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Iodosalicylates and iodobenzoates supplied to tomato plants affect the antioxidative and sugar metabolism differently than potassium iodide

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ABSTRACT

Iodine is considered as a beneficial element for plants. As compared to the mineral form of iodine, the effect of organoiodine compounds on physiological and biochemical processes in plants is weakly recognized. This study describes the influence of different forms of iodine – mineral as KI and organic as iodosalicylates and iodobenzoates on the antioxidative and sugar metabolism of tomato plants. Plants were treated with KI and with the following organoiodine compounds: 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA) and 4-iodobenzoic acid (4-IBeA). The effect of salicylic acid (SA) and benzoic acid (BeA) on plants was also tested. The plants revealed a lower tolerance to 3,5-diISA, 4-IBeA and slightly to BeA as compared to control. Tested compounds did not affect the content of ascorbic (AA) and dehydroascorbic (DHA) acid. All tested compounds, with the exception of 2-IBeA, did not affect the content of phenols, phenylpropanoids and anthocyanins in leaves. Tested compounds variously modified the activity of catalase (CAT), ascorbic peroxidase (APX) and peroxidase (POX) in leaves and roots. The content of soluble sugars in tomato leaves and roots varied depending on the combination, with a noticeable tendency to increase after the application of organoiodine compounds.

Key words: antioxidant enzymes, index of tolerance, iodine biofortification, non-enzymatic antioxidants, organoiodine compounds

Abbreviations:

2-IBeA – 2-iodobenzoic acid, 3,5-diISA – 3,5-diiodosalicylic acid, 4-IBeA – 4-iodobenzoic acid, 5-ISA – 5-iodosalicylic acid, AA – ascorbic acid, APX – ascorbate peroxidase, BeA – benzoic acid, CAT – catalase, DHA – dehydroascorbic acid, KI – potassium iodide, POX – guaiacol peroxidase, ROS – reactive oxygen species, SA – salicylic acid

INTRODUCTION

There are numerous pathways of reactive oxygen species (ROS) formation in plants including its generation during the photosynthesis, cell respiration (Tripathy and Oemüller, 2012) or stress processes (Gill and Tuteja, 2010). ROS are mainly the products of various metabolic routes localized in chloroplasts, mitochondria and peroxysomes (Del Rio et al., 2006; Navrot et al., 2007). ROS may negatively affect proteins, lipids, sugars and nucleic acids in plants causing the oxidative stress (Gill and Tuteja, 2010). Antioxidant system that protects plant cells from oxidative stress involves

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both enzymatic and non-enzymatic mechanisms (Eraslan et al., 2007). The following enzymes play important roles in the neutralization of ROS in plant cells: superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), ascorbate peroxidase (APX) and others (Iba, 2002). CAT and APX reveal high affinity to hydrogen peroxide /H_2O_2/ (Sofo et al., 2015; König et al., 2002). A typical reaction of catalase is H_2O_2 dismutation into water and molecular oxygen (Zamocky et al., 2008). On the other hand, APX utilizes two molecules of ascorbate to reduce H_2O_2 to H_2O with the formation of monodehydroascorbic acid /MDHA/ (Welinder, 1992). Guaiacol peroxidase is responsible for the reduction of H_2O_2 with the oxidation of oxidizing hydrogen donors including guaiacol or pyrogallol (Onsa et al., 2004). The following compounds are included in the group of non-enzymatic antioxidants: ascorbic acid (AA), glutathione (GSH), carotenoids and phenolics. Antioxidants that are present in plants interact with cellular components and are cofactors for numerous enzymes (De Pinto and De Gara, 2004).

Iodine is not a plant mineral nutrient but is included by numerous researchers into the group of the beneficial elements for plant growth and development. Iodine is also considered to play a substantial role in the antioxidant system of numerous species of aquatic plants (Medrano-Macías et al., 2016).

The current model of iodine prophylaxis is based on the enrichment of table salt with that element. Despite the wide-spread usage of iodized salt in the daily diet the problem of iodine deficiency still concerns many countries around the world and it is estimated 2 billion people ingest an insufficient amount of iodine (Mottiar, 2013). The insufficient supply of that element may contribute to the miscarriage, hypothyroidism or impaired development of the nervous system in the newborns (Melse-Boonstra and Jaiswal, 2010; Walker et al., 2007; Zimmermann, 2011). The World Health Organization (WHO) recommends developing alternative sources of iodine in the daily diet (WHO, 2014). One of the possible solutions may include the production of crop plants with the increased accumulation of iodine in the edible parts (Medrano-Macías et al., 2016).

The research conducted by Blasco et al. (2008, 2011) indicates that plant enrichment with mineral forms of iodine (KI, KIO_3) may modify the activity of selected antioxidant enzymes as well as the content of ascorbic acid and phenolics that are involved in the detoxification of free radicals. The antioxidant properties of lettuce plants vary depending on the chemical form of iodine (Blasco et al., 2008, 2011). The application of I^- anions during lettuce cultivation decreased the activity of SOD, improved the activity of CAT and L-galactose dehydrogenase as well as increased the content of ascorbic acid (AA) and glutathione (GSH) in the leaves. Introduction of the IO_3^- into the lettuce plants led to the increase of SOD, APX and CAT activity (Blasco et al., 2011).

The research conducted by Gupta et al. (2015) revealed a positive effect of iodine in the form of IO_3^- on the antioxidant system in *Glycine max* L. plants subjected to heavy metal stress. After the application of various concentrations of IO_3^- (20, 40 or 80 μM), the increase of SOD, APX and GSH reductase was noted in the plants treated with Cd. A gradual increase of the content of AA and GSH was also demonstrated after the application of 100 mM $CdCl_2$ together with the increasing IO_3^- dose (Gupta et al., 2015). Other studies revealed the increase in the antioxidant activity of tomato fruits after the application of 5 mM KI (Kiferle et al., 2013).

The studies conducted so far have been mainly focused on the application and the effect of mineral forms of iodine on plants (Medrano-Macías et al., 2016; Gonzali et al., 2017). Needs to be taken into account are present, that in soil mineral (e.g. I^- and IO_3^-) and organic compounds of iodine with that element bound covalently to the aromatic ring. Up to now it has been considered that iodine bound to the soil organic matter (including organoiodine compounds) is not available for plants. However, our previous studies not only have described 5-iodosalicylic acid (5-ISA) as a iodine source for plants but also showed that 5-ISA may modify the chemical composition of plants differently the inorganic iodine (Smoleń et al., 2017). These observations provide a novel perspective on iodine uptake by plants. Plant response to organoiodine compounds: iodosalicylates and iodobenzoates on the physiological and biochemical level has not yet been recognized. The studies presented in the work bring about some substantial insight onto that issue.

The aim of the work was to determine the influence of organoiodine compounds (iodosalicylates and iodobenzoates, as compared to the mineral form of iodine – KI) on the activity of selected antioxidant enzymes and the content of L-ascorbic acid (AA), dehydroascorbic acid (DHA), soluble sugars and

phenolic compounds in the leaves and roots of tomato plants at the early stage of vegetative growth.

