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SELECTED ASPECTS OF NITROGEN METABOLISM AND QUALITY OF FIELD-GROWN LETTUCE (*Lactuca sativa* L.) DEPENDING ON THE DIVERSIFIED FERTILIZATION WITH IODINE AND SELENIUM COMPOUNDS

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Abstract. Iodine and selenium together fulfill important functional roles in organisms of humans and animals. Conducting simultaneous biofortification (enrichment) of plants with these elements is justified as combined endemic deficiency of I and Se (hidden hunger) is often encountered. Relatively little is known about the interaction between I and Se in plants, not only with respect to their accumulative efficiency, but also its influence on mineral nutrition or biological quality of crop. The study (conducted in 2012–2014) included soil fertilization of lettuce cv. 'Valeska' with I and Se in the following combinations: control, KI, KIO_3, Na_2SeO_4, Na_2SeO_3, KI + Na_2SeO_4, KIO_3 + Na_2SeO_4, KI + Na_2SeO_3, KIO_3 + Na_2SeO_3. Iodine and selenium were applied twice: before sowing and as a top-dressing (each of 2.5 kg $I \cdot ha^{-1}$ + 0.5 kg $Se \cdot ha^{-1}$) – a total dose of 5 kg $I \cdot ha^{-1}$ and 1 kg $Se \cdot ha^{-1}$ was used. Diversified weather conditions significantly modified the impact fertilization with I and Se had on tested aspects of nitrogen metabolism and biological quality of lettuce – except for the lettuce heads mass and the sucrose content. Based on the results concerning average lettuce weight as well as the level of sugars, phenolic compounds, phenylpropanoids, flavonols and antioxidizing activity it was concluded that the application of Na_2SeO_4 alone or together with iodine acted as a stress factor for cultivated plants.

Key words: biofortification, biological quality, nitrate, phenolic compounds

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INTRODUCTION

Lettuce is one of the "model" species used in the basic research conducted on field crops [Dzida et al. 2012, Pitura and Michałojć 2012, Borowski et al. 2014, Sirtautas et al. 2014], including the biofortification of plants with mineral components [Blasco et al. 2010, Hawrylak-Nowak 2013] or vitamins and health-related compounds [Nunes et al. 2009].

Iodine and selenium are the nutrients necessary for the proper functioning of human and animal organisms. About 30 and 15% of people throughout the world suffer from I and Se deficiency in food, respectively. Nearly two third of the population has insufficient amount of I and Se (but not scarce) in food [Hirschi 2009, White and Broadley 2009]. Biofortification (enrichment) of crops is one of the simplest methods to counteract this problem.

Neither of the two discussed elements meets the criterion of indispensability for plants, though selenium may affect them positively [Kopsell and Kopsell 2007]. This causes, that using of crop plants as vectors of I and Se for the food chain is a complex problem. Development of agricultural practices for plant fertilization with these elements requires laborious basic research work to be carried out first.

Since 80-ies of the previous century, mineral fertilizers have been enriched with selenium in Finland [Eurola et al. 2003]. The programme of soil fertilization with selenium has also been implemented in Malawi [Chilimba et al. 2012]. No such strategies concerning iodine have been however developed with the exception of fertigation of rice paddies with the solution of KIO_3 in the province of Xinjiang (China) [Ren et al. 2008].

In recent years, a dozen or so results of research concerning biofortification of lettuce with selenium [Ríos et al. 2008, 2010] and iodine [Blasco et al. 2010, Voogt et al. 2010, Voogt and Jackson 2010] were published. In principal, these were the works carried out in the hydroponics, soilless systems or as pot culture experiments. Analyzing the number of available literature in the databases, we have to state that the impact of selenium on plants is relatively better identified than that of iodine.

Research works treating on the simultaneous application of iodine and selenium are unique. So far, they have been conducted in the hydroponically grown spinach [Zhu et al. 2004] or lettuce [Rakoczy 2014, Smoleń et al. 2014 a]. As for the field cultivation, Mao et al. [2014] analyzed the impact of simultaneous fertilization with Se + Zn + I on the yield and the content of Se, Zn, I, B, Cu, Fe and Mn in wheat, maize, soybean, potato, canola and cabbage. Unfortunately, this work documents only one-year results. There is no information documenting the *sensu stricto* interaction of iodine with selenium on plants cultivated in field for several years – thereby exposed to interchangeable soil and weather conditions.

The aim of the study was to evaluate the influence of iodine and selenium fertilization (in their different chemical forms) on the mass of lettuce heads, selected aspects of nitrogen metabolism and the parameters shaping nutritious and health-related quality of lettuce grown in the field condition.

MATERIAL AND METHODS

Plant material and treatments. A field study with lettuce cv. 'Valeska' was conducted in the Experimental Station (50°07'910 N, 19°84'764 E) of University of Agriculture in Krakow, Poland. In 2012–2014 lettuce was grown during spring season on different parts of the same field of the experimental station.

Seeds were sown during the first ten days of March (in each year) in a greenhouse, where seedlings were produced. On 18, 23 and 15 April in the subsequent years seedlings were planted into the soil in rows 30 cm apart with 30 cm plant spacing.

Lettuce was cultivated on heavy soil (heavy clay: 24% sand, 23% dust and 53% loam). In individual years, chemical parameters of the soil prior to the cultivation of lettuce were within the range: organic matter 2.33–2.56%, pH_{H_2O} 7.19–7.45, EC 0.10–0.11 mS cm^{-1}, Eh from +189.3 to +276.0 mV, $N\text{-}NH_4 + N\text{-}NO_3$ 8.4–13.4 $mg \cdot dm^{-3}$, P 12.8–59.5 $mg \cdot dm^{-3}$, K 139.1–181.4 $mg \cdot dm^{-3}$, Mg 114.0–194.2 $mg \cdot dm^{-3}$, Ca 1 651.8–2 089.3 $mg \cdot dm^{-3}$ and S 22.1–42.9 $mg \cdot dm^{-3}$.

