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The quality of carrot (*Daucus carota* L.) cultivated in the field depending on iodine and selenium fertilization

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ABSTRACT

The aim of the study was to evaluate the influence of various chemical forms of iodine (I^- and IO_3^-) and selenium (SeO_3^{2-} and SeO_4^{2-}) on the nutritional and health-promoting quality of carrot (*Daucus carota* L.) storage roots. The experiment (conducted in 2012-2014) comprised the soil fertilization of carrot 'Kazan' F_1 in the following combinations: 1. Control, 2. KI, 3. KIO_3, 4. Na_2SeO_4, 5. Na_2SeO_3, 6. KI + Na_2SeO_4, 7. KIO_3 + Na_2SeO_4, 8. KI + Na_2SeO_3, 9. KIO_3+ Na_2SeO_3. Iodine and selenium were applied twice: before sowing and as top dressing in a total dose of 5 kg I ha^{-1} and 1 kg Se ha^{-1}. No significant influence of iodine and selenium fertilization was noted with respect to average root weight and leaf yield. Each year, the application of KI + Na_2SeO_4 negatively affected the content of glucose and total sugars in carrot. An increased sucrose level was noted in the roots of plants treated with KIO_3+ Na_2SeO_4, with a total sugar concentration comparable to the control. Irrespective of the year, carrots fertilized with KI were characterized by the highest accumulation of nitrates (III) – NO_2^- in roots. The simultaneous introduction of iodine and selenium compounds (KI + Na_2SeO_4, KIO_3 + Na_2SeO_4, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3) into the soil reduced the content of nitrates (III) in carrot as compared to combinations with the individual application of these compounds. The influence of the tested factors on other analysed parameters (the content of dry weight, nitrates (V), chlorides, oxalates, citrates, free amino acids, carotenoids, phenolic compounds, phenylpropanoids, flavonols and anthocyanins as well as free radical scavenging activity (DPPH) was rather year-dependent.

Key words: biofortification, carotenoids, citrates, DPPH , oxalates, phenolic compounds, sugars

Abbreviations:

GS-GOGAT – glutamine synthetase-glutamate synthase; NiR – nitrite reductase; NR – nitrate reductase; PAR – photosynthetically active radiation; SeMet – selenomethionine; SeCys – selenocysteine

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INTRODUCTION

Carrot is one of the most popular crop species both in Poland and worldwide and its nutritional and health-promoting quality is of great importance, particularly with respect to the production of baby food (Cebulak and Sady 2000, Rożek et al. 2000, Sady and Cebulak 2000).

The biofortification (enrichment) of crop plants with mineral nutrients (Fe, Zn, Se, I, Mg, Ca) is one of the low-cost but effective methods of their introduction into the food chain. It is particularly recommended for areas with substantial deficiency of micronutrients in soils (White and Broadley 2009, Przybysz et al. 2016).

Selenium and iodine are not essential nutrients for plants – yet selenium is included in the group of beneficial elements (Kopsell and Kopsell 2007, Kabata-Pendias 2011). However, both elements play important roles in human and animal organisms. A common situation is an occurrence of a simultaneous deficiency or hidden hunger for iodine and selenium in numerous regions of the world (White and Broadley 2009).

Many studies have documented the possibility of increasing the accumulation of iodine (Blasco et al. 2010, Kato et al. 2013, Lawson et al. 2015) and selenium in plants (Ríos et al. 2010, Hawrylak-Nowak et al. 2015). However, the problem of simultaneous plant fertilization with these elements has not been often described. Such an approach requires the development of optimal agro-technical rules for conducting double biofortification of crop plants with iodine and selenium.

So far, studies on the simultaneous application of iodine and selenium have mainly been conducted in soilless and hydroponic systems with the cultivation of spinach (Zhu et al. 2004) or lettuce (Smoleń et al. 2014 a). In field conditions, a single one-year experiment was carried out with the application of Se + Zn + I fertilization during the cultivation of wheat, maize, soybean, potato, canola and cabbage (Mao et al. 2014). The above-mentioned works document solely the efficiency of biofortification, *i.e.* improved uptake and accumulation of iodine and selenium by crop plants, but lack the description of the effect of its increased content on the nutritional and health-promoting quality of biofortified crops. Only Smoleń et al. (2014a) have presented the spectrum of effects exerted by the simultaneous application of I and Se – foliarly or through the nutrient medium – on the uptake and accumulation of macro- and micronutrients by lettuce plants. Double iodine and selenium biofortification of carrot plants has not yet been studied, therefore, no information is available as to whether this process is even possible. Conducting such studies may help to fill the information gap with respect to the influence of iodine and selenium application on the nutritional quality of carrot storage roots. It is all the more important as the soil application of iodine alone (also when combined with nitrogen fertilization) diversely changes the nutritional and health-promoting quality of carrot roots. It also affects the tested parameters after storage (Smoleń et al. 2014b).

Iodine and selenium uptake activates the metabolic processes responsible for the conversion of these elements into organic compounds, including the biosynthesis of seleno-amino acids: selenomethionine (SeMet) and selenocysteine (SeCys) (Zhu et al. 2009, Winkel at al. 2015, Longchamp et al. 2015), iodine-containing proteins (Kabata-Pendias 2011) or the emission of volatile iodine (CH_3I – Rhew et al. 2003) or selenium compounds: dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) (Zhu et al. 2009, Winkel et al. 2015). All of these processes require a substantial input of energy and may impair the production and accumulation of sugars or secondary metabolites, including those responsible for organoleptic, nutritional and health-promoting values (nutraceuticals). In the case of carrot storage roots, the following parameters should be taken into account: the content of sugars (fructose, glucose, sucrose, total soluble sugars), free amino acids, carotenoids, phenolic compounds as well as free radical scavenging activity (Barańska et al. 2005, 2006, Quilitzsch et al. 2005, Barański et al. 2012, Smoleń et al. 2014b).

The research hypothesis stated that conducting simultaneous fertilization with iodine and selenium would allow us to obtain a carrot yield with improved or unchanged quality. The aim of the study was to evaluate the influence of soil fertilization with various chemical forms of iodine (I^- and IO_3^-) and selenium (SeO_3^{2-} and SeO_4^{2-}) on the nutritional and health-promoting qualities of storage roots of carrot (*Daucus carota* L.)