MATERIAL AND METHODS

Plant material and treatments

The experiment with 'Kmicic' tomato (*Solanum lycopersicum* L.*)* plants was conducted in a greenhouse of the Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow (50°05'04.1"N 19°57'02.1"E) in February-April, 2016. Tomato seeds were sown into boxes containing peat substrate: sand mixed in volumetric ratio of 1:1. In March, tomato plants with 2-3 true leaves were transferred into the 1.5 dm^3 pots filled with perlite. Plant watering was conducted in the doses of the nutrient solution that would prevent its leaching from pots. One week after transplanting, the plants were watered every day with the nutrient solution (EC 1.73 mS cm^{-1}) containing the following macronutrients (in mg dm^{-3}): 190 N (190 N-NO_3), 40 P, 210 K, 50 Mg, 160 Ca, 50 S. Microelements were introduced with the multi-element fertilizer 'Mikro plus' (Intermag, Olkusz, Poland) in the following amounts (in mg dm^{-3}): 0.028 B, 0.015 Cu, 0.24 Fe, 0.1 Mn, 0.054 Mo, 0.35 Zn. The pH of the nutrient solution was set at 5.8 using 38% nitric acid. Plants were exposed to sunlight only.

Plants were treated with the salicylic acid (25 µM SA), benzoic acid (25 µM BeA) and iodine compounds added into the nutrient solution in the concentration of 25 µM I. Iodine was applied in the mineral form (potassium iodide, KI) and as the following organoiodine compounds: 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA) and 4-iodobenzoic acid (4-IBeA) (Sigma-Aldrich, Poland). The control plants received nutrient solution without iodine compounds, SA or BeA (Tab. 1). The application of the organic form of iodine during plant cultivation is the subject of a patent application (Sady et al., 2014). The tested compounds were dissolved in the initial nutrient solution and 200 ml was applied to each pot every other day for two weeks. On the alternate days, the plants in all treatments were watered using the control nutrient solution (without the addition of iodine compounds, SA and BeA). All the tested compounds were first introduced into the nutrient solution when plants reached the stage of 4-5 true leaves, *i.e.* on 30.03.2016 with in total seven applications. The last treatment of iodine compounds, SA and BeA was performed on 11.04.2016. Plant sampling for biomass (upper parts of plants and root system) and biochemical analyses was conducted the day after the last treatment.

Table 1. Total amount of iodine applied per plant and iodine content in nutrient solution

Treatments	Iodine content in the nutrient solution (mg I dm^{-3})	Total amount of iodine applied per plant (mg I)**
Control	0.09*	0.36
KI	3.17	4.78
SA	0.09*	0.36
BeA	0.09*	0.36
5-ISA	3.17	4.78
3,5-diISA	3.17	4.78
2-IBeA	3.17	4.78
4-IBeA	3.17	4.78

*the content of iodine in the control nutrient solution results from the presence of its trace amounts in used mineral fertilizers
**including iodine content in the control nutrient solution

The total amount of introduced compounds and the calculated iodine dose per plant are presented in Table 1. In the initial nutrient solution, a trace amount of iodine was determined at a level of 0.09 mg I dm^{-3}. The total amount of control solution introduced during the whole period of cultivation (before, during and after the applications of tested compounds) per plant was 4000 ml. Each treatment consisted of four replicates with four plants per replicate (16 plants per treatment). The total number of plants in the experiment was 128.

Index of tolerance

The weight (biomass) of leaves and roots was measured for the calculation of index of tolerance towards iodine. The index of tolerance was calculated as the mass ratio of leaves and roots of plants subjected to tested compounds and of the control plants and expressed as a percentage [Index of tolerance = (Average weight of plant's treated by tested compounds / Average weight of plant's for control) × 100%] (Hawrylak-Nowak, 2008).

Process of sample preparation

After biometric analysis of plants whole plants were washed three times in distilled water and then leaves and roots were separated from plants by hand. Then, chemical and biochemical analysis were performed in the leaves and roots samples. The detailed analysis procedures are described below.

Iodine content analysis and calculation of iodine uptake by tomato plants

Iodine content in tomato plants was determined in dry samples (dried at 50°C in a laboratory dryer

with forced air circulation and ground in variable speed rotor mill Pulverisette 14, FRITSCH using 0.5 mm sieve) according to the following procedure: 0.5 g of plant sample (shoot system or roots), 10 ml of double-distilled water and 1 ml of 25% TMAH (tetramethylammonium hydroxide) were put into 30 ml falcon tubes. After mixing, samples were incubated for 3 hours at 70°C. After incubation, samples were cooled to the temperature of approximately 20°C, filled to 30 ml with double-distilled water and mixed. Samples were then centrifuged for 15 min at 4 500 rpm, 5°C. The measurements of iodine content using ICP-OES spectrometer were conducted in the supernatant without its decanting (PN-EN 15111 – 2008; Smoleń et al., 2016). The iodine uptake by one plant was calculate from formula: Iodine uptake (mg I) = [Iodine content (mg kg^{-1} f.w.) × weight of one plant (g)] / 1000.

Ascorbic and dehydroascorbic acids assay

The content of L-ascorbic acid (AA) in fresh leaves and roots samples was analyzed after the homogenization of 20 g of fresh plant tissue in 80 ml of 2% oxalate acid. Homogenates were centrifuged for 15 min at 4 500 rpm, 5°C. Supernatants were collected, further centrifuged for 10 min at 10 000 rpm and analyzed using Beckman PA 800 Plus capillary electrophoresis (CE) system with DAD detection. Capillaries of i.d. 50 µm, o.d. 365 µm and total length of 50 cm (40 cm to detector) were used. A negative power supply of -25 kV was applied. The running buffer solution containing 30 mM NaH_2PO_4, 15 mM $Na_2B_4O_7$ and 0.2 mM CTAB (pH 8.80) was prepared as proposed by Zhao et al. (2011).

Dehydroascorbic acid (DHA) in leaves and roots was analyzed according to Dresler et al. (2013) method with 50 mM DTT (dithiothreitol) added after the second centrifugation. Determination of AA and DHA content was conducted in two separate runs with the use of CE technique. The amount of DHA was calculated by subtracting the initial AA from the total AA obtained after DHA reduction (Dresler et al., 2013).

Enzyme assays

The activity of CAT, POX and APX enzymes was determined in tomato leaves and roots. Fresh plant material was collected and flooded with extraction buffer containing: 100 mM phosphate buffer (KH_2PO_4:K_2HPO_4), pH 7.5, 1 mM EDTA and 1% PVP. In order to analyze the activity of APX, 1 mM ascorbic acid was also added. Prepared samples were homogenized and centrifuged for 15 min at 4 500 rpm, 5°C. Supernatants were collected and further centrifuged for 10 min at 10 000 rpm, 2 °C (Lin et al., 2002). The activity of CAT, POX and APX was measured in the supernatants obtained after the second centrifugation.

CAT activity was assayed according to Beers and Sizer (1952) with modifications. The assay mixture (3 ml) contained: 100 mM phosphate buffer (pH 7.5), 200 mM H_2O_2 and enzyme extract. The optical density of the solution was measured at 240 nm 45 s and 60 s after the addition of enzyme extract into the mixture (extinction coefficient = 39.4 mM^{-1} cm^{-1}). Enzyme specific activity was expressed as µmol of oxidized H_2O_2 mg min^{-1} per protein.

POX activity was assayed according to Reuveni et al. (1992) with modifications The assay mixture (2 ml) consisted of 15 mM phosphate buffer (pH 6.5), 10 mM guaiacol (o-metoxyphenol), enzyme extract and 1 mM H_2O_2 as an initiator. The absorbance of the reaction mixture was determined for 3 min at 470 nm by Hitachi Spectrophotometer (extinction coefficient = 26.6 mM^{-1} cm^{-1}). POX activity was estimated by the increase in absorbance of guaiacol at 470 nm and was expressed as µmol of oxidized guaiacol mg min^{-1} per protein.