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: before planting seedlings and as a top-dressing (in the first phase of heads formation – first ten days of May) in a total dose of 5 kg $I \cdot ha^{-1}$ and 1 kg $Se \cdot ha^{-1}$ (presowing and top-dressing as 2.5 kg $I \cdot ha^{-1}$ + 0.5 kg $Se \cdot ha^{-1}$), respectively. Fertilization with iodine and selenium was performed using the analytically pure reagents: KI or KIO_3 (P.O.Ch., Poland) as well as Na_2SeO_4 or Na_2SeO_3 (Sigma-Aldrich, Germany).

Presowing fertilization with iodine, selenium and N, P, K was performed one day prior to lettuce planting. Doses of P and K were calculated basing on the soil analysis results − P and K contents were supplemented to the level optimal for lettuce P–70 and K–200 (in $mg \cdot dm^{-3}$ of soil). Presowing nitrogen was applied as ammonium nitrate in a dose of 50 mg $N \cdot dm^{-3}$ soil, i.e., 50% of N dose. Presowing fertilization with Mg, Ca and S was not performed, because their content in soil covered the nutritional requirements of lettuce. The remaining 50% of nitrogen dose was applied with the top-dressing application of iodine and selenium, after which plants were irrigated with water in a dose of ca. 15 mm of rain.

The experiment was arranged in a split-plot design with four replications. Each experimental treatment was randomized in four repetitions on 5 m × 1.5 m (7.5 m^2) plots. The total area used for experiment was 270 m^2.

During the harvest (30 May, 11 and 3 June in the 2012, 2013 and 2014 respectively), the average head weight was measured from sixteen lettuce plants from each plot/repetition. For further analyses, eight lettuce were collected from each repetition. During the lettuce harvest, samples of soil were collected from the topsoil (0–30 cm), separately for each object of the experiment. In this work, results of the lettuce head weight are shown, however information concerning the crop height will be presented in a separate publication.

Plant analysis. In the lettuce samples, the content of nitrates(V) (NO_3^-), nitrates(III) (NO_2^-) and chlorides (Cl^-) was determined by the capillary electrophoresis technique, ammonium ions by FIA technique, free amino acids by spectrophotometric method after

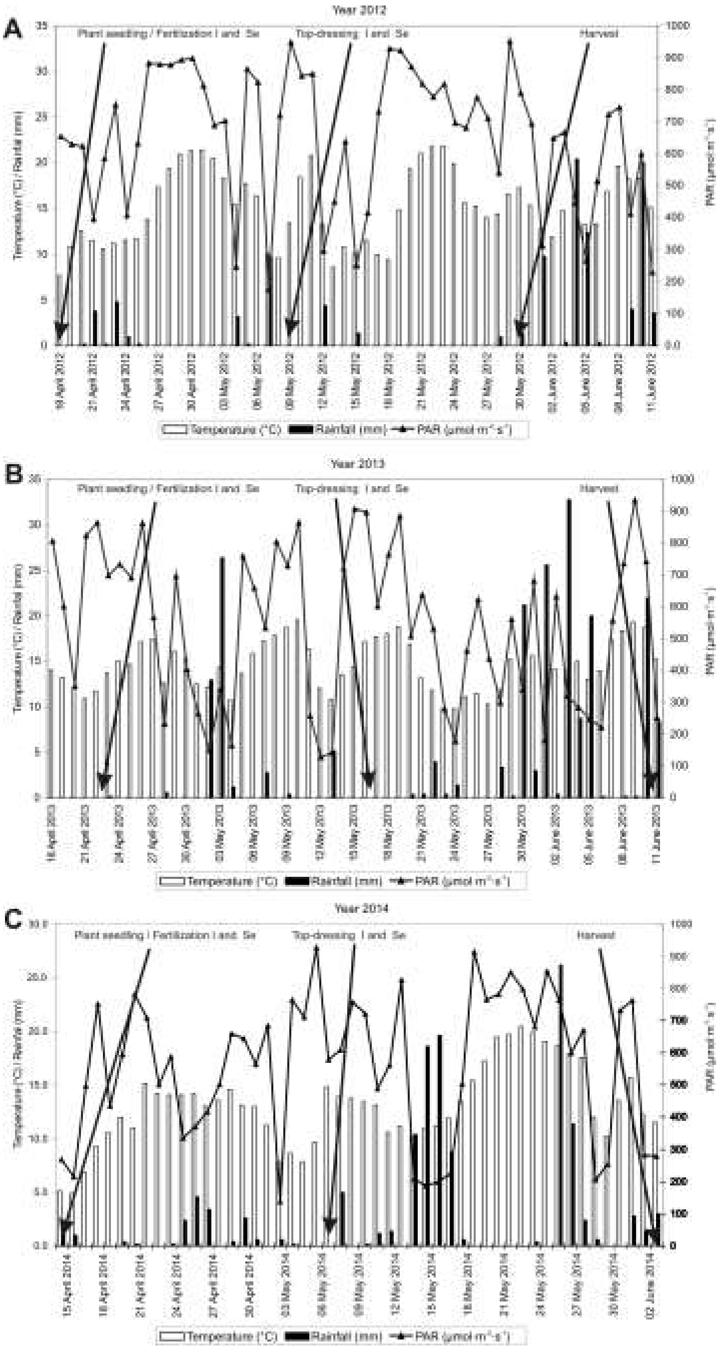

Fig 1. Weather conditions in the period of cultivation of lettuce in 2012 (A), 2013 (B) and 2014 (C). The figure shows also the times marked for planting, presowing and top-dressing fertilization with I and Se and for the harvest of lettuce

reaction with ninhydrin, N-total by Kjeldahl method and ascorbic acid by capillary electrophoresis technique after extraction of samples with 2% oxalic acid. Moreover, the content of total phenols, phenylpropanoids, flavonols and anthocyanins was determined by spectrophotometric method after reaction with 0.1% HCl solved in ethanol. The level of glucose, fructose and sucrose was determined by HPLC technique and its sum was expressed as "total sugars concentration". Free radical scavenging activity of lettuce was additionally determined on the basis of the sample reaction with diphenylpicrylhydrazyl (DPPH) – as measured after 30 minutes.