MATERIAL AND METHODS

Plant material and treatments

In the years 2012-2014, the field cultivation of carrot (*Daucus carota* L.) cultivar Kazan F_1 was conducted in Marszowice (50°18’6 N, 20°09’1 E) near Krakow, Poland. Each year, carrot was grown

Table 1. Selected physical and chemical properties of the 0-30 cm soil layer prior to the experiment in 2012-2014 (n = 4)

Parameter	2012	2013	2014
pH_{H_2O}	6.30	7.77	6.10
EC (mS cm^{-1})	0.13	0.12	0.04
Eh (mV)	+ 220.0	+ 257.9	+ 233.5
Organic matter (%)	2.11	2.48	2.25
Particle size fraction (in %): sand / silt / loam	4 / 47 / 49	2 / 48 / 50	4 / 47 / 49
Soil texture class	Silty clay (heavy soil)	Silty clay (heavy soil)	Silty clay (heavy soil)

on the same farm but on different sites within a single soil complex.

Carrot was cultivated on heavy soil of a silty clay type, with a comparable content of main soil separates within the years 2012-2014 (Tab. 1). The chemical properties of the soil in the subsequent years are presented in Table 1.

The study included soil fertilization with iodine and selenium in the following combinations: 1. Control, 2. KI, 3. KIO_3, 4. Na_2SeO_4, 5. Na_2SeO_3, 6. KI + Na_2SeO_4, 7. KIO_3 + Na_2SeO_4, 8. KI + Na_2SeO_3, 9. KIO_3 + Na_2SeO_3. Iodine and selenium were applied twice: prior to seed sowing (before ridge formation) and as top-dressing (at canopy closure), each at a dose of 2.5 kg I ha^{-1} + 0.5 kg Se ha^{-1}. Pre-sowing fertilization with iodine and selenium was conducted in the third 10-day period of April (12 April 2012, 18 April 2013 and 04 April 2014), and as top-dressing in the third 10-day period of June (29 June 2012, 3 July 2013 and 4 July 2014). The total amount of iodine and selenium introduced into the soil was 5 kg I ha^{-1} and 1 kg Se ha^{-1}, respectively. Iodine and selenium were applied as KI and KIO_3 (Avantor Performance Materials, Poland) as well as Na_2SeO_4 and Na_2SeO_3 (Sigma-Aldrich, Germany); all compounds were of analytical grade. The experiment was arranged in a split-plot design. Each treatment was randomized in four repetitions on 4 m × 6 m (24 m^2) plots. The total area of the experiment was 864 m^2.

Based on the results of the soil chemical analysis, N, P and K fertilization was conducted (along with iodine and selenium application) one day prior to ridge formation in order to supplement nutrient contents to the level optimal for carrot: N-100, P-80 and K-200 (in mg dm^{-3} of soil). Nitrogen was applied as urea, phosphorus as ammonium phosphate, and potassium as 60% potassium salt.

Carrots were cultivated in single rows on 40 cm-wide and 30 cm-high ridges at a seeding rate of 37 seeds m^{-1} (approximately 600,000 seeds per hectare). The seeds were sown on 19 April 2012, 25 April 2013 and 05 April 2014. Carrot roots were harvested on 26 September 2012, 11 September 2013 and 9 September 2014. During harvest, the yield of carrot leaves and storage roots as well as plant density per hectare were determined (detailed data is presented in the publication by Smoleń et al. 2016a). Based on these measurements, the average leaf yield per plant as well as the average weight of the roots were calculated.

At harvest, samples of approximately 10 kg of carrot storage roots were chosen from each of the four plots (replications) for laboratory analysis.

Plant analysis

Dry matter content in carrot storage roots was assayed at 105°C. The total content of carotenoids in fresh carrot roots was analysed using a β-carotene standard curve after sample extraction with acetone/n-hexane (4:6; v/v). The determination of phenols, phenylpropanoids, flavonols and anthocyanins in carrot was conducted in 80% methanol extracts using the spectrophotometric method described by Fakumoto and Mazza (2000). Free radical scavenging activity of the prepared plant extracts was evaluated on the basis of sample reaction with diphenylpicrylhydrazyl (DPPH) (Pekkarinen et al. 1999).

The content of free amino acids, sucrose, glucose and fructose was assessed in ethanol-preserved samples. The level of free amino acids was determined spectrophotometrically after a reaction with ninhydrin. The contents of sucrose, glucose and fructose were analysed using RP-HPLC. The assessment of the level of these compounds was conducted in room temperature using a Knauer system (Germany). Samples (10 µl) were injected into an amine LiChrospher RP 100-10 NH_2 250 × 4 mm column. The eluent contained the mixture of acetonitrile/water (87:13 v/v) and the flow rate was adjusted to 1.3 ml min^{-1}. Detection was conducted using a Smartline RI 2300 refractometric detector (Bogdanov 2002).

The contents of nitrates (V), nitrates (III), chlorides, oxalates and citrates in carrot leaves and roots were analysed using capillary electrophoresis. Plant samples were dried in a dryer with forced air circulation at 70°C and ground in a laboratory grinder. Extraction was conducted according to the following procedure: 0.1 g of air-dried plant samples with 20 ml of extraction solution were put into 30 ml falcon tubes. After mixing, samples were incubated for 1 hour at 90°C, cooled to a temperature of approximately 20°C, mixed thoroughly and centrifuged for 15 min at 4,500 rpm. The supernatants were filtered through a 0.25 µm cellulose acetate membrane filter and analysed using a PA 800 Plus capillary electrophoresis system (Beckman Coulter, USA) with DAD detection. For the measurements, a silica capillary tube with an i.d. of 75 µm, o.d. of 365 µm and a total length of 50 cm was used. A negative power supply of -4 kV was applied. The running buffer solution was prepared as proposed by Zhao et al. (2011), containing 30 mmol NaH_2PO_4, 15 mmol $Na_2B_4O_7$ and 0.2 mmol CTAB (pH 8.80).