APX activity was measured according to Nakano and Asada (1981). The assay mixture (2 ml) consisted of 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, 5 mM ascorbate, enzyme extract and 0.1 mM H_2O_2 as an initiator. APX activity was estimated by the reduction in ascorbate concentration. The absorbance was continuously read for 3 min by Hitachi Spectrophotometer at 290 nm (extinction coefficient = 2.8 mM^{-1} cm^{-1}). Enzyme specific activity was expressed as µmol of oxidized ascorbate mg min^{-1} per protein.

The content of protein was determined according to the Lowry method with bovine serum albumin as a standard (Waterborg, 2002).

Determination of the soluble sugars in plant leaves and roots

In the root and leaf extracts prepared for enzymatic assays (CAT, POX) the content of glucose, fructose and sucrose was also assessed by capillary electrophoresis (PA 800 Plus system with DAD detection). The capillary of ø 25 µm and total length of 60.5 cm (50 cm to detector) was used. A positive power supply of 30 kV was applied, and the temperature of detection was set to 18°C. The running buffer solution contained 3.6 mM Na_2HPO_4

and 0.2 mM β-cyclodextrin, pH was set to 12.7 using 130 mM NaOH. The sum of the glucose, fructose and sucrose concentrations was expressed as total sugar content (Smoleń et al., 2016).

Content of phenolic compounds, phenylpropanoids, flavonols and anthocyanins

According to the method Fakumoto and Mazza (2000) the sum of compounds classified in the group of phenolic compounds, phenylpropanoids, flavonols and anthocyanins was analyzed. After root and leaf sample preservation in 96% ethanol (using the reflux condenser), these compounds were analyzed spectrophotometrically after sample reaction with 0.1% HCl dissolved in ethanol (Fukumoto and Mazza, 2000). Reaction mixture consisted of 0.25 ml of plant extract with 0.25 ml of 0.1 % HCl in 96 % ethanol and 4.5 ml of 2 % HCl. The absorbance was measured at 280 nm using chlorogenic acid as a standard, 320 nm – caffeic acid as standard, 360 nm – quercetin as standard and 520 nm – cyanidin as standard for sum of phenols, phenylpropanoids, flavonols and anthocyanins respectively (Leja et al., 2013).

Statistical analysis

All data were statistically verified using ANOVA module of Statistica 12.0 PL program at significance level $p \leq 0.05$. In the case of significant effects, homogenous mean groups were distinguished on the basis of Tukey test.

RESULTS

Index of tolerance

In comparison to the control, leaf biomass substantially decreased only in plants treated with 3,5-diISA and 4-IBeA what was reflected by the respective values of the tolerance index, namely 46.1% and 45.1% (Figs 1 and 2A). It was also found that tomato roots were less sensitive to the application of these compounds than leaves (Fig. 2B). The application of BeA to a lesser extent decreased the value of the tolerance index for leaves and roots. In the case of other iodine compounds (mineral KI, 5-ISA and 2-IBeA) the values of the tolerance index for leaves increased. The application of tested compounds did not affect the biomass of roots in tomato plants.

Content of iodine and uptake of this element by tomato plants

The detailed results on the content of iodine in leaves and roots have been presented in our earlier publication (Halka et al., 2018). The iodine content for roots samples (in mg I kg^{-1} d.w.) was about 0.5 – 0.6 for control, SA and BeA treatments; 76.0 after 3,5-diISA; 137.9 – KI; 208.5 – 5-ISA; 236.5 – 4-IBeA and 314.0 after 2-IBeA application, as well as for leaves samples: 0.5 for control, SA and BeA;

Figure 1. Tomato plants after tested compounds treatment

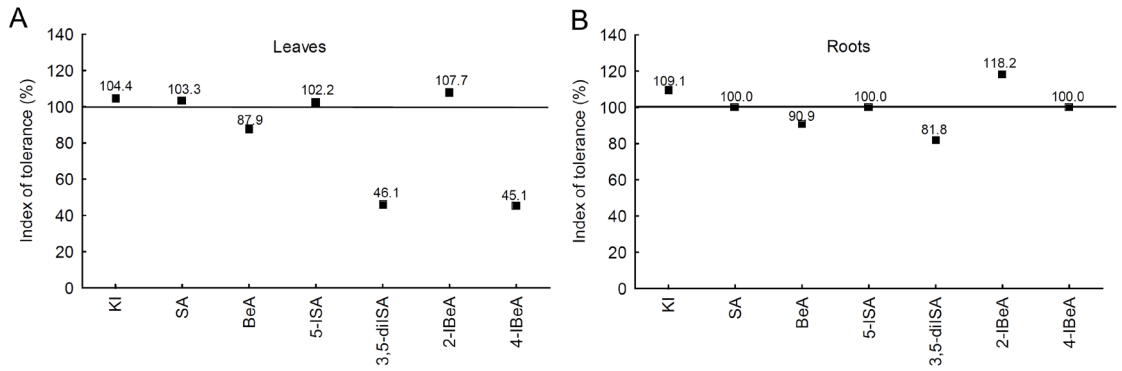

Figure 2. The values of index of tolerance for leaves (A) and roots (B) of tomato plants in relation to the control plants (100%) (n = 4)

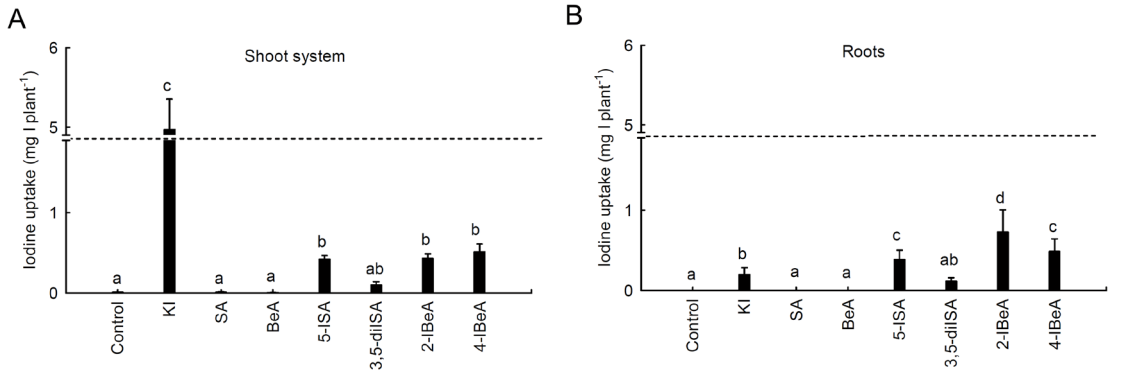

Figure 3. Iodine uptake by shoot system (A) and roots (B) of tomato plants − parameter calculated on the basis of iodine content and plant biomass. Means followed by the same letters are not significantly different for $p < 0.05$. Bars indicate standard error (n = 4)

