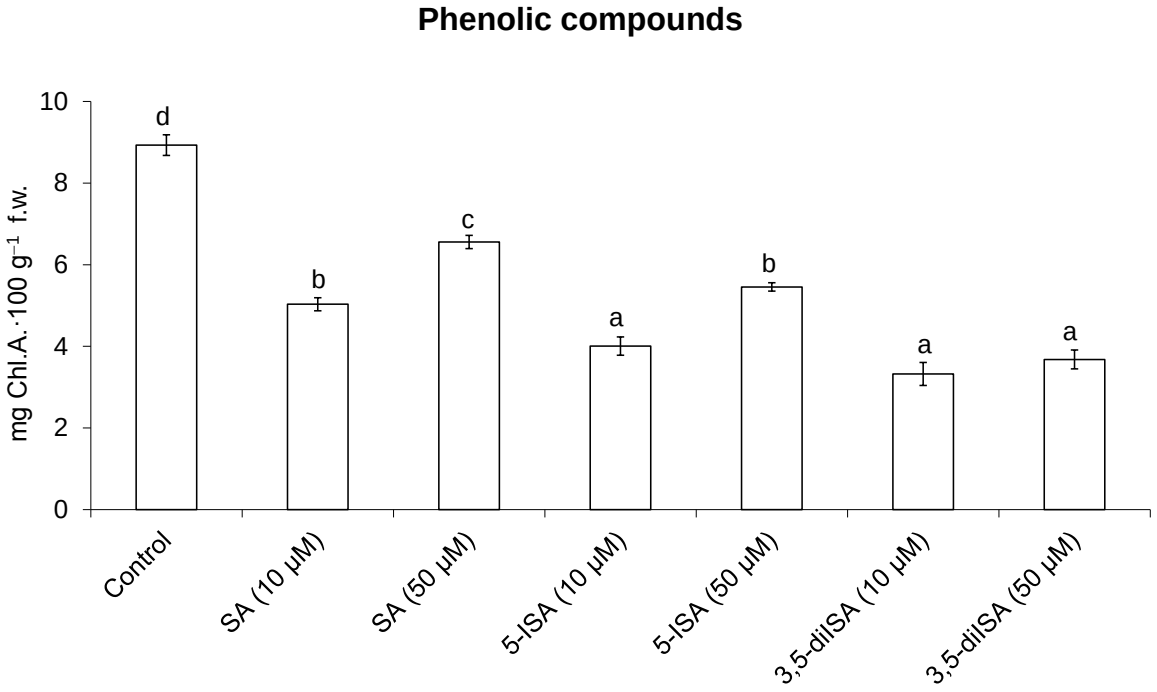


**Fig. 5.** The content of phenolic compounds in the leaves of tomato seedlings grown in the nutrient solution containing SA or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