Meteorological data

Each year, carrot was grown from April to September. During that period, the total amount of rainfall was 293.4, 428.5 and 437.9 mm, whereas the mean daily air temperature was 16.1, 15.2 and 15.5°C in 2012, 2013 and 2014, respectively (Fig. 1 – data from the HOBO Weather Station). The average daily PAR value during the time from

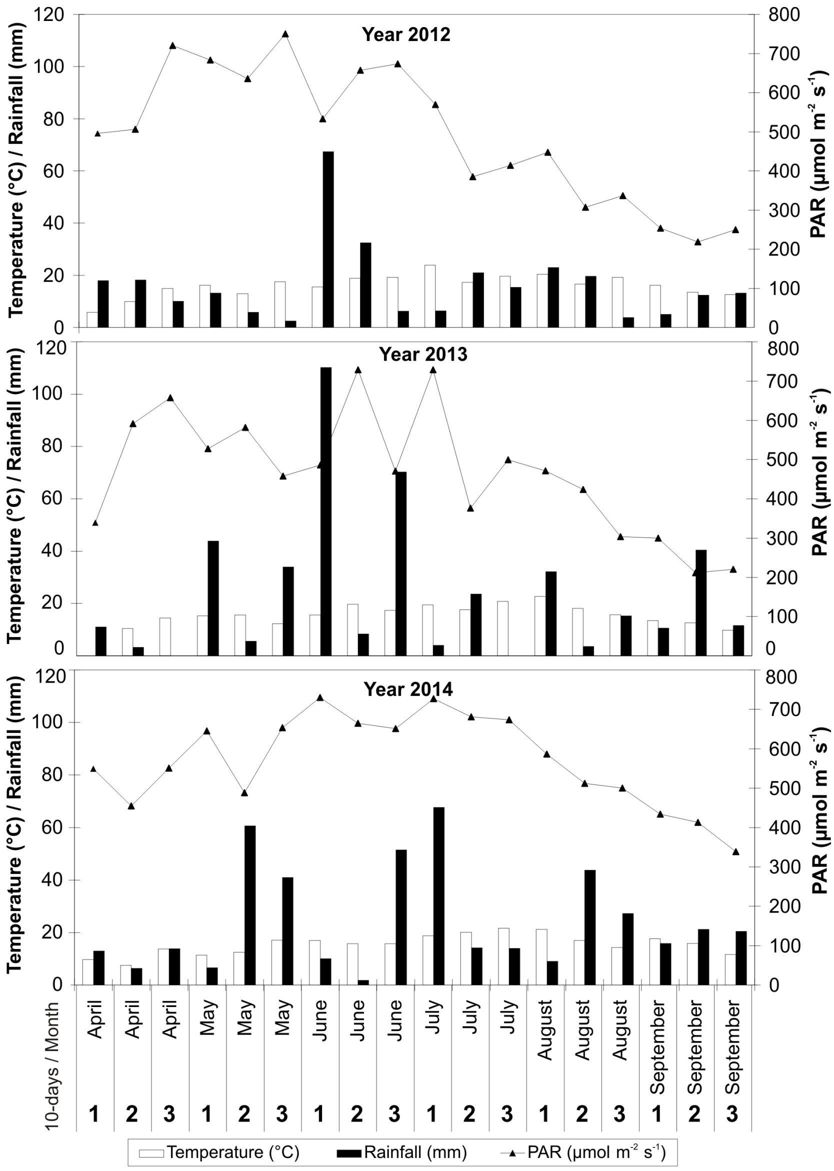

Figure 1. Meteorological data for carrot cultivation in 2012, 2013 and 2014

carrot planting in the field to the harvest was 491.0, 465.3 and 569.6 µmol $m^{-2} s^{-1}$ in 2012, 2013 and 2014, respectively.

In 2012, the most unfavourable conditions with respect to the amount and distribution of rainfall occurred, as compared to the years 2013-2014 (Fig. 1). The total amount of rainfall from April to September 2012 was 141.6 mm lower, and in the years 2013-2014, it fluctuated around the average for the period of 1971-2000 (435 mm – GUS 2005). From April to September of 2012, a higher mean daily air temperature was recorded than in the same period in 2013 and 2014 it was 1.8°C higher than the mean from the years 1971-2000 (14.3°C, GUS 2005) and was caused by heat spells occurring in the summer months. More precisely, in the years 1971-2000, the average monthly air temperature was 8.0°C in April, 13.4°C in May, 16.2°C in June, 17.8°C in July, 17.5°C in August and 13.2°C in September. In the same period, the average monthly rainfall in the area under study was 50 mm in April, 74 mm in May, 94 mm in June, 81 mm in July, 76 mm in August and 60 mm in September (GUS 2005).

With respect to PAR, its highest total was noted in 2014 and was evenly distributed throughout the entire period of carrot cultivation (Fig. 1). In the years 2012-2013, large fluctuations in PAR values in the subsequent ten-day periods from April to mid-August were recorded.

Data analysis

All data were subjected to an analysis of variance using the ANOVA module of Statistica 10.0 PL. The Tukey test was used for determining the significance between the means. The significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Biomass

Despite the diverse weather conditions in 2012-2014, the tested combinations with iodine and selenium fertilization did not significantly affect the average weight of a single root as well as leaf yield per plant (Fig. 2) in any year of the study.

Generally, the lowest yield of carrot leaves and roots was noted in 2012 due to less favourable weather conditions than those occurring in 2013-2014 – particularly with respect to the smaller amount of rainfall and higher air temperature. The year 2014 was characterized by the most optimal conditions for carrot cultivation and in that year the highest values of leaf yield and average weight of root were noted.

Detailed data concerning the effects of iodine and selenium fertilization on various parameters of carrot yield (including, among others, biological, total and marketable yield) are presented elsewhere (Smoleń et al. 2016a). In the mentioned study, the results of the level of iodine and selenium accumulation in carrot leaves and storage roots are also included.

Using tested doses of both elements, we did not observe any symptoms of their negative effect on carrot plants even despite the substantial levels of iodine and selenium accumulation in leaves and storage roots (Smoleń et al. 2016a). In the other study conducted by our team using the same doses and chemical forms of iodine and selenium, a toxicity of Na_2SeO_4 applied alone or together with iodine on lettuce plants was revealed (Smoleń et al. 2015). This may indicate that carrot is a crop species less sensitive than lettuce to high doses of Na_2SeO_4 when applied alone or together with iodine. It needs