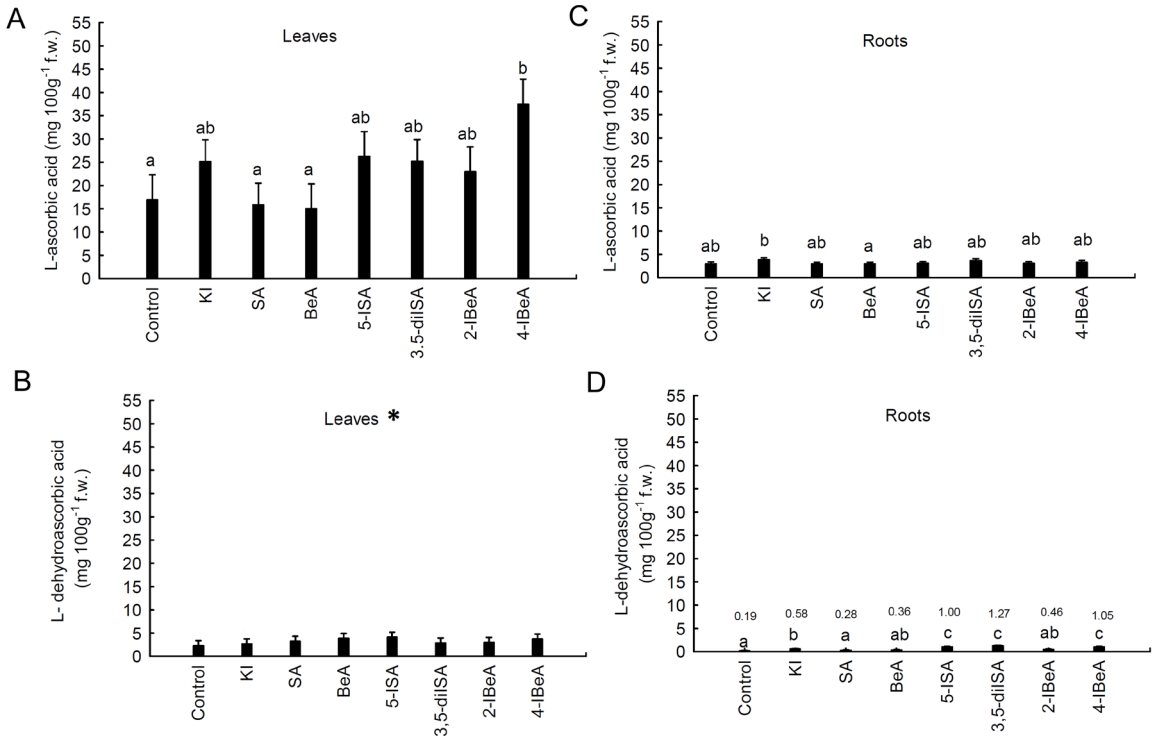

Figure 4. Content of L-ascorbic /AA/ (A, C) and L-dehydroascorbic /DHA/ (B, D) acids in tomato leaves and roots. Means followed by the different letters are not significantly different for $p < 0.05$. Bars indicate standard error (n = 4)

*not significant differences

2.2 for 3,5-diISA; 14.1 for 5-ISA and 4-IBeA; 64.7 for 4-IBeA and 308.5 for KI treatments, respectively (Halka et al., 2018). Iodine compounds application had an influence on iodine uptake by shoot system and roots of tomato plants (Fig. 3). Iodine after KI application was mainly accumulated in shoot system (Fig. 3A), but after organoiodine compounds application in roots (Fig. 3B). In shoot system the iodine uptake after KI application was 49 times higher than after 3,5-diISA, 12 times after 5-ISA and 2-IBeA and 9 times after 4-IBeA application (Fig. 3A). In roots the highest iodine uptake was observed after 2-IBeA treatment (Fig. 3B).

Ascorbic and dehydroascorbic acids

In the roots and leaves of tomato plants treated with the tested compounds the content of the reduced form of vitamin C (AA) exceeded the content of DHA (Fig. 4A-D). Only the application of 4-IBeA significantly increased the level of AA in leaves as compared to the control (Fig. 4A). As for roots, the content of DHA increased mostly in the plants treated with 3,5-diISA, 4-IBeA and 5-ISA (approximately 6-time increase), followed by the application of KI, for which a three-time increase in DHA content was noted (Fig. 4D). For the remaining iodine compounds, the level of DHA

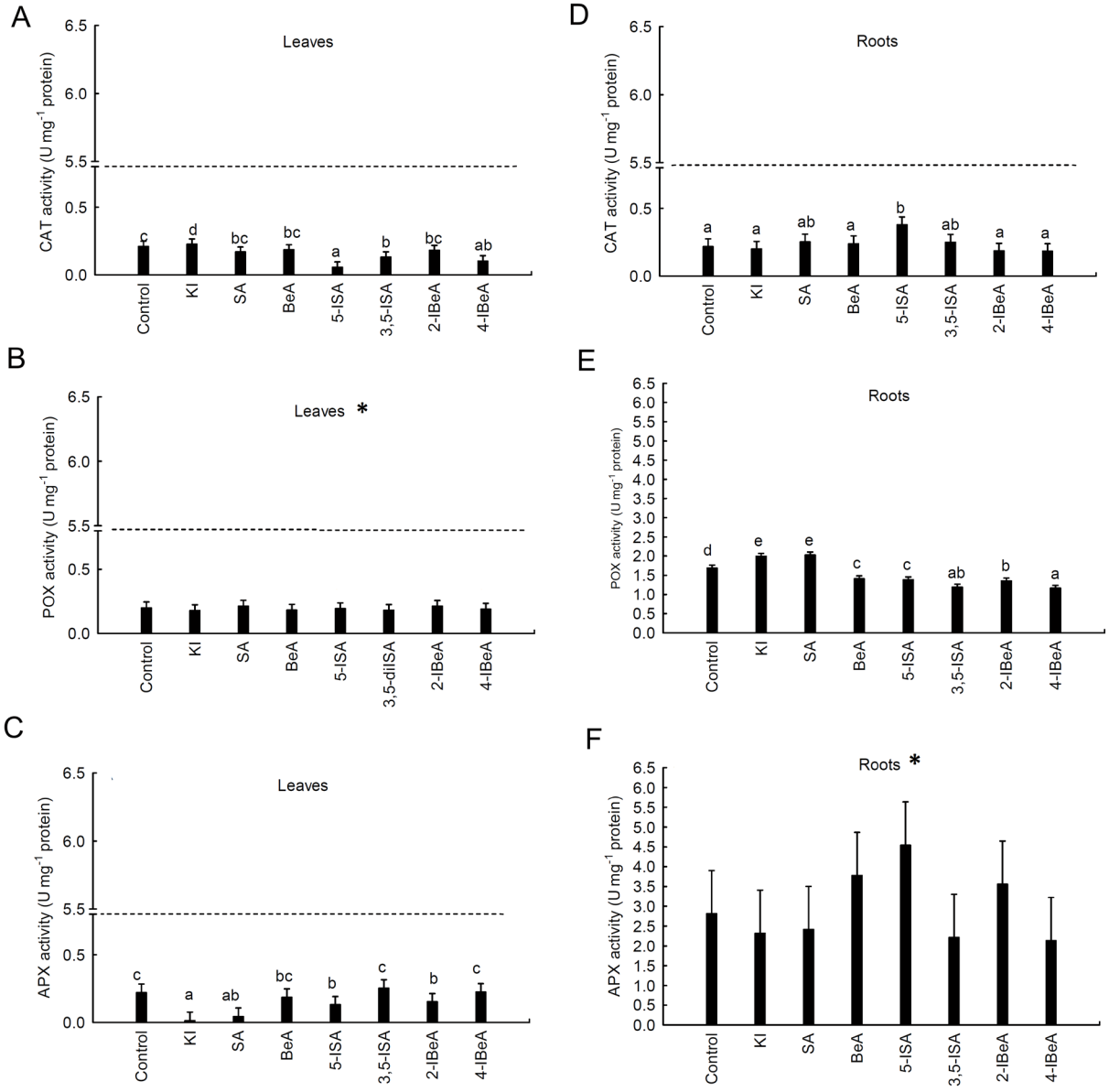

Figure 5. Catalase /CAT/ (A, D), guaiacol peroxidase (/POX/ (B, E) and ascorbate peroxidase /APX/ (C, F) activity in tomato leaves and roots. Means followed by the same letters are not significantly different for $p < 0.05$. Bars indicate standard error (n = 4)

*not significant differences

in tomato roots was comparable to the control values. What is more, the tested compounds did not substantially affect the level of AA in roots and DHA in the leaves (Figs 4C and 4B).