Soil analysis. Prior to plant cultivation in the 0–30 cm soil layer pH, EC, Eh as well as the content of: organic matter, N (N-NO_3 and N-NH_4), P, K, Mg, Ca and S were analyzed. Obtained results were used for the characterization of soil and presented in the subchapter "Plant material and treatments". In soil samples mixed with water (1:2 vol/vol, soil:H_2O) measurement of pH and redox potential (Eh) was conducted potentiometrically as well as total soil salinity (EC) using conductivity meter. The organic matter in soil was assayed by the Tiurin method. For the assessment of the content of available forms of N, P, K, Mg, Ca and S soil extraction with 0.03 M acetic acid was employed. Determination of N-NO_3 and N-NH_4 was conducted by FIA technique while P, K, Mg, Ca and S was analyzed using ICP-OES spectrometer.

After cultivation of lettuce, the content of mineral forms of nitrogen: N-NH_4 and N-NO_3 in the 0–30 cm soil layer was determined by FIA techniques after extraction of 0.03 M with acetic acid.

Meteorological data. The most adverse weather conditions for lettuce growing were observed in 2012 (fig. 1). In 2012 in relation to the years 2013–2014, the smallest rain precipitation and the highest daily average temperature of air and the value of PAR were noted. It is worth mention that in 2013 and 2014, few days before the harvest of lettuce the heavy rainfall occurred.

From the time of lettuce planting in field (end of April) to its harvest (end of May/beginning of June) the average daily PAR value was 656.7, 537.8 and 562.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In turn, the overall amount of rainfall was 31.6, 203.8 and 132.2 mm, respectively for 2012, 2013 and 2014. The daily average air temperature for the whole May was 15.6, 14.0 and 13.8 °C, respectively for 2012, 2013 and 2014. For comparison, in the years 1971–2000 the total monthly average precipitation in the region of the research was: 50 mm in April, 74 mm in May and 94 mm in June [GUS (Central Statistical Office of Poland) 2005]. On the other hand, in the years 1971–2000, the monthly average air temperature was: 8.0°C in April, 13.4°C in May and 16.2°C in June.

Data analysis. Obtained results were statistically verified by two-way ANOVA module of Statistica 8.0 PL program for significance level $P < 0.05$. Changes of any significance were assessed with the use of variance analysis. In case of significant changes homogenous groups were determined on the basis of Tukey's test.

RESULTS AND DISCUSSION

The applied combinations of fertilization with the compounds of iodine and selenium had significant impact on the weight of lettuce heads and the content of nitrates(V), nitrates(III), ammonium ions, chlorides, amino acids, N-total (tab. 1), glucose, fructose, sucrose, total sugars (tab. 2), ascorbic acid, phenolic compounds, phenylpropanoids, flavonols and anthocyanins as well as on free radical scavenging activity (tab. 3) in lettuce. With the exception of head weight (fig. 2 A) and the content of sucrose (fig. 3 B) in lettuce the impact of the tested factors on the presented quality features of the crop was different in individual years – significant values for the treatments × year of study correlation (fig. 2 B–G, fig. 3 A, B and D, fig. 4 A–F). Also the content of mineral nitrogen in soil ($N\text{-}NH_4$ and $N\text{-}NO_3$ forms) after lettuce cultivation depended on applied fertilization with iodine and selenium (tab. 4) and varied within the years of the study (fig. 5 A and B).

Lettuce head weight, selected aspects of nitrogen metabolism and the content of chlorides in plants. In comparison with the control and other treatments, fertilization with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 caused significant reduction of the average weight of lettuce head (tab. 1). The strongest effect was observed in 2012 (fig. 2 A), when the lowest precipitation and the highest air temperatures and PAR radiation in the period of lettuce cultivation were noted. Our results are in agreement with the reports indicating the more toxic impact on plants of selenium in the form of SeO_4^{2-} than SeO_3^{2-} [Kopsell and Kopsell 2007]. On the other hand, Ríos et al. [2008, 2010] demonstrated that in cultivation of lettuce in the soilless system, the form of SeO_4^{2-} was better absorbed and, at the same time, less toxic for plants than SeO_3^{2-}. In the research of Hawrylak-Nowak et al. [2015] cucumber plants cultivated in hydroponics showed higher sensitivity to SeO_3^{2-} than to SeO_4^{2-} – importantly, accumulation of selenium in the shoots and roots was lower with the application of SeO_3^{2-} than SeO_4^{2-}.

Lack of negative impact of both tested iodine forms on the weight of lettuce heads proves that the applied total dose of 5 kg $I \cdot ha^{-1}$ was within the limits tolerated by lettuce. Certainly, toxic impact of iodine on lettuce was restricted by strong sorption of this element in soil [Fordyce et al. 2000, Ishikawa et al. 2010, Nath et al. 2010, Hong et al. 2012] what reduces its availability and uptake by plants [Johanson 2000, Dai et al. 2009, Nath et al. 2010].