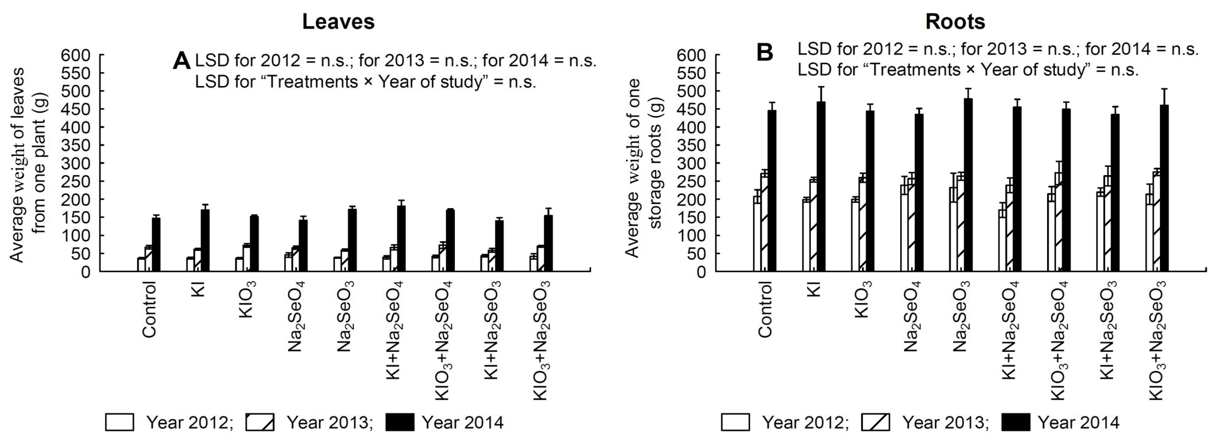

Figure 2. Average weight of leaves per plant (A) and one storage root (B) in each year of the study (2012-2014) depending on the applied I and Se fertilization. Bars indicate standard error (n = 4)

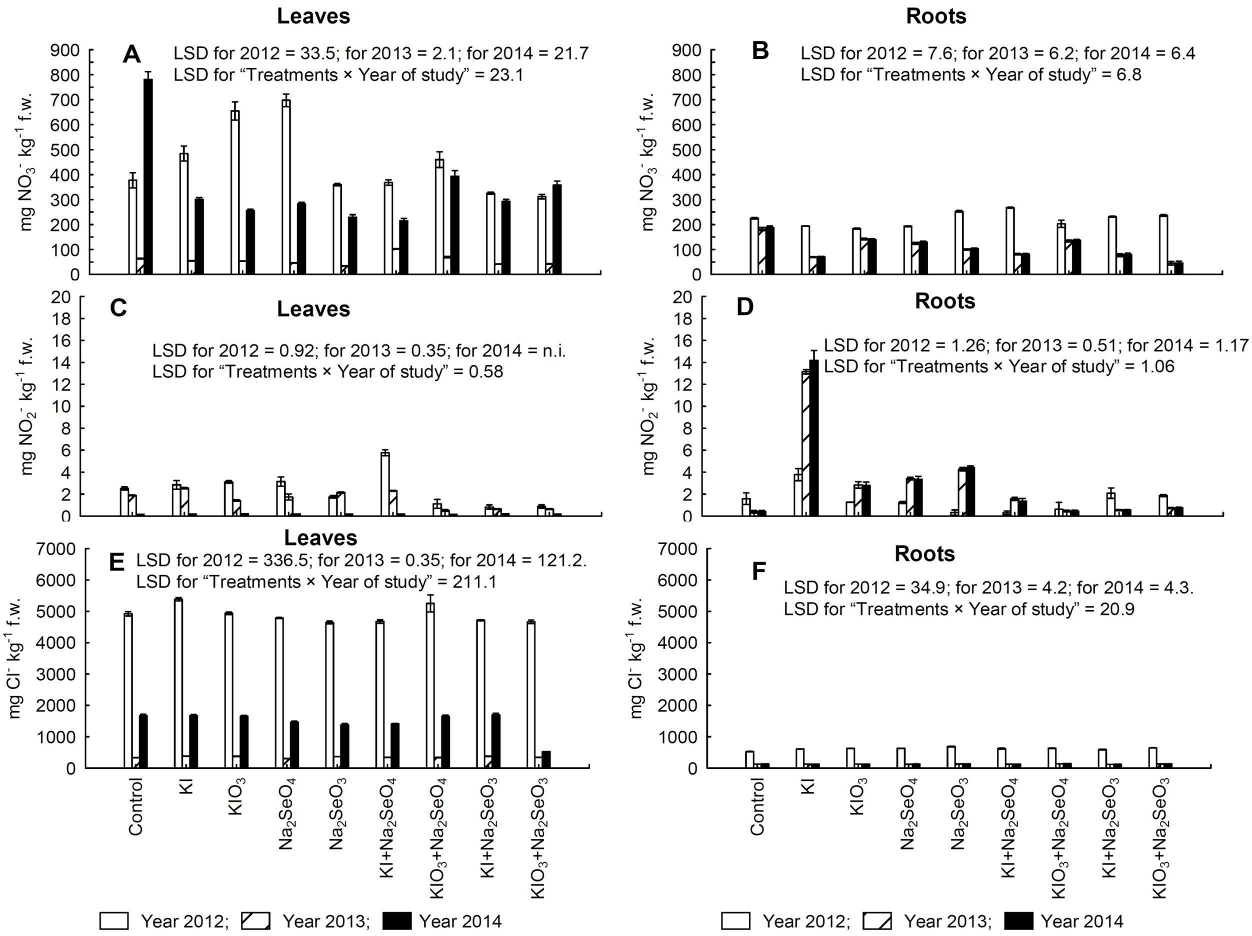

Figure 3. Content of nitrates (V) (A and B), nitrates (III) (C and D) and chlorides (E and F) in leaves (A, C and E) and storage roots (B, D and F) in each year of the study (2012-2014) depending on the applied I and Se fertilization. Bars indicate standard error (n = 4)

to be underlined that plant tolerance to selenates (IV) and selenates (VI) strongly depends on the cultivation system, whether hydroponics or in the field, with complex processes of sorption occurring in the latter (Ríos et al. 2010, Hawrylak-Nowak et al. 2015, Smoleń et al. 2015, 2016a). Also in the case of iodine, carrot is far more tolerant to high doses of this element than other vegetable species, including spinach, pak choi and celery (Dai et al. 2004).

The content of nitrates (III), nitrates (V), chlorides, oxalates and citrates in carrot plants

The content of nitrates (III), nitrates (V), chlorides, oxalates and citrates in carrot leaves and roots was significantly affected by the tested factors, with the exception of nitrate (III) level in leaves in 2014 (Fig. 3 A-F and Fig. 4 A-D). Various weather conditions occurring during carrot cultivation contributed, however, to diverse quantitative relations between combinations regarding these parameters – a significant ‘treatments × year of study’ interaction (Fig. 3 A-F and Fig. 4 A-D).