Enzyme activity

A significant effect of the tested compounds was noted with respect to the activity of CAT in leaves and roots, POX in roots and APX in leaves (Fig. 5A-F).

All tested compounds, with the exception of mineral form KI, caused a decrease in CAT activity in leaves as compared to the control plants (Fig. 5A). The greatest reduction of CAT activity in leaves was noted in plants treated with 5-ISA (by 73%), 4-IBeA (by 51%) and 3,5-diISA (by 38%). On the

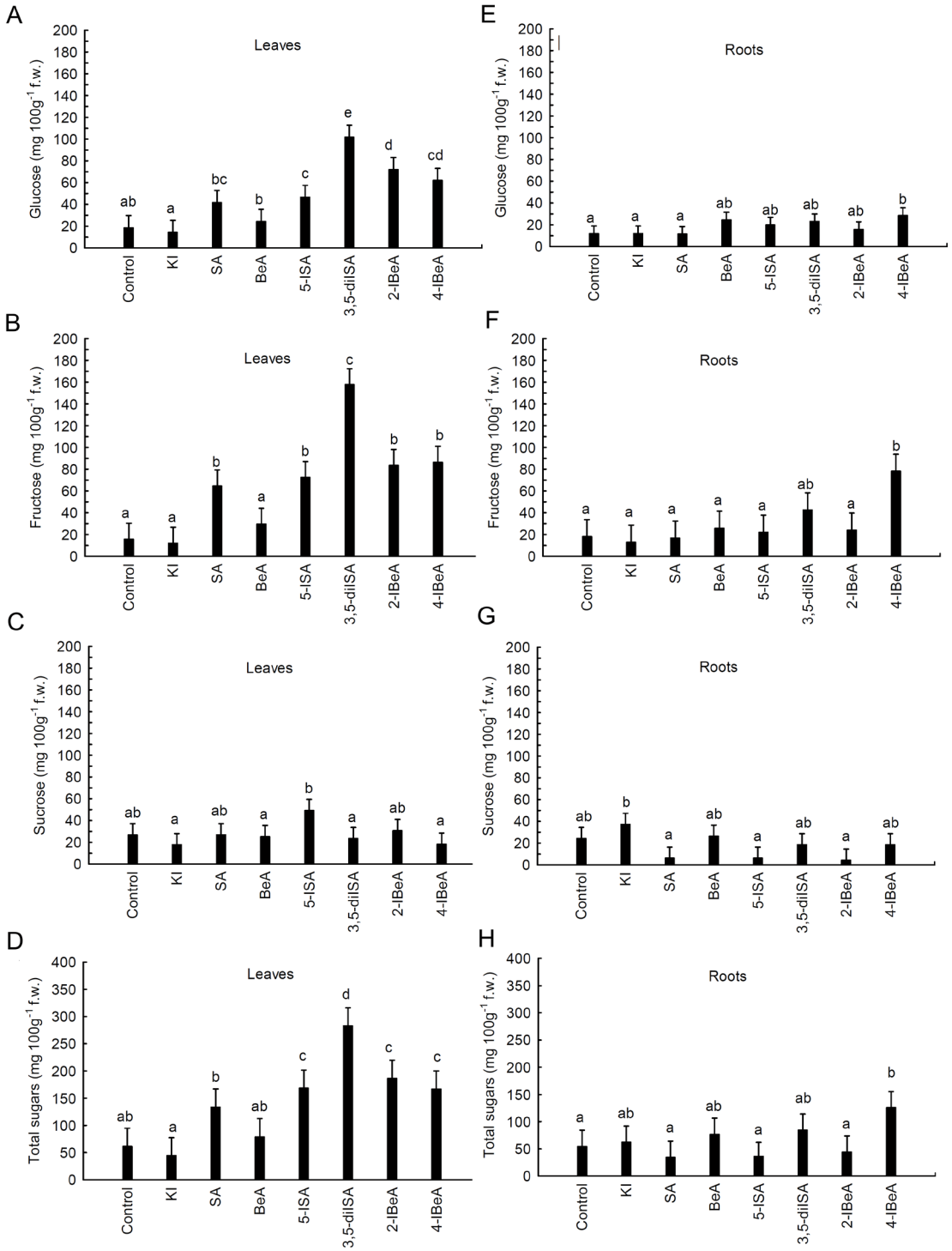

Figure 6. Content of glucose (A, E), fructose (B, F), sucrose (C, G) and total sugars (D, H) in tomato leaves and roots. Means followed by the same letters are not significantly different for $p < 0.05$. Bars indicate standard error (n = 4)

other hand, the activity of CAT in roots improved only after the application of 5-ISA − by 73% as compared to the control (Fig. 5D). The application of 5-ISA during plant cultivation resulted in the highest CAT activity in roots, the lowest in leaves.

The tested iodine compounds as well as SA and BeA had no effect on POX activity in leaves of tomato plants (Fig. 5B). As for roots, the application of SA and KI led to a significant increase in POX activity - approximately by 19% as compared to the control (Fig. 5E). Other applied compounds reduced POX activity in roots with the greatest decrease noted for 4-IBeA (by 30.8%) and 3,5-diISA (by 29.2%) treatment.

All tested compounds, except for 3,5-diISA and 4-IBeA, reduced APX activity in leaves of tomato plants (Fig. 5C). The most substantial decrease of parameter (by 94%) was caused by KI application followed by SA (by 80%), 5-ISA (by 41%) and 2-IBeA (by 31.5%). Despite the lack of statistical significance was observed a tendency of increasing APX activity in tomato roots after the application of 5-ISA (Fig. 5F).

Content of soluble sugars

In tomato leaves and roots the content of glucose, fructose, sucrose and total sugars varied depending on the compound introduced into the nutrient solution (Fig. 6A-H). The highest content of sucrose was noted in leaves of plants grown in the presence of 5-ISA – however this value didn't differ significantly from the control (Figs 6C and 6G).

As for other analyzed sugars, the application of all tested organoiodine compounds (5-ISA, 3,5-diISA, 2-IBeA, 4-IBeA) significantly increased the level of glucose, fructose and total sugars in tomato leaves as compared to the control as well as the application of KI and BeA (Figs 6A, 6B and 6D). The highest total content of sugars in leaves was noted in the combination with 3,5-diISA. The application of SA also contributed to a significant increase of the content of glucose, fructose and total sugars in tomato leaves. On the other hand, after the application of 4-IBeA the level of glucose, fructose and total sugars increased in the tomato roots (Figs 6E, 6F and 6H). The content of sugars in the roots of plants treated with other tested compounds did not vary from the control value.

It needs to be underlined that in the leaves and roots of tomato from the control and KI combination the content of sucrose slightly exceeded that of glucose and fructose. In all the plants subjected to organoiodine compounds and SA, the predominant sugars occurring in leaves were fructose and glucose which mostly contributed to the increase of total sugar content in leaves (Figs 6A, 6B and 6D).