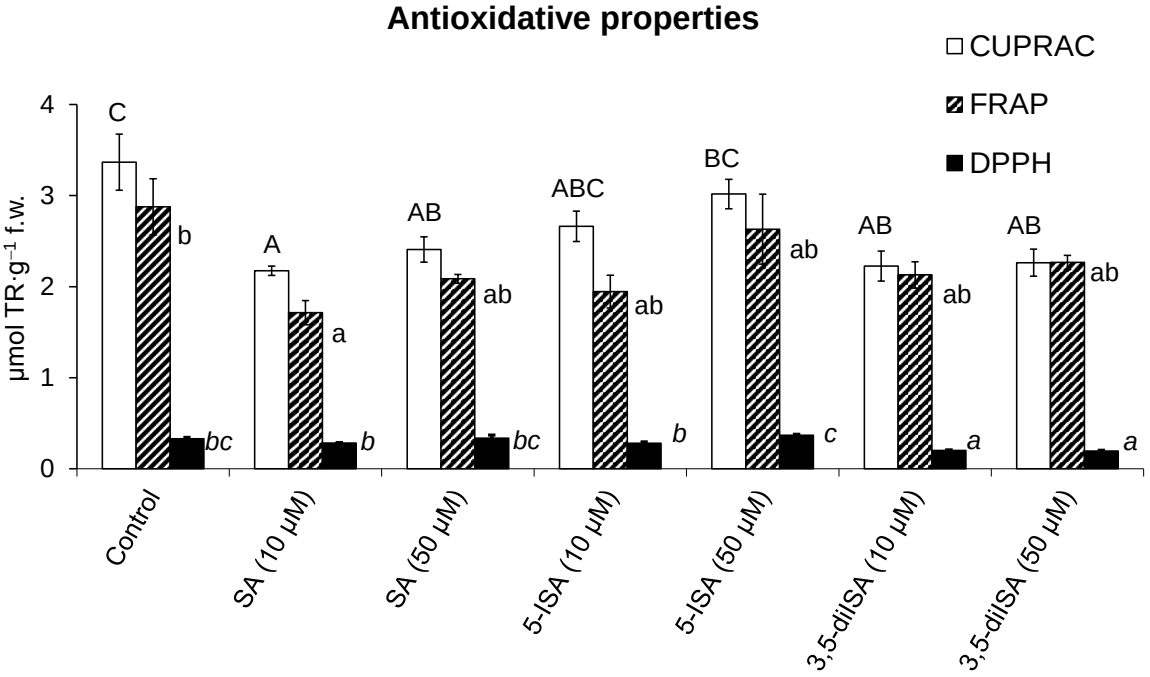


**Fig. 6.** Antioxidant capacity of leaves of tomato seedlings grown in the nutrient solution containing SA or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

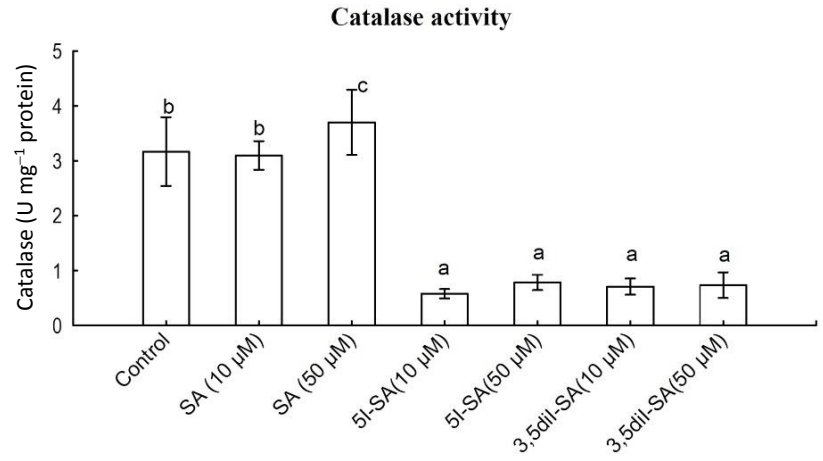


**Fig. 7.** CAT activity in the leaves of tomato seedlings grown in the presence of SA or its iododerivatives. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