It seems important that the decrease of the average weight of lettuce heads was also accompanied by a significant reduction of N-total content in the lettuce fertilized with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 (tab. 1). This relationship was observed in 2013 and 2014, respectively when the excessive and multi-year near-average rainfall occurred (fig. 2G). In 2012, the plants from those three treatments were characterized by the highest content of N-total and additionally the NO_3^- ions (fig. 2B and G). It resulted from strong inhibition of plant growth, which in turn caused the accumulation of NO_3^- and N-total in the lettuce tissues. It seems that the impact of iodine and selenium on nitrogen status of lettuce depends on the cultivation conditions. In the two-year research conducted by Smoleń et al. [2014 a] no significant correlation was demonstrated between the foliar application of I and Se and diversified content of these elements in the nutrient solution with respect to N-total content in the lettuce grown in the autumn season in the NFT hydroponic system. In those studies no disturbances in the functioning of the nitrogen nutrition appeared when fertilization with I and Se was used. Importantly, iodine and selenium were applied in concentrations safe for plants i.e. 1 mg $I \cdot dm^{-3}$ and 0.5–1.5 mg $Se \cdot dm^{-3}$ in the nutrient medium and 0.05% I and 0.005% Se for foliar spray-

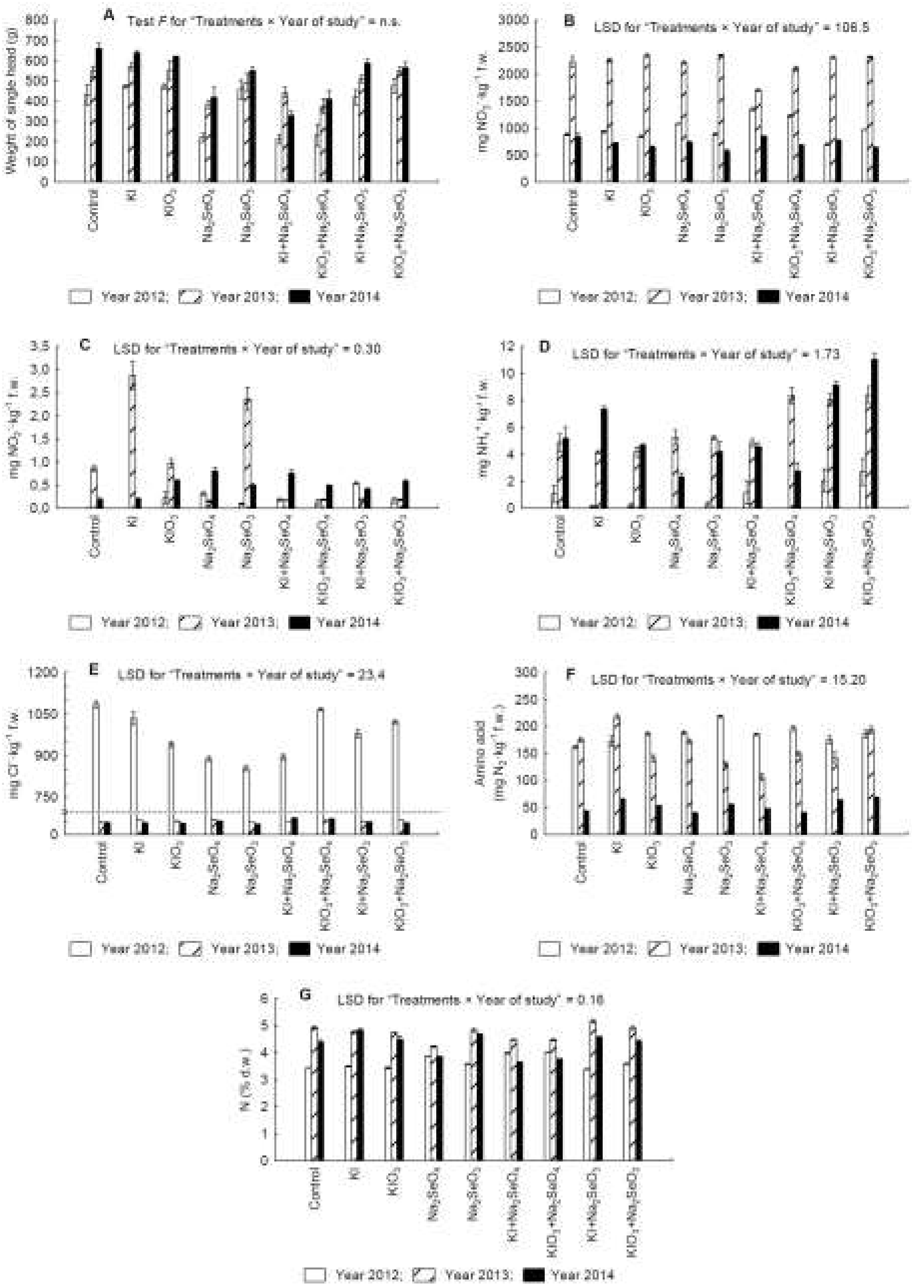

Fig. 2. Weight of single lettuce head (A) and content of nitrate(V) (B), nitrate(III) (C), ammonium ions (D), chlorides (E), amino acid (F) and N-total in lettuce (G) in the years 2012–2014. Means followed by the same letters are not significantly different for $P < 0.05$

ing [Smoleń et al. 2014 a]. On the other hand, Rakoczy [2014] demonstrated that during cultivation of lettuce on mineral wool in a plastic tunnel an increase of N-total content in lettuce occurred after foliar application of KI, KI + Na_2SeO_4, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3 (0.001% I and 0.0001% Se) as compared to the control.

Antagonistic interactions may occur between iodine and chlorine as chloride channels are easily permeable to iodides [Roberts 2006]. In tomato cultivation chlorides (Cl^-) caused the decrease of total N, but no NO_3^- in plants [Komosa and Górniak 2012]. In our study significantly higher levels of Cl^- in 2012 (fig. 2E) was observed. Average values from three years of research indicate to the comparable accumulation of NO_3^- ions in lettuce in all tested treatments in relation to the control. However, it is significant that in each year of the research, fertilization with iodine and selenium had different and unique impact on the content of nitrates(V) in lettuce (fig. 2B).