Content of phenolic compounds

Substantially higher accumulation of phenolic compounds including: phenylpropanoids, flavonoids and anthocyanins was noted in the leaves than roots of tomato plants (Fig. 7A-H). The level of these compounds varied significantly between tested combinations, with the exception of flavonoid content in leaves (Fig. 7C).

The sum of phenolics as well as phenylpropanoids and anthocyanins in the leaves of tomato from all tested combinations was similar to the control value (Figs 7A, 7B and 7D). It is worth noticing that the application of 2-IBeA resulted in the lowest accumulation of the sum of phenolics, phenylpropanoids and anthocyanins in tomato leaves, even below the control values (by 16%, 11% and 8%, respectively).

In the case of tomato roots, the lowest level of phenolic compounds including phenylpropanoids, flavonoids and anthocyanins was noted in plants subjected to the mineral iodine (KI, Fig. 7E-H). The highest content of these compounds was found in plants treated with 4-IBeA and 3,5-diISA. However, only the level of phenols and phenylpropanoids in roots of plants treated with 4-IBeA and 3,5-diISA was statistically higher than in the control.

DISCUSSION

Iodine uptake and tolerance of tomato plants to iodine compounds

Studies conducted by other authors revealed that the application of 10 mM KI and KIO_3 during plant cultivation does not lead to plant damage or decrease of the yield (Landini et al., 2011). Exceeding the dose causes negative symptoms of toxicity including: leaf chlorosis, epinasties or plant withering (Landini et al., 2011; Kiferle et al., 2013). In the present study a negative effect on the growth of tomato leaves was observed for 3,5-diISA and 4-IBeA. The growth of roots was also impaired after the application of 3,5-diISA. All other iodine compounds (KI, 5-ISA, 2-IBeA) applied in a 25 μmol I dose did not affect the growth and development of tomato plants. The obtained results indicate a different level of plant sensitivity to 3,5-diISA and 4-IBeA as compared to KI, 5-ISA and 2-IBeA. It may be the outcome of differing mechanisms of the uptake and metabolism of these compounds in plants as documented in the previous

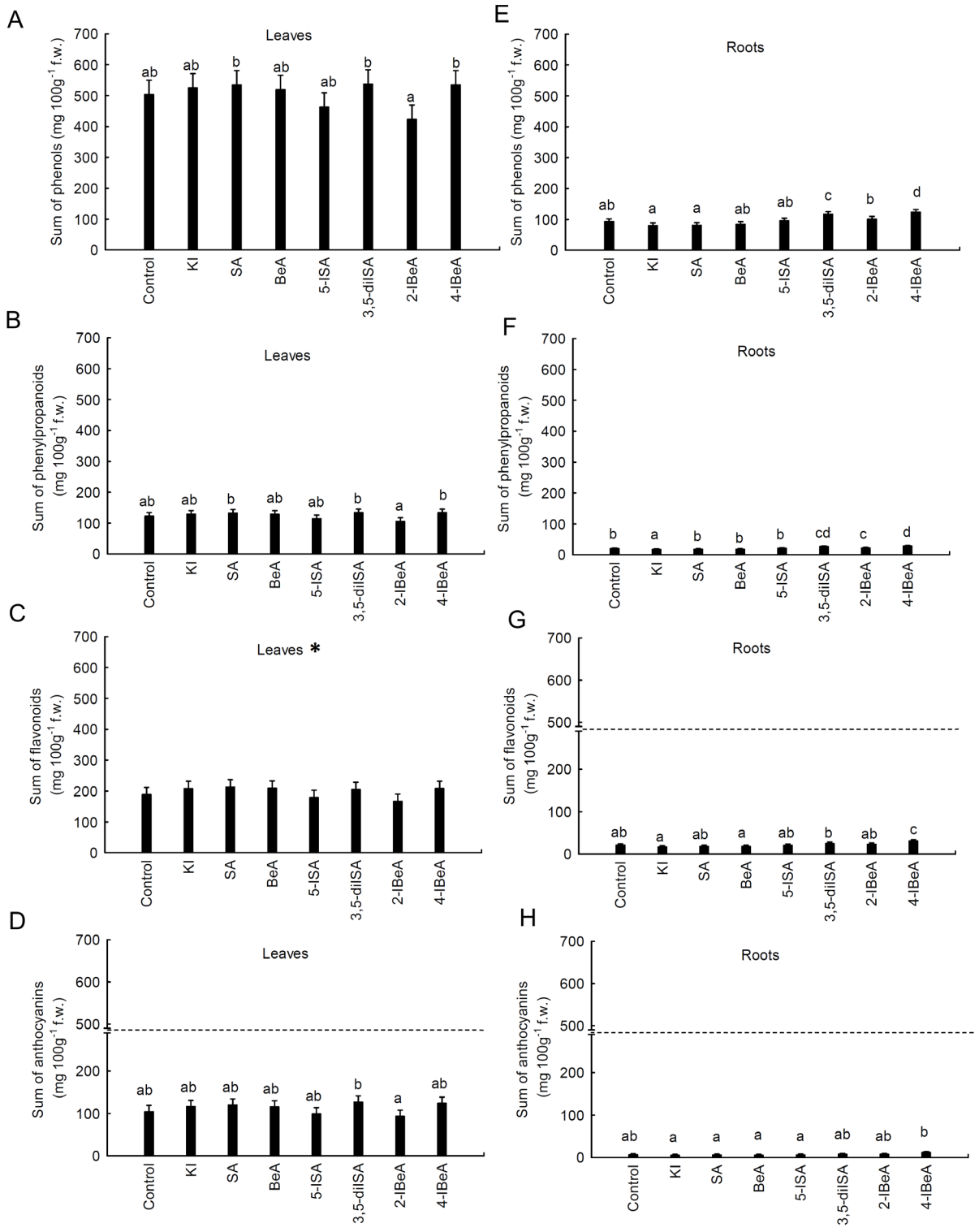

Figure 7. Content of total phenols (A, E), phenylpropanoids (B, F), flavonoids (C, G) and anthocyanins (D, H) in tomato leaves and roots. Means followed by the same letters are not significantly different for $p < 0.05$. Bars indicate standard error (n = 4)

*not significant differences

report covering the results of the molecular studies and iodine content in plants (Halka et al., 2018).

In our study all iodine compounds increased iodine level in shoot system and in the roots of tomato plants. KI was mainly accumulated in upper parts of plant and organic forms was found mainly in roots. Probably it can be related to the simpler KI structure and its faster movement to shoot system from roots compared to iodosalicylates and iodobenzoates, which are containing aromatic

rings in their structure (Halka et al., 2018). We also observed that organoiodine compounds, which contains one iodine in their structure (5-ISA, 2-IBeA and 4-IBeA) were taking up better than 3,5-diISA which has two atoms of iodine connected to aromatic rings. It can be concluded that iodine uptake depends on the structure of the iodine compound.

AA and DHA content in tomato plants after iodine biofortification

The imbalance between ROS formation and the efficiency of plant detoxification system is a primary cause of oxidative stress (Tripathy and Oelmüller, 2012). Various types of ROS, such as: superoxide, hydrogen peroxide, hydroxyl radicals, singlet oxygen are generated under the influence of numerous environmental factors as well as during metabolic processes in plant cell organelles (Smirnoff and Wheeler, 2000).