Generally, a higher content of nitrates (V) and chlorides was found in leaves than in roots, while the latter organs were characterized by an increased accumulation of nitrates (III). With respect to oxalates and citrates, their content remained at the same level in both leaves and roots (Fig. 3 A-F and Fig. 4 A-D). In each year of the study, adverse effects of the applied iodine and selenium were revealed with respect to the leaf content of nitrates (V) as well as leaf and carrot levels of nitrates (III) and chlorides in individual combinations of the study.

Studies on protein isolates revealed that iodate form of iodine (IO_3^-) can be an alternative electron acceptor for nitrate reductase (NR) enzyme (Barber and Notton 1990). We did not find any clear effect of soil fertilization with I and Se on the nitrate (V) content in plants. In other words, iodine application in the form of IO_3^- did not contribute to excessive accumulation of these ions in plant tissues. Such a relationship was previously proposed based on the findings of marine algae studies, suggesting the

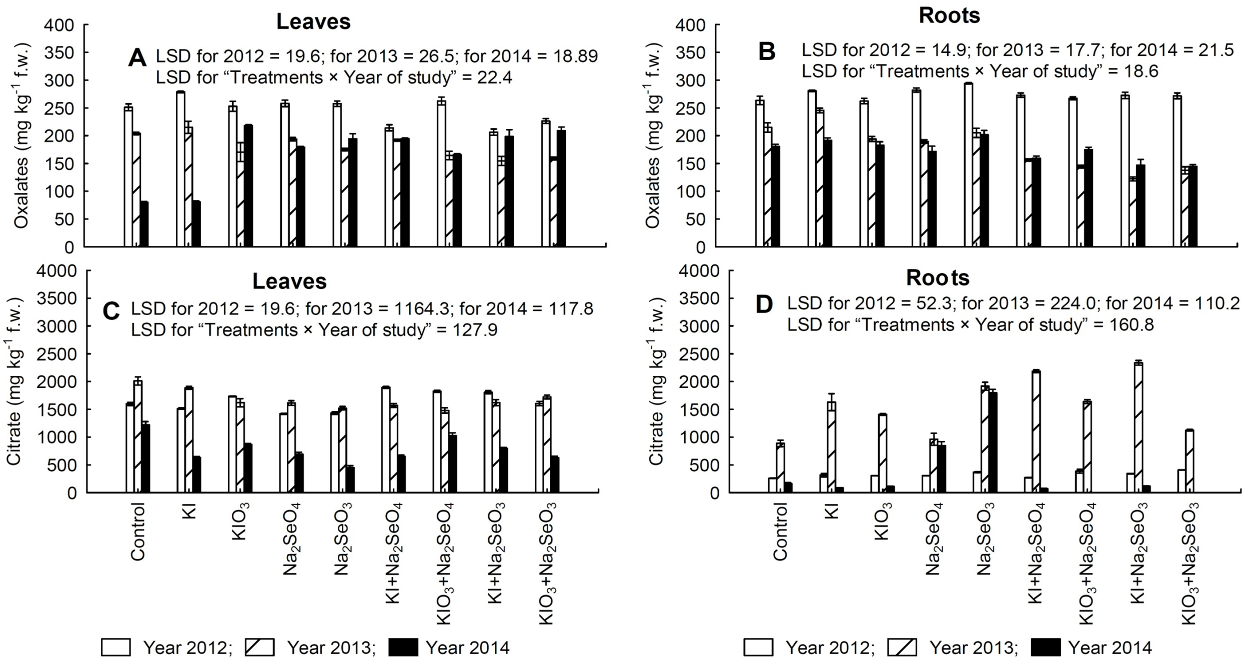

Figure 4. Content of oxalates (A and B) and citrates (C and D) in leaves (A and C) and storage roots (B and D) in each year of the study (2012-2014) depending on the applied I and Se fertilization. Bars indicate standard error (n = 4)

participation of nitrate reductase in IO_3^- reduction to I^- (Hung et al. 2005). According to these authors, IO_3^- application weakens the process of nitrate reduction, leading to excessive accumulation of nitrates (V). The lack of a clear relationship between the application of KIO_3 (alone or together with selenium) on nitrate (V) content, observed in the present study, could have been caused by IO_3^- reduction to the I_2 or I^- that occurs in the soil (Ashworth et al. 2003, Yuita et al. 2005, Ashworth and Shaw 2006) or root surface, as found in rice plants (Kato et al. 2013). Generally, the content of nitrates (III) in vegetables remains at a very low level – below 5 mg NO_2^- kg^{-1} (Tamme et al. 2006) – and increases during improper post-harvest and post-production storage (Nabrzyski and Gajewska 1993). In comparison to the control, an increase of the content of nitrates (III) in the roots of carrots fertilized with KI, KIO_3, Na_2SeO_3, Na_2SeO_4 was noticeable in the entire three-year period. On the other hand, simultaneous application of iodides and iodates with both selenium forms significantly lowered nitrate (III) accumulation in carrot roots when compared to the application of iodine (KI and KIO_3) or selenium (Na_2SeO_3 and Na_2SeO_4) alone (Fig. 3 D). Such relationships were not observed in the leaves (Fig. 3 C). In comparison to the control and other combinations, the highest content of nitrates (III) was noted in the storage roots of carrot fertilized with KI and exceeded 12 mg NO_2^- kg f.w. in 2013 and 2014 (Fig. 3 D). These results indicate that the application of iodides (I^-) inhibited the reduction of NO_2^- to NH_4^+, which is controlled by nitrite reductase (NiR) – unlike the nitrate reductase (NR) enzyme responsible for NO_3^- to NO_2^- reduction. Basing on literature data, the direct physiological and biochemical causes of increased NO_2^- accumulation after KI or KIO_3 application cannot be established (Dai et al. 2004, Smoleń et. al 2011a,b, 2014b). Interestingly, in our study KI fertilization did not substantially affect the content of free amino acids in carrot storage roots. The biosynthesis of amino acids is indirectly dependent on the NO_3^- reduction to NH_4^+ (through NO_2^-). Ammonium cations are further assimilated in the GS-GOGAT cycle leading to the production of organic nitrogen compounds – amino acids (Blasco et al. 2010, 2011). In the studies conducted by Smoleń et al. (2011b), simultaneous application of KI with various nitrogen fertilizers [$Ca(NO_3)_2$ + KI or $(NH_4)_2SO_4$ + KI] decreased the level of N-total in carrot storage roots (lower nitrogen nutrition status) as compared to respective combinations with KIO_3 application [$Ca(NO_3)_2$ + KIO_3 or $(NH_4)_2SO_4$ + KIO_3]. Additionally, Smoleń et al. (2011b) indicate that iodine form (I^-) impairs the nitrogen metabolism of plants to a greater extent than iodates (IO_3^-). Blasco et al. (2012) observed that increasing doses of I^- substantially decreased N-total content on lettuce (in soilless cultivation), which was not noted for IO_3^- application. On the other hand, Smoleń et al. (2014a) did not reveal any influence of KIO_3 and