Table 1. Weight of single lettuce head and content of nitrate(V), nitrate(III), ammonium ions, chlorides, amino acid and N-total in lettuce (means for 2012–2014)

Treatments (n = 12)	Weight of single head (g)	$(mg \cdot kg^{-1} f.w.)$					N (% d.w.)
		NO_3^-	NO_2^-	NH_4^+	Cl^-	amino acid (in calculation on N_2)	
Control	546.1 b	1318.9 ab	0.36 a	3.7 ab	392.3 f	127.0 b	4.26 bc
KI	561.1 b	1308.3 ab	1.03 c	3.9 b	377.5 ef	152.0 c	4.36 cd
KIO_3	548.8 b	1280.2 a	0.60 b	3.0 ab	345.3 bc	127.0 b	4.23 b
Na_2SeO_4	340.3 a	1342.0 b	0.43 ab	2.5 a	331.4 b	134.0 b	3.99 a
Na_2SeO_3	499.9 b	1275.3 a	0.99 c	3.2 ab	313.6 a	135.0 b	4.37 cd
KI + Na_2SeO_4	328.1 a	1300.7 ab	0.38 ab	3.5 ab	336.2 b	113.0 a	4.04 a
KIO_3 + Na_2SeO_4	339.6 a	1339.0 b	0.27 a	3.7 ab	393.4 f	129.0 b	4.09 a
KI + Na_2SeO_3	505.2 b	1262.5 a	0.38 ab	6.4 c	359.4 cd	127.0 b	4.38 d
KIO_3 + Na_2SeO_3	529.5 b	1313.2 ab	0.31 a	7.4 c	374.3 de	149.0 c	4.32 bcd

Means followed by the same letters are not significantly different for $P < 0.05$

In the individual years of research, no clear effect of fertilization with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 on the content of NO_2^-, NH_4^+, amino acids or chlorides in lettuce was seen (fig. 2. C–F). *De facto* these three discussed treatments did not have negative impact on the content of ammonium ions and free amino acids in lettuce – with the exception of KI + Na_2SeO_4 in case of amino acids (average from three years of the study; tab. 1). When ammonium ions and free amino acids contents are concerned, a different, diversified reaction of plants on the fertilization with selenium and iodine compounds was observed in each year of the research. Application of selenium compounds, (mainly Na_2SeO_3 and then Na_2SeO_4 and KI + Na_2SeO_4) caused a significant reduction in the chlorides content in lettuce (tab. 1). This relation was observed mainly in 2012, when the plants grew in the conditions of water shortage in soil and higher than average air temperature. It is worth mentioning, that in comparison with other treatments, significantly increased content of NO_2^- ion was found in lettuce treated with KI or Na_2SeO_3 separately (tab. 1). However, this observation was to a great extent determined by the results obtained in 2013 (fig. 2 C). The plants cultivated in that year also showed higher content of nitrates(V) than in the other years. In 2013, adverse weather conditions prevailed during cultivation of lettuce – in terms of abnormal amount and distribution of rainfall, lower insolation and lower daily air temperature. This could cause weakening of the nitrates(V) reduction process through the nitrate reductase, as its activity largely depends on the photosynthetic capacity of plants [Campbell 1999].

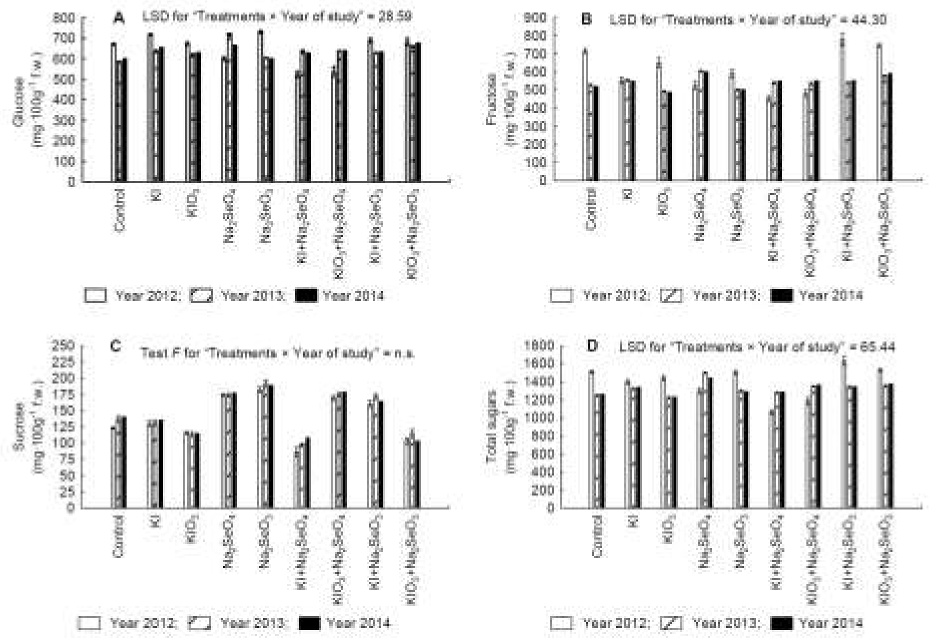

Fig. 3. Content of glucose (A), fructose (B), sucrose (C) and total sugars (sum of glucose, fructose and sucrose) (D) in the years 2012–2014. Means followed by the same letters are not significantly different for $P < 0.05$

Soil background for nitrogen nutrition of plants – the effect of iodine and selenium fertilization on the content of mineral nitrogen in soil. It is interesting that after the application of Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4, higher content of N-NO_3 in soil (as compared to other combinations – mean values from the three years of the study, tab. 4) was noted. This was valid for the years 2012 and 2014 with the exception of KIO_3 + Na_2SeO_4 in 2012 (fig. 5 B). In 2012, the highest content of ions NH_4^+ in soil (fig. 4 A) was also determined after fertilization with Na_2SeO_4. These results indicate that the application of selenate(VI) can exert (to a certain degree) the stabilizing effect of mineral nitrogen in the soil. Interestingly, such influence was obtained both in the conditions of drought (2012) and with the near-average (multi-year) distribution of rainfall (2014). The primary cause of this effect may be the slowdown of the nitrification process or limitation of the denitrification process when using relatively high dose of SeO_4^{2}-. Moreover, weather conditions in 2013 could also contribute to the lower content of N-NO_3 in soil after lettuce cultivation from the combinations with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 treatments, as noted in 2012 and 2014. This was probably caused by N-NO_3 leaching in to the soil profile.