AA is one of the most important antioxidative compounds that can interact with various already mentioned forms of ROS (Smirnoff and Wheeler, 2000). In plant cells, AA is mainly present in the cytoplasm but can also be distributed through cell membrane to apoplast. Due to its apoplastic localization AA plays a significant role in stress signaling, redox homeostasis and the regulation of oxidative stress as well as physiological and biochemical plant response to various conditions, including abiotic stress (Akram et al., 2017).

Studies conducted by Blasco et al. (2011) revealed that the application of inorganic iodine as I^- and IO_3^- may improve the accumulation of L-ascorbic acid (AA) and its oxidized form (DHA) in lettuce plants. The greatest increase of AA and DHA content was noted after the addition of 80 μM I as I^- into the nutrient solution. The results of our study also revealed the increase of AA and DHA level in tomato leaves and roots after the application of KI (Fig. 4). It is also worth mentioning that all applied organoiodine compounds, with the exception of 5-ISA, increased the accumulation of AA in leaves (Fig. 4A) with no effect on its content in roots (Fig. 4C).

In plants AA is synthesized in a few metabolic routes. One of the most important is the Smirnoff-Wheeler or D-mannose/L-galactose pathway with D-glucose as a precursor (Wheeler et al., 1998). Possibly, the increase in AA content in tomato leaves after the application of tested iodine compounds, in particular organoiodine compounds, was related to the observed higher levels of glucose.

AA is a reducing agent that may be oxidized and then conversed into dehydroascorbic acid (DHA) by losing two electrons. DHA can be reduced back to AA by some enzymatic pathways, including dehydroascorbate reductase (DHAR), or through GSH-independent pathway (Akram et al., 2017). The content of reduced form of ascorbic acid (AA) is usually higher than DHA (Deutsch 1997). Two enzymes are involved in the AA metabolism that leads to the formation of DHA, namely: ascorbate oxidase (AO) and ascorbate peroxidase /APX/ (Akram et al., 2017). None of the tested compounds changed the level of DHA in leaves as compared to the control (Fig. 4B). In the roots, however, the content of DHA increased after the application of all organoiodine compounds (mostly for 5-ISA, 3,5-diISA, 4-IBeA), KI and BeA (Fig. 4D). It can be concluded that the mentioned organoiodine compounds may have directed the synthesis of DHA into the AO pathway as no modification of APX activity in roots was noted.

Basically the content and AA/DHA ratio in the leaves and roots of tomato plants was not affected by the applied iodine compound. It seems significant that with the applied doses these compounds did not reduce the level of oxidized and reduced form of L-ascorbic acid (DHA and AA) in vegetative parts of tomato plants.

Sugars – secondary metabolites as antioxidants

It is recognized that soluble sugars occurring in the plants cells may act as ROS scavengers, particularly when present in very high concentrations (Van den Ende and Valluru, 2009). There is some evidence to suggest that synergistic interaction occurs between sugars or sugar-like compounds and phenolics resulting in its participation in the antioxidative metabolism in plants as well as plant resistance to various stress factors (Bolouri-Moghaddam et al., 2010). Various environmental factors including: light, water or pathogens, may decrease the level of soluble sugars in plant tissues as well as limit the photosynthetic efficiency (Journet et al., 1986). Sucrose and glucose play a significant role as substrates for cell respiration (Gupta and Kaur, 2005) or as osmolytes that help to maintain the homeostasis when fructose is directed to the synthesis of secondary metabolites (Hilal et al., 2004). Glucose along with fructose are the substrates for sucrose biosynthesis that takes place in the cytosole (Lunn and MacRae, 2003). After its synthesis in the green parts of the plants, sucrose is transported through phloem into the below-ground

organs where it undergoes degradation into glucose and fructose (Kühn et al., 1999).

Some studies indicate that iodine compounds may modify the accumulation of selected sugars in plants (Blasco et al., 2011; Smoleń et al., 2015). Application of mineral iodine in the form of KI in a dose of 7.88 μM I decreased the level of fructose in tomato fruits with no effect on the glucose concentration (Smoleń et al., 2015). In other studies application of 40 μM KI increased the level of fructose in lettuce leaves. The level of sucrose and glucose in leaves was additionally modified when KI was applied in 20 or 40 μM dose (Blasco et al., 2011). When iodine was applied as IO_3^- the level of soluble sugars remained comparable to the control both in tomato fruits (Smoleń et al., 2015) and lettuce leaves (Blasco et al., 2011). In the current studies the application of KI in a concentration of 25 μM I contributed to a decrease in the content of glucose and sucrose in leaves but no changes in the level of fructose in both leaves and roots of tomato were noted (Fig. 6). However, the introduction of the mineral form of iodine (KI) increased the sucrose content in tomato roots what may support the hypothesis of a stimulation action of KI on sucrose transport through phloem into the below-ground parts of the plant.

A substantial increase in the level of fructose and glucose (but not sucrose) in the leaves of tomato was observed in all combinations with the application of organoiodine compounds that are the derivatives of SA or BeA (Figs 6A, 6B and 6D) – for the roots such dependency was noted only for the fructose after the application of 4-IBeA. It may indicate that the organoiodine compounds (5-ISA, 3,5-diISA and 2-IBeA) may enhance sucrose hydrolysis into fructose and glucose in leaves with no particular effect on supplying assimilates to roots. An increased amount of glucose and fructose that is formed in the leaves due to the application of 5-ISA, 3,5-diSA and 2-IBeA is most likely consumed in various metabolic processes in leaves excluding the synthesis of phenolic compounds. The level of total phenolics, phenylpropanoids, flavonoids and anthocyanins in tomato leaves was not affected by the application of any tested compounds.

Phenolic compounds in tomato plants

Phenolic compounds are included into the most important non-enzymatic antioxidants showing a wide spectrum of action. An increase of the level of phenolics is frequently associated with plant response to mechanical damage, pathogens or other stress factors (Sikora et al., 2008). In general, phenolic compounds are plant secondary metabolites that are formed through pentose phosphate, shikimate and phenylpropanoid pathways (Randihr et al., 2004). Flavonoids are synthesized through phenylpropanoid pathway that converts phenylalanine into 4-cumaroyl-CaA that is directed into the route of flavonoid synthesis. The process of flavonoid biosynthesis is regulated by numerous enzymes of various classes: isomerases, reductases, hydroxylases and other (Martens et al., 2010). Anthocyanins are synthesized in the same metabolic route as flavonoids from three molecules of malonyl-CoA and one molecule of 4-cumaroyl-CoA (Zhang et al., 2014).

Some works on plant biofortification with iodine have revealed the influence of mineral forms of iodine such as I^- and IO_3^- on the level of particular groups of phenolic compounds in plants (Blasco et al., 2008; Kapusta-Duch et al., 2017). Studies conducted by Blasco et al. (2008) on lettuce plants indicate that the application of mineral forms of iodine may increase the total content of phenolics in plant cells as well as of flavonoids and anthocyanins. The greatest increase of total phenolics and flavonoids was observed after iodine application in the form of I^- and dose of 120 and 160 μM. The highest concentration of anthocyanins in plants was noted in the combination with I^- applied in the concentration of 80 μM. Introduction of iodates (IO_3^-) into the nutrient solution, particularly in relatively high doses, also increased the level of phenolic compounds in lettuce (Blasco et al., 2008). In the studies conducted by Kapusta-Duch et al. (2017) a significant decrease in the content of phenolic was noted in carrot plants treated with KI.