Na_2SeO_4 or KIO_3 + Na_2SeO_4 applied foliarly or through the nutrient solution on the level of nitrogen nutrition of lettuce leaves and roots. A mechanism of antagonistic action of the I^- form of iodine on the uptake and metabolism of nitrogen (NO_3^- and NH_4^+) is yet to be described. Little information was given by Blasco et al. (2010, 2011). In their study, the application of IO_3^- (as opposed to I^-) stimulated NO_3^- reduction, enhanced NH_4^+ assimilation in the GS-GOGAT cycle and improved the process of photosynthesis in lettuce plants.

In that aspect, the observation of substantially reduced accumulation of NO_2^- in carrot roots after the simultaneous application of KI with Na_2SeO_4 or Na_2SeO_3 as compared to KI applied alone is interesting. It may indicate that the presence of selenium at IV and VI oxidation states (and the products of their conversion) limits the inhibiting influence of iodides on the functioning of the NiR enzyme. In our studies, the application of KI and KIO_3 similarly increased N-total content in carrot storage roots as compared to the control (detailed data concerning total nitrogen content is not presented). In leaves, the application of KIO_3 increased the content of the N-total to a greater extent than KI when compared to the control combination. In our previous studies, we did not note any significant influence of one-time pre-sowing fertilization with KI and KIO_3 on nitrate (III) content in carrot (also cultivar Kazan F_1) cultivated in a pot experiment (Smoleń et al. 2009). It can therefore be concluded that the effect of iodine fertilization on nitrate (III) accumulation or N-total content in carrot plants strongly depends on the cultivation method but also on iodide availability to plants. In the case of top-dressing application of KI (as tested in our study), it could have been greater than that of one-time pre-sowing introduction (Smoleń et al. 2009, 2011a, b).

The content of oxalates and citrates in plants is related to calcium nutrition. Carrot and other species from the Apiaceae family are characterized by a low content of oxalates (Wińska-Krysiak 2006). The level of citrate synthase expression (formally the first enzyme in that cycle, responsible for the formation of citrate) is directly dependent on the growth of carrot cells (Koyama et al. 1999). The accumulation of citrates in carrot is correlated with the H^+ efflux, possibly resulting from the action of H^+-ATPase located on the plasma membrane (Ohno et al. 2003). In 2012, a lower content of calcium was noted in carrot roots than in 2013 and 2014 (of 0.052 and 0.030 Ca % d.w, respectively – detailed data not presented). The problems related to calcium uptake by plants could have impaired water balance in plants as Ca^{2+} cations, along with K^+, play an important role in plant cell osmoregulation. In 2012, which had a lower amount of rainfall and higher air temperature than in 2013-2014, relatively higher contents of nitrates (V), chlorides and oxalates were noted in carrot leaves and roots as well as of nitrates (III) in leaves as compared to the other years of the study (Fig. 3 A, B, C, E, F and Fig. 4 A-B). A shortage of water in soil might have contributed to such results, more negatively affecting metabolic processes (related to cell respiration) in storage roots rather than leaves. It is substantiated by much lower contents of calcium and citric acid (a basic compound of the Krebs cycle) in roots in 2012 than in 2013-2014. However, no such relationships were observed in the leaves (Fig. 4 C and D).

The content of oxalates (White and Broadley 2009) and citrates (Eticha et al. 2010) is genotype-dependent. Jaworska and Kmiecik (1999) revealed that the concentration of total and soluble oxalates in spinach and New Zealand spinach is strongly related to climatic conditions throughout the cultivation period, however. Smoleń et al. (2016b) observed that oxalate and citrate levels in lettuce were substantially modified by soil and climate conditions in individual years rather than the applied iodine and selenium fertilization. The results obtained in the present study also indicate that the contents of the aforementioned compounds in carrot leaves and roots was affected more by such conditions than the tested combinations. The lack of an unequivocal influence of I, Se as well as I + Se fertilization on the oxalate content in carrot plants needs to be considered as a positive outcome, however. According to White and Broadley (2009), plant biofortification with mineral elements should increase the content of nutraceutics, simultaneously limiting the accumulation of antinutrients such as oxalates, polyphenolics (tannins) or phytates in crops.

The content of sugars in carrot storage roots

A statistically significant effect of the tested combinations with iodine and selenium fertilization was noted with respect to the content of glucose, fructose, sucrose and total sugars in carrot storage roots – also a significant 'treatments × year of study' interaction of these parameters was observed (Fig. 5 A-D). In 2014 a significantly higher content of glucose, fructose and total sugars was noted in carrots from all combinations (with the exception of KIO_3 + Na_2SeO_4) when compared to the years