Table 2. Concentration of glucose, fructose, sucrose sum of these as total sugars in lettuce (means for 2012–2014)

Treatments (n = 12)	(mg·100 g^{-1}f.w.)			
	glucose	fructose	sucrose	total sugars
Control	617.2 b	587.7 d	133.5 c	1338.4 bc
KI	668.9 de	552.4 bc	132.1 c	1353.5 c
KIO_3	638.6 bc	543.0 abc	114.6 b	1296.2 b
Na_2SeO_4	659.1 cde	576.2 cd	174.7 d	1410.0 de
Na_2SeO_3	644.7 c	530.9 ab	187.2 e	1362.8 cd
KI + Na_2SeO_4	596.0 a	513.2 a	96.8 a	1206.0 a
KIO_3 + Na_2SeO_4	603.2 ab	521.2 ab	174.2 d	1298.6 b
KI + Na_2SeO_3	650.4 cd	623.1 e	165.1 d	1438.6 e
KIO_3 + Na_2SeO_3	673.0 e	636.4 e	107.2 b	1416.5 e

Means followed by the same letters are not significantly different for $P < 0.05$

Content of sugars in lettuce. Fertilization with KI + Na_2SeO_4 had adverse impact on the content of glucose, fructose, sucrose and total sugars in lettuce (tab. 2) and it was observed with the greatest intensity in 2012 (fig. 3 A–D). What is important, decrease of sucrose content in lettuce after the application of KI + Na_2SeO_4 took place in each of the research years (fig. 3 C). Treatment with KIO_3 + Na_2SeO_4 (tab. 2) also had adverse impact on the glucose and fructose content but not on sucrose and total sugars content in lettuce – this was the case only in 2012. Fertilization with Na_2SeO_4 alone did not have such negative impact on the content of sugars as its application combined with iodine (KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4). This indicates that in the conditions of rainfall shortages, high temperatures and large number of hot and sunny days, adverse interaction between I^-/IO_3^- and SeO_4^{2-} occurs, in scope of significant decrease of carbohydrates content in lettuce. In literature, there is no information, which could explain such relations. One of the mechanisms of detoxification of plants from selenium and iodine is volatilization of methylated forms of both elements by leaves: CH_3I [Rhew et al. 2003] and $(CH_3)_2Se$ [Terry et al. 1992]. In that process assimilates produced during photosynthesis and cellular respiration are used. We suppose that the mechanism of I and Se methylation could be the direct cause of the decrease of carbohydrates content in lettuce, especially in the adverse environmental conditions in 2012. In excess, I^- ions are more toxic for plants than IO_3^- [Blasco et al. 2010, Kato et al. 2013] and SeO_4^{2-} ions than SeO_3^{2-} [Kopsell and Kopsell 2007]. It is possible that this directs plant metabolism to methylation, in the first place of iodine taken up as I^-, than IO_3^-, and in the next stage of selenium: SeO_4^{2-} over SeO_3^{2-}. It is here, where we also seek the cause of significant decrease of sugars content in lettuce, to the greater extent after fertilization with KI + Na_2SeO_4 than KIO_3 + Na_2SeO_4.