The increase of total content of phenolics in tomato roots noted after the application of 4-IBeA and 3,5-diISA was most likely related to its toxicity is further confirmed by decreased values of index of tolerance for leaves and roots, as in the case of 4-IBeA. Interestingly, the mineral form of iodine, *i.e.* KI, did not contribute to any changes in the content of phenolic compounds, phenylpropanoids, flavonoids and anthocyanins in the leaves and roots. It may have been related to a greater tolerance of tomato plants to KI as compared to organoiodine compounds. The experiment conducted by Golubkina et al. (2018) in enrichment *Brassica juncea* L. with iodine (KI) and selenium (Na_2SeO_4) separately and together showed the increase of flavonoids in leaves (Golubkina et al, 2018). That's

show that the content of some phenolic compounds may depend on the plant species.

Enzymatic antioxidants in tomato leaves and roots

Antioxidant system of plant cell comprises not only of antioxidant compounds (such as ascorbic acid or phenolic compounds) but also specific enzymes such as: superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), ascorbate peroxidase (APX) and others (Iba, 2002).

Salicylic acid is included into the compounds that function as endogenous signaling and activating factors for the plant defense system against various stress conditions. In tobacco SA may bind to soluble SA-binding proteins (SABP) with a size of 280 kDa (Chen and Klessig, 1991; Chen et al., 1993). SABP converse harmful H_2O_2 to H_2O and O_2 exhibiting the similar activity as CAT. It may also be related to the decrease of CAT activity in plant cells (Conrath et al., 1995). The latter study also showed that SA, as well as its derivatives, when applied a 1 mM dose, may significantly inhibit CAT activity in tobacco plants *in vivo* and *in vitro*, at the same time stimulating plant defense system against pathogen infection. It has been also revealed that triggering that system may be related to the expression of PR-1 gene that codes pathogenesis-related protein at the same time limiting the activity of CAT. It has also been suggested that SA and its derivatives share some common features that enable its binding to SABP as well as allow to inhibit the activity of CAT (Conrath et al., 1995). According to some authors, the loss of CAT activity is a common response of numerous plant species subjected to oxidative stress and is strictly related to SA accumulation in plants (Shim et al., 2003).

Blasco et al. (2011) and Gupta et al. (2015) revealed that the introduction of mineral I^- or IO_3^- into the nutrient solution may modify the activity of selected antioxidative enzymes in plants. In the studies conducted by Blasco et al. (2011) application of I^- or IO_3^- a concentration of 20, 40 and 80 μM during lettuce cultivation significantly improved CAT activity with the highest increase noted for the treatment with 80 μM IO_3^-. An increase in APX activity was noted in lettuce grown in the nutrient solution supplemented with 20, 40 and 80 μM IO_3^- as well as 40 and 80 μM I^-. However, for the lowest concentration of I^- (20 μM) the activity of APX decreased (Blasco et al., 2011). Gupta et al. (2015) found that the treatment with IO_3^- of soybean subjected to heavy metal stress also improved the activity of CAT and APX particularly when iodine was applied in 20 and 40 μM dose.

In the present studies application of 25 μM I as KI increased the activity of CAT in leaves and of POX in tomato roots (Fig. 5A, 5E). No modification of CAT and APX activity in roots as well as of POX in leaves was noted in tomato plants subjected to KI with a significant decrease of APX activity in leaves (Fig. 5). Organoiodine compounds such as 2-IBeA and 4-IBeA as well as BeA did not modify the activity of CAT while all the remaining compounds slightly increased CAT activity in tomato roots (Fig. 5D). Introduction of SA, BeA and organoiodine compounds into the nutrient solution decreased the activity of CAT in tomato leaves (Fig. 5A).

It is considered that SA and its derivatives may contribute to the decrease of APX in leaves (Durner and Klessig, 1995). It has also been confirmed in our studies by the decrease of APX activity in leaves after the application of SA, 5-ISA and 2-IBeA. Interestingly, no such effect was noted for 3,5-ISA and 4-IBeA what may suggest that the latter compounds undergo other metabolic pathways than SA, 5-ISA and 2-IBeA. Also 3,5-ISA and 4-IBeA may not act as intermediates in the regulation of APX activity – in contrast to SA, 5-ISA and 2-IBeA.

The studies on tobacco plants conducted by Durner and Klessig (1995) showed that SA and its derivatives, including halogen ones, may decrease the activity of APX in plant cells in the similar manner as CAT with a simultaneous induction of defense-related genes and the improvement of tobacco resistance to tobacco mosaic virus. Interestingly, the decrease of POX activity may not even be noted (Durner and Klessig, 1995). This observation was further confirmed in the present studies but only with respect to the leaves (Fig. 5B). The decrease of POX in the roots was noted for the plants treated with BeA and all tested organoiodine compounds (5-ISA, 3,5-diISA, 2-IBeA and 4-IBeA). The increase in the root activity of APX was observed in the plants from the combination with the mineral iodine form, *i.e.* KI and SA (Fig. 5D). It may be assumed that BeA as well as organoiodine compounds may affect the activity of POX in tomato roots in a different manner than SA and KI. The analyzed iododerivatives of SA and BeA may limit the activity of POX similarly to that of CAT. SA is a weak inhibitor of POX as it can not compete with the most suitable substrates for these peroxidases, such as guaiacol, pyrogallol or hydroquinone (Durner and Klessig, 1995). BeA and the tested

organoiodine compounds have higher affinity to POX in the roots of tomato plants than SA.

It needs to be mentioned that a negative effect of 3,5-diISA and 4-IBeA on the plant growth and development (as described by the values of index of tolerance) was not further reflected by the modified activity of any of analyzed enzymes (CAT, POX, APX) in the leaves and roots. Therefore, the stress conditions caused by 3,5-diISA and 4-IBeA triggered other defense mechanisms than these engaging CAT, POX and APX enzymes.

CONCLUSIONS

The uptake mechanism of organoiodine compounds by higher plants is not fully understood. Our study showed that only 3,5-diISA and 4-IBeA had a negative effect on plant growth and development, while the remaining compounds were tolerated by tomato plants at an early stage of growth. KI was mainly accumulated in shoot system but iodosalicylates and iodobenzoates in root system. This observation may be associated with different mechanisms of the uptake and accumulation of iodine compounds.

The tested compounds modified the CAT and APX activity, but had no effect on POX activity in leaves. The decrease of CAT and APX activity, as observed for some treatments, may be closely related to the simultaneous activation of the plant defence system in other metabolic pathways. Importantly, the negative effect of 3,5-diISA and 4-IBeA on the plant growth and development didn't affect the CAT activity in roots, POX in leaves and APX activity in leaves and roots. However, the increased synthesis of phenolic compounds was noted for these treatments.

Organoiodine compounds led to increased fructose and glucose content in leaves, and only 4-IBeA caused increase of fructose in the roots of tomato plants. This proves that 5-ISA, 3,5-diSA and 2-IBeA may stimulate sucrose hydrolysis to fructose and glucose in the leaves, with no significant effect on the level of assimilates in the roots.

Our study showed that iodosalicylates and iodobenzoates can be taking up by tomato seedlings and in the future can be a good source of iodine for tomato biofortification. In addition, the use of this organoiodine compounds for iodine biofortification of tomato plants can also have a positive health-promoting properties. However, further research is needed for greater consumer confidence and safety.

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AUTHOR CONTRIBUTIONS

H. M., S. S. – were responsible for planning the experiment, conducting plant cultivation, analysis of plant material, results interpretation and writing the manuscript. L.-S. I. – conducted chemical analyses as well as manuscript preparation. Sady W. – was involved in writing and editing of manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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