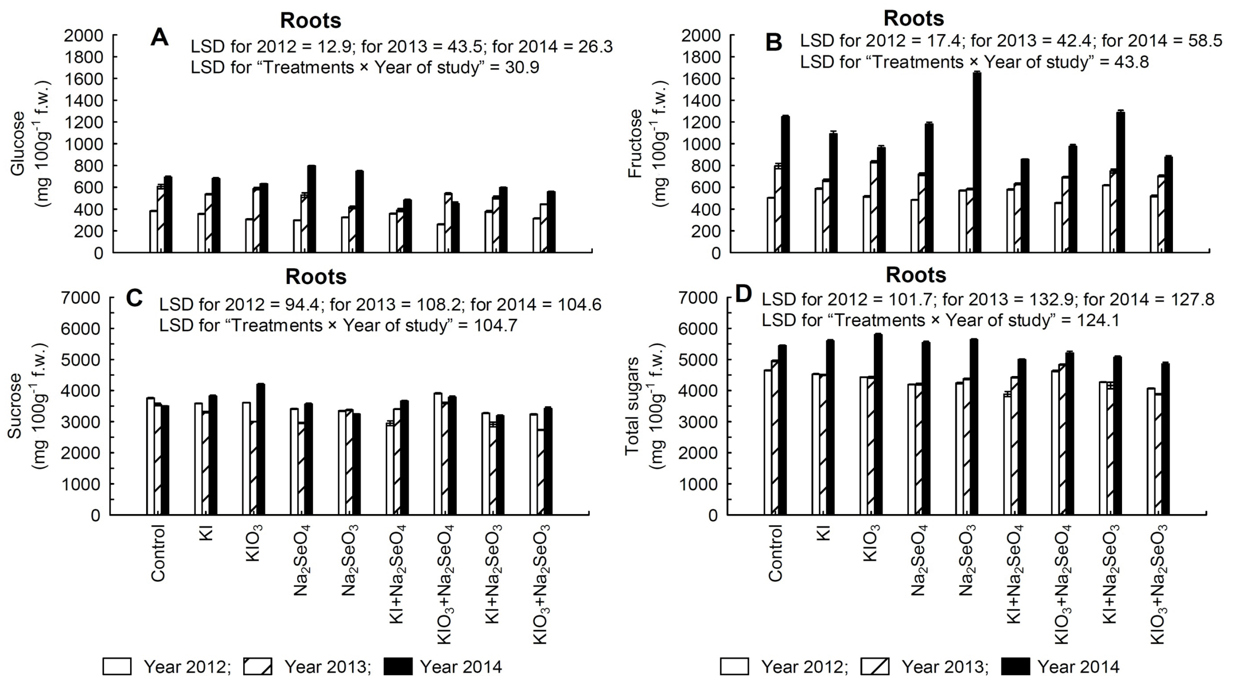

Figure 5. Content of glucose (A), fructose (B), sucrose (C) and total sugars (the sum of the three – D) in storage roots in each year of the study (2012-2014) depending on the applied I and Se fertilization. Bars indicate standard error (n = 4)

2012 and 2013. Most likely, it was favoured by the most evenly distributed rainfall (followed by optimal soil humidity) as well as PAR and temperature values noted in that year. In 2012 and 2013 a significant decrease in the content of sucrose and total sugars was observed in all combinations when compared to the control, with the exception of carrots fertilized with KIO_3 + Na_2SeO_4. Among all of the analysed sugars, the most similar values within the three years of the study were found for sucrose, irrespective of diverse weather conditions (Fig. 5 C).

Each year, soil fertilization with KI + Na_2SeO_4 reduced the content of glucose and total sugars in carrot as compared to the control and other combinations (Fig. 5 A and D). It additionally lowered the accumulation of sucrose (in 2012, Fig. 5 C) and fructose in roots (in 2014, Fig. 5 B). It needs to be underlined that, irrespective of the year, simultaneous application of Na_2SeO_4 with KIO_3 contributed to a significant increase of sucrose content, while the total concentration of sugars in carrot roots remained at a level comparable to the control (Fig. 5 C and D). In the remaining combinations with iodine and selenium application (apart from Na_2SeO_4 + KI/KIO_3), diverse effects ('*in plus*' or '*in minus*') were noted with respect to the content of tested sugars in roots (Fig. 5 A-D). In other studies conducted according to the same experimental design by Smoleń et al. (2015), simultaneous application of Na_2SeO_4 with KI significantly decreased sucrose content in lettuce, while fertilization with the same Se form applied with KIO_3 increased it. This observation, therefore, confirms the possibility of obtaining diverse plant reactions, with respect to sugar accumulation, depending on the iodine form when applied together with selenates (VI).

Other quality parameters of carrot storage roots

The nutritional and health-promoting quality of carrot storage roots is affected by numerous endo- and exogenous factors (Suojala 2000), as well as on the cultivar and harvest date (Gajewski et al. 2009b, Szymczak et al. 2009). Exogenous factors occurring during the cultivation, particularly climatic (Rosenfeld et al. 1998a,b,c), soil and agro-technical ones have a substantial effect on the growth, shape and size of storage roots along with their chemical composition (Suojala 2000, Gajewski et al. 2009a, 2010).

In each year of the study, a significant but diverse influence of applied iodine and selenium fertilization was observed with respect to the contents of dry matter, free amino acids and carotenoids in carrot (Fig. 6 A, B and C). In one or two years of the experiment, the tested factors significantly affected the level of phenolic compounds (in 2012 and 2013, Fig. 6 E), phenylpropanoids (in 2013, Fig. 6 F) and flavonols (in 2012, Fig. 6 G), as well as free radical scavenging activity (DPPH, in 2013, Fig. 6 D).