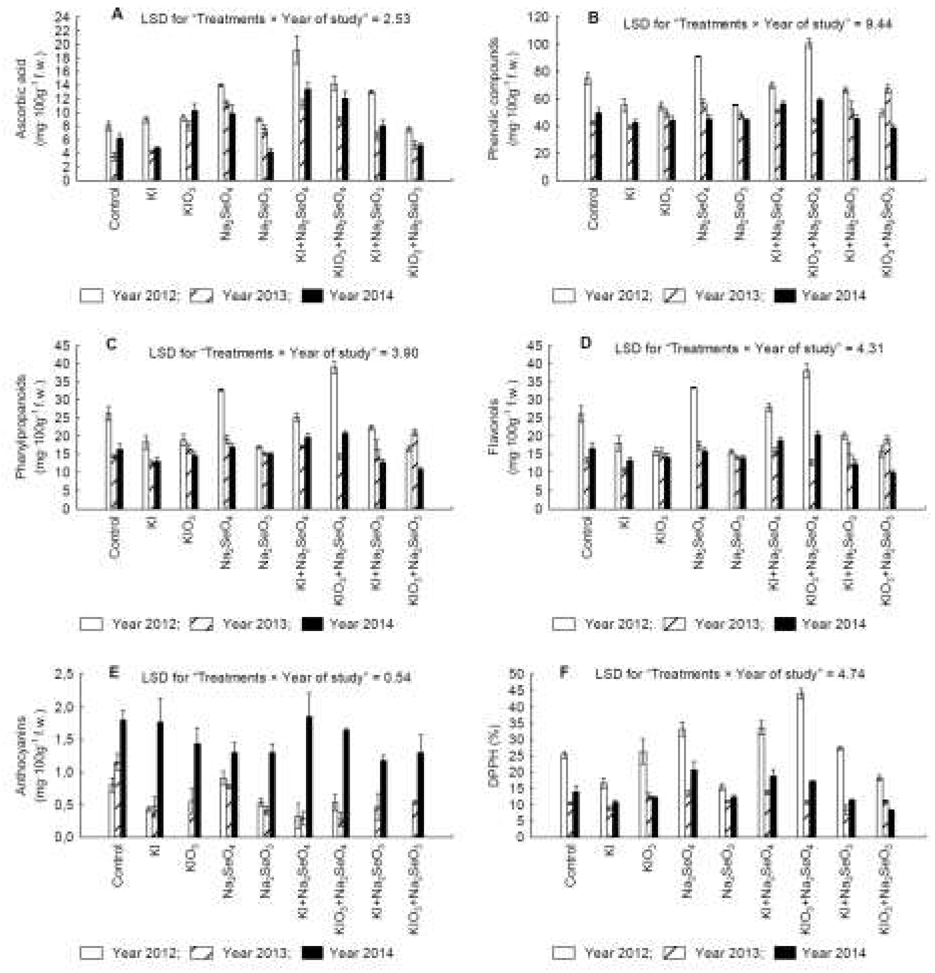

Fig. 4. Concentration of ascorbic acid (A), phenolic compounds (B), phenylpropanoids (C), flavonols (D), anthocyanins (E) and free radical scavenging activity (% DPPH) (F) in lettuce in the years 2012–2014. Means followed by the same letters are not significantly different for $P < 0.05$

Content of ascorbic acid, phenolic compounds, phenylpropanoids and flavonols as well as scavenging activity of free radical DPPH of lettuce. Accumulation of phenolic compounds in plants is observed as the reaction of plants on biotic and abiotic stress factors [Michalak 2006, Korkina 2007], including high selenium [Ríos et al. 2008] and iodine doses [Blasco et al. 2010]. As already mentioned, only the application of Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 induced toxicity symptoms on lettuce plants. Stress effect of selenate(VI) on lettuce is confirmed by the increased content of ascorbic acid, phenolic compounds, phenylpropanoids and flavonols as well as by the higher level of scavenging activity of free radical DPPH (tab. 3). These relations were observed at their highest degree in 2012 especially for treatments with Na_2SeO_4 and KIO_3 + Na_2SeO_4 (fig. 4 A, B, C, D and F). In principle, all tested combinations with the application of Na_2SeO_4 with iodine had negative impact on the content of anthocyanins in lettuce (means for 2012–2014 – tab. 3 and results from 2012 and 2013 – fig. 4 E).

Table 3. Concentration of ascorbic acid, phenolic compounds, phenylpropanoids, flavonols, anthocyanins and free radical scavenging activity (% DPPH) in lettuce (means for 2012–2014)

Treatments (n = 12)	(mg·100 g^{-1}f.w.)					DPPH (%)
	ascorbic acid	phenolic compounds	phenylpropanoids	flavonols	anthocyanins	
Control	5.9 a	55.7 bc	19.0 bc	18.7 bc	1.25 c	16.6 c
KI	6.0 a	45.8 a	14.6 a	14.0 a	0.89 abc	12.0 a
KIO_3	9.2 b	49.5 ab	16.7 ab	15.1 a	0.67 ab	17.0 c
Na_2SeO_4	11.7 c	64.4 de	23.0 de	22.2 d	1.00 bc	22.5 d
Na_2SeO_3	6.9 a	49.6 ab	15.8 a	14.6 a	0.75 ab	12.9 ab
KI + Na_2SeO_4	14.6 d	59.1 cd	20.7 cd	20.8 cd	0.83 ab	22.1 d
KIO_3 + Na_2SeO_4	11.8 c	68.0 e	24.7 e	23.8 d	0.82 ab	24.0 d
KI + Na_2SeO_3	9.2 b	54.8 bc	17.1 ab	15.8 ab	0.55 a	15.8 bc
KIO_3 + Na_2SeO_3	6.0 a	51.9 ab	16.1 ab	14.9 a	0.61 ab	12.5 ab

Means followed by the same letters are not significantly different for $P < 0.05$

Presented reaction of plants to toxic effect of SeO_4^{2-} (applied separately and in combination with KI and KIO_3) on lettuce resulted from the application of relatively high dose of selenium. In the treatment with selenium applied in the form of SeO_4^{2-} nearly eightfold higher content of Se in lettuce was observed in relation to the application of SeO_3^{2-} (142.4 and 17.0 mg Se·kg^{-1} d.w., respectively for Na_2SeO_4 and Na_2SeO_3). Detailed results of the determination of iodine, selenium and SeMet and SeCys content in lettuce will be discussed in a separate publication.

Compared with the control, the fertilization using selenium(IV) (Na_2SeO_3, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3) did not cause, or caused much smaller changes than selenate(VI), as far as the content of ascorbic acid, phenolic compounds, phenylpro-

Table 4. Concentration of mineral nitrogen form in soil after lettuce cultivation (means for 20112–2014)

Treatments (n = 12)	(mg dm^{-3} soil)	
	N-NH_4^+	N-NO_3
Control	0.95 a	23.5 bc
KI	1.92 b	26.7 c
KIO_3	1.99 b	20.3 abc
Na_2SeO_4	1.91 b	40.9 e
Na_2SeO_3	0.86 a	20.0 abc
KI + Na_2SeO_4	1.02 a	36.7 d
KIO_3 + Na_2SeO_4	0.70 a	39.6 d
KI + Na_2SeO_3	1.07 a	15.7 a
KIO_3 + Na_2SeO_3	1.38 ab	17.9 ab

Means followed by the same letters are not significantly different for P < 0.05

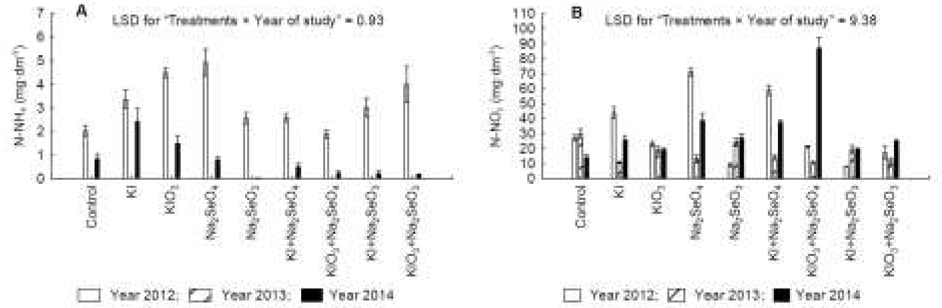

Fig. 5. Concentration of N-NH_4 (A) and N-NO_3 (B) in soil after lettuce cultivation in the years 2012–2014. Means followed by the same letters are not significantly different for P < 0.05

panoids, flavonols, anthocyanins and antioxidative activity in lettuce are concerned (tab. 3 and fig. 4 A–F). This indicates that fertilization with Na_2SeO_3, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3 did not have so far-reaching negative impact on the synthesis of these secondary compounds of plant metabolism as Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4. In the years 2013–2014, very high diversification in the contents of ascorbic acid, phenolic compounds, phenylpropanoids, flavonols, anthocyanins and antioxidative activity was observed after the applied fertilization with Na_2SeO_3, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3.

During the three-year research by Smoleń et al. [2014 b] on iodine biofortification of carrot, the observed impact of weather conditions on the content of dry matter, sugars, phenolic compounds and carotenoids in the storage roots of carrot fertilized with KI and KIO_3 in combination with $Ca(NO_3)_2$ or $(NH_4)_2SO_4$ was insignificant. Unlike carrot, lettuce is the shallow rooted plant and has much shorter cultivation period. Therefore, it is not surprising that in our research we have observed significant impact of weather conditions (extremely different in the particular years of the research) for the determined quality parameters of the crop as affected by iodine and selenium application.

Water deficit in soil, heat waves and high insolation in 2012 were additional factors, which enhanced stress effect of Na_2SeO_4 on the lettuce plants. It seems that in the conditions of optimal or excessive water content in soil in the years 2013–2014, the adverse impact of Na_2SeO_4 and its interaction with iodine compounds on lettuce were limited. This concerns metabolic and physiological processes responsible for the synthesis and accumulation of all compounds analyzed in lettuce (tab. 1–3, fig. 2–4). It did not, however, prevent the impairment of plant growth, which should be mainly attributed to the toxic effect of selenium(VI) rather than to its interaction with I^- and IO_3^- ions.

CONCLUSIONS

Extremely different course of weather conditions in the research years significantly modified the impact of fertilization with I and Se on nitrogen metabolism and the chemical composition of lettuce affecting its quality – with exception of the average weight of lettuce heads and the content of sucrose.

Application of Na_2SeO_4, separately or simultaneously with KI and KIO_3, induced stress effect on lettuce decreasing the average weight of heads each year as well as increasing the content of phenolic compounds, phenylpropanoids and flavonols followed by improved antioxidative activity in lettuce.

Simultaneous fertilization with KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 had particularly adverse impact on the content of total sugars in lettuce – and sucrose for fertilization with KI + Na_2SeO_4 – which was not observed when applying Na_2SeO_4 alone.

During drought and heatwaves in 2012, fertilization with Na_2SeO_4 KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 caused the increase, and in the years 2013–2014 (the years with the excessive and moderate rainfall) the decrease of N-total content in lettuce.

Fertilization with Na_2SeO_3, KI + Na_2SeO_3 and KIO_3 + Na_2SeO_3 did not cause so negative reaction or triggered much smaller changes than Na_2SeO_4 (applied separately and in combination with KI and KIO_3) with respect to the average weight of lettuce heads as well as determined parameters of nitrogen metabolism and biological quality of lettuce.

Each year the applied fertilization with iodine and selenium had different and unique impact on the content of NO_3^-, NO_2^-, NH_4^+, amino acids or chlorides in lettuce.

The determined content of N-NH_4 and N-NO_3 in soil after lettuce cultivation depended on the applied treatments with iodine and selenium as well as the course of weather conditions in individual years of the study.

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WYBRANE ASPEKTY METABOLIZMU AZOTU ORAZ JAKOŚĆ SAŁATY (*Lactuca sativa* L.) GRUNTOWEJ W ZALEŻNOŚCI OD ZRÓŻNICOWANEGO NAWOŻENIA ZWIĄZKAMI JODU I SELENU

Streszczenie. Jod i selen razem pełnią ważne funkcje w organizmach ludzi i zwierząt. Prowadzenie równoczesnej biofortyfikacji (wzbogacenia) roślin w te pierwiastki jest więc uzasadnione – często spotyka się problemy łącznego endemicznego niedoboru (lub ukrytego głodu) I i Se w żywności. Stosunkowo niewiele wiadomo na temat interakcji pomiędzy I i Se w roślinach. Nie tylko w zakresie efektywności ich akumulacji, ale również wpływu I i Se na gospodarkę mineralną roślin czy jakość biologiczna plonu. Badania przeprowadzone w latach 2012–2014 obejmowały nawożenie doglebowe jodem i selenem sałaty odmiany 'Valeska' z uwzględnieniem następujących kombinacji: kontrola, KI, KIO_3, Na_2SeO_4, Na_2SeO_3, KI + Na_2SeO_4, KIO_3 + Na_2SeO_4, KI + Na_2SeO_3, KIO_3 + Na_2SeO_3. Jod i selen były aplikowane dwukrotnie: przedsiewnie i w nawożeniu pogłównym (każde po 2,5 kg $I \cdot ha^{-1}$ + 0,5 kg $Se \cdot ha^{-1}$) – zastosowano całkowitą dawkę 5 kg $I \cdot ha^{-1}$ i 1 kg $Se \cdot ha^{-1}$. Z wyjątkiem masy główek