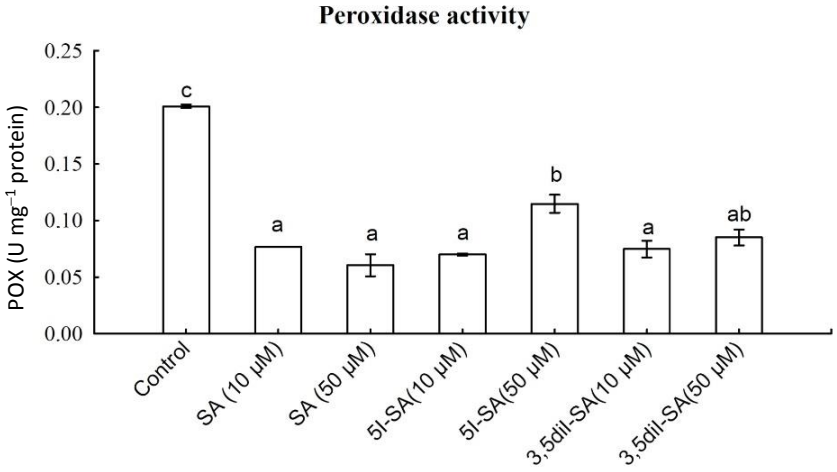


**Fig. 8.** POX activity in the leaves of tomato seedlings grown in the presence of SA or its iododerivatives in the nutrient solution. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

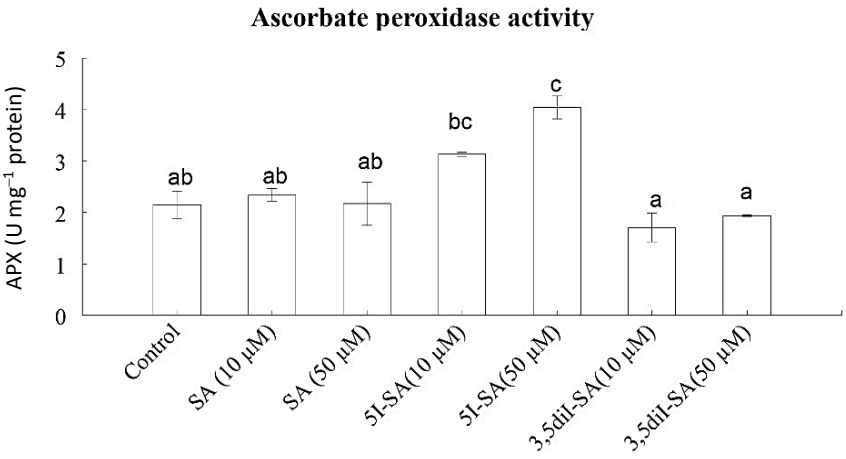


**Fig. 9.** APX actvity in the leaves of tomato seedlings grown in the presence of SA or its iododerivatives in the nutrient solution. Bars indicate standard error. Means followed by the same letter are not significantly different at $p < 0.05$. (n = 3)

The decrease in the content of antioxidative compounds under the influence of the tested iodosalicylic acids (as well as SA) was followed by the decrease of antioxidant potential of leaf extracts measured by CUPRAC, FRAP and DPPH assays (Fig. 6). Values similar to the control were noted only in the leaves of tomato cultivated in the presence of 50 μM 5I-SA in the nutrient solution. Interestingly, the most significant decrease of antioxidant potential measured by DPPH scavenging assay was noted for leaves of plants grown in the nutrient solution containing 3,5diI-SA. Substantially lower values of antioxidant potential measured with the use of DPPH radical, as compared to CUPRAC and FRAP, may confirm the higher selectivity of that method [Giovanelli and Buratti 2009]. DPPH scavenging assay describes the ability of compounds or plant extracts to react ('scavenge') with free radicals (in this case – DPPH), while CUPRAC and FRAP – ability to reduce metal ions present in complexes [Tirzitis and Bartosz 2010]. It also needs to be taken into account that the reaction of an antioxidant with DPPH radical is reversible what may lead to low readings of antioxidant capacity for numerous compounds present in plant extracts [Mishra et al. 2012]. What is more, one of the most important factors affecting the efficiency of antioxidants towards DPPH scavenging is steric accessibility to the radical site of DPPH [Prior et al. 2005]. In general, low-molecular weight antioxidants exhibit higher capacity to react with DPPH that those containing i.e. multiple phenolic rings [Xie and Schaich 2014]. Apart from that, the observed results indicating that exogenous organoiodine compounds may reduce the FRAP, CUPRAC and DPPH scavenging potential of tomato seedlings need further documentation.

**The activity of antioxidant enzymes in leaves of tomato seedlings.** It is assumed that one of the major mechanisms determining salicylic acid as a factor triggering systemic acquired resistance and stress tolerance is related to its binding to the catalase protein leading to the reduction of its enzymatic activity [e.g. Janda et al. 1999, Hayat et al. 2010]. Catalase is responsible for the regulation (together with peroxidases) of intracellular concentration of hydrogen peroxide [Willekens et al. 1995] by converting $H_2O_2$ into water and oxygen. Its decreased activity leads to the accumulation of hydrogen peroxide, which activates selected genes responsible for the induction of plant resistance and antioxidant potential of a plant [Knörzer et al. 1999]. It may be therefore said that salicylic acid induces transient oxidative stress that acts as a hardening factor, but the dose of salicylic acid is of substantial importance. It has been revealed that the application of SA in a dose below 1 mM does not in fact reduce the activity of catalase in tobacco [Willekens et al. 1994], corn [Guan and Scandalios 1995] or *Arabidopsis thaliana* [Summermatter et al. 1995]. In the present studies no inhibition of CAT activity was noted in response to the application of both tested doses of SA i.e. 5 and 50 μM SA (Fig. 7) what most likely is related to the above-mentioned effect of SA. At the same time both tested iododerivatives of SA (5I-SA, 3,5diI-SA) caused an approximately three-fold decrease in CAT activity as compared to control as well as the combinations with SA application.

Apart from catalase, also peroxidases (including guaiacol peroxidase, POX and ascorbate peroxidase, APX) take part in the control of $H_2O_2$ accumulation in plant cells [Blokhina et al. 2003]. In the present studies the application of SA and its iododerivatives variously modified the activity of peroxidases in the leaves of 30-day-old tomato seedlings. In all tested combinations, irrespective of applied compounds and its dose, there was noted a decrease in POX activity as compared to the control (Fig. 8). On the other hand, a substantial reduction of APX activity followed only the application of 3,5diI-SA during tomato cultivation (Fig. 9). Growth of tomato seedlings in the presence of 5I-SA in the nutrient solution (particularly in its higher dose) increased the activity of ascorbate peroxidase in leaves. Our findings concerning POX activity are in agreement with some reports of decreased peroxidase activity due to exogenous application of salicylic acid [Durner and Klessig 1995] and may imply a similar action of iodosalicylates in that matter. It needs to be underlined that the observed effect strongly depended on the analyzed type of peroxidase and applied SA dose [Durner and Klessig 1995]. For example, in the case of leaves of corn subjected to 0.5 mM SA no change of APX activity was noted while POX activity increased [Janda et al. 1999]. The obtained diversity with respect to the influence of salicylic acid and its iododerivatives on the modification of the activity of antioxidant enzymes implies the need for further studies that may suggest the mechanism of action of tested compounds.

## CONCLUSIONS

In conclusion, it has been revealed that a low dose of 5I-SA may improve the growth of tomato seedlings. The presence of tested iodosalicylic acids in the nutrient solution may decrease the content of antioxidant compounds in tomato seedlings such as salicylic acid, L-ascorbic acid and phenolics as well as reduce antioxidative potential of tomato leaf extracts. A diverse modification of the activity of antioxidant enzymes due to the application of iododerivatives of salicylic acid also occurred. The modifying influence of 5I-SA and 3,5diI-SA on analyzed parameters of antioxidant systems of tomato plants is not related to phytotoxicity of tested compounds as no negative symptoms regarding plant growth and development were observed. The ability of the higher plants to absorb and accumulate iodine from organoiodine compounds has also been confirmed. In addition to the lack of evident toxicity symptoms, the possibility for applying iodosalicylic acids for biofortification purposes arises. To our knowledge it is one of the first documentations of organoiodine compounds affecting the antioxidative system in higher plants what opens the possibilities for further studies to be conducted also on a molecular level.

## ACKNOWLEDGEMENTS

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