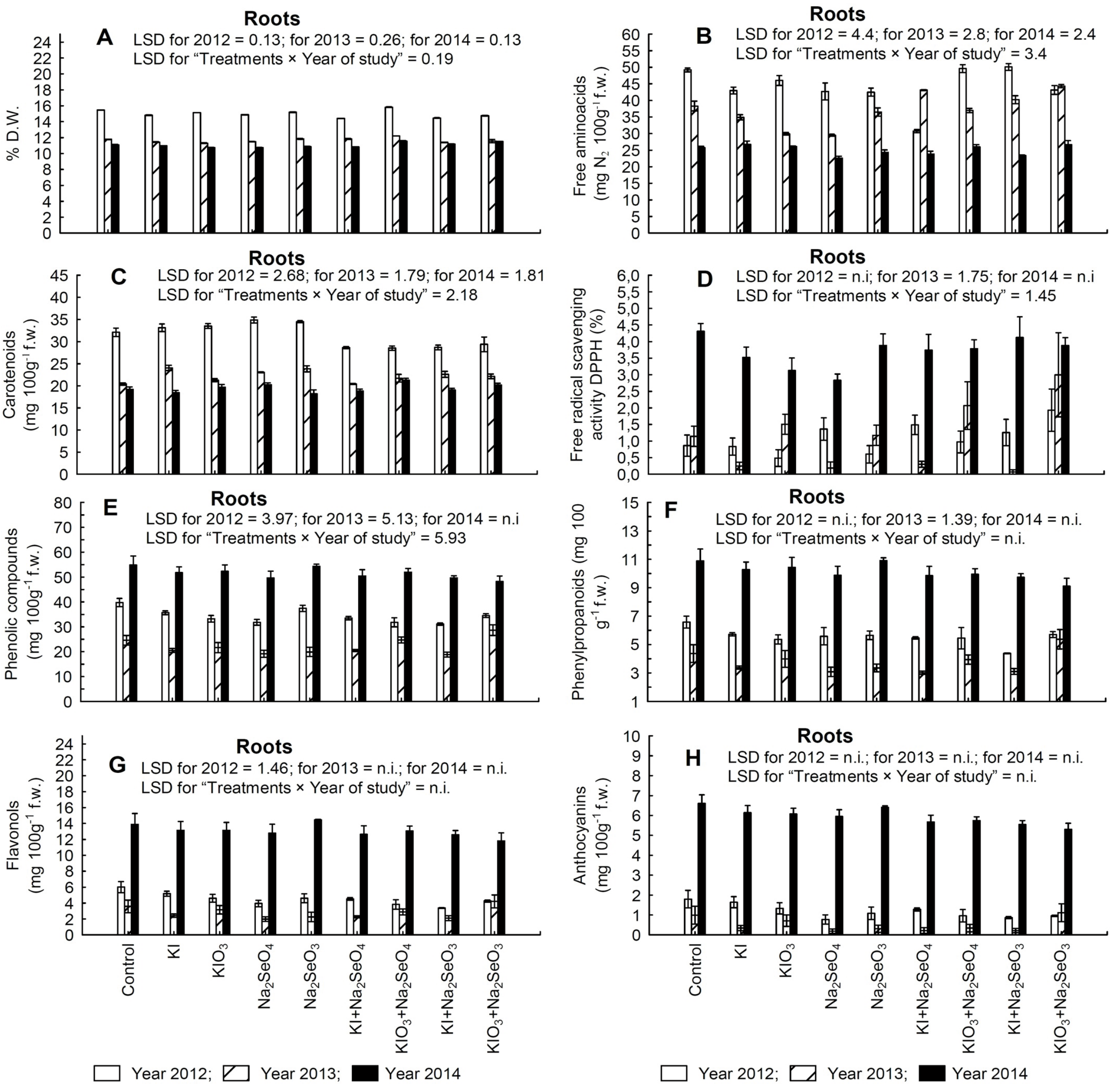

Figure 6. Content of dry weight (A), free amino acid (B), carotenoids (C) and free radical scavenging activity (DPPH) (D) as well as the content of phenolic compounds (E), phenylpropanoids (F), flavonols (G) and anthocyanins (H) in storage roots in each year of the study (2012-2014) depending on the applied I and Se fertilization. Bars indicate standard error (n = 4)

Among the described quality parameters, a statistically significant ‘treatments × year of study’ interaction was noted for the contents of dry matter, free amino acids, carotenoids and phenolic compounds as well as on the free radical scavenging activity of carrot storage roots (Fig. 6 A-E). The content of anthocyanins in carrot was in no case affected by the tested factors, as well as the ‘treatments × year of study’ interaction (Fig. 5 H). The content of phenylpropanoids and flavonols (Fig. 6 F and G) in carrot roots did not vary significantly between the combinations within the entire period of the study (Fig. 6 F and G).

Gajewski et al. (2011) indicated that a diminished amount of PAR radiation lowers the quality of carrot storage roots, mainly by a significant increase of nitrate (V) accumulation and a decrease in dry matter, soluble solids and β-carotene contents. The proposed relationships were, however, strongly dependent on other weather parameters. Our studies revealed a significant increase in the contents of dry matter, free amino acids and carotenoids in carrot roots cultivated in the year with the lowest amount of rainfall in the summer months and much higher PAR radiation (2012, Fig. 6 A, B and C). On the other hand, carrots grown in 2014 (with the most optimal weather conditions for their cultivation) were characterized by substantially higher levels of phenolic compounds, phenylpropanoids, anthocyanins and free radical scavenging activity (DPPH) as compared to the years 2012 and 2013 (Fig. 6 D-H).

Analysing the influence of soil fertilization with iodine and selenium compounds on the described quality parameters of carrot, a significant decrease of carotenoid content in plant roots fertilized simultaneously with iodine and selenium was clearly distinguished in 2012 when compared to its separate application (Fig. 6 C). Additionally, a significant increase of free radical scavenging activity (DPPH) was observed after simultaneous introduction of iodine and selenium as compared to fertilization with KI, KIO_3, Na_2SeO_3 and Na_2SeO_4 alone (Fig. 6 D). However, these relationships were not revealed in other years of the study. Taking the above into account, it can be assumed that the values of the parameters in carrot cultivated in 2012-2013 and described in this subchapter depended to a greater degree on the soil and weather conditions than on the applied fertilization with iodine and selenium.

Smoleń et al. (2015) revealed that soil fertilization with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 induced a stress response in lettuce plants, reflected as decreased head weight and sugar content (except for Na_2SeO_4 applied alone) accompanied by an increased level of phenolic compounds, phenylpropanoids, flavonols and antioxidant activity. Accumulation of phenolic compounds in plants is described as one of the mechanisms of plant reaction to biotic and abiotic stress factors (Michalak 2006, Korkina 2007), including high selenium (Ríos et al. 2008) and iodine doses (Blasco et al. 2010). In terms of plant physiology and biochemistry, no clear and unequivocal effect of the tested I, Se and I + Se fertilization on the described quality parameters of carrot storage roots can be considered positive. It confirms the lack of a strong negative impact and toxicity of iodine and selenium (applied in relatively high doses) to carrot biomass and chemical composition that characterizes its quality. Importantly, it was observed even despite a substantial increase of I and Se content in carrot plants – detailed data presented in the publication by Smoleń et al. (2016a).

CONCLUSIONS

1. Despite diverse weather conditions in each year of the study, no significant influence of iodine and selenium application was found with respect to average weight of a single root and leaf yield from one carrot plant.
2. The influence of the simultaneous application of selenates and iodine on sugar accumulation in carrot storage roots depended on the chemical form of iodine.
3. Only in the case of nitrite (III) accumulation was a clear effect of iodine and selenium interaction revealed and noted in all three years of the study. It was manifested by a significant decrease in its content in storage roots after simultaneous fertilization with iodine and selenium as compared to the application of each of the elements alone.

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AUTHOR CONTRIBUTIONS

S.S. – designed and supervised the experiment, analysed and discussed the results, wrote the manuscript; Ł.S. – supervised the field cultivation of carrot, performed analyses using a ICP-OES spectrometer, helped with manuscript preparation; I.L.-S. – performed statistical analyses, helped with manuscript preparation; R.R. – conducted field cultivation and chemical analyses using capillary electrophoresis; M.L.-S. – performed chemical analysis using the HPLC technique, helped with manuscript preparation; A.K., E.P., R.B.-K., A.K., J.K.-D. – performed the experiment, consulted and corrected the manuscript; W.S. – designed the general idea of the project, helped with manuscript preparation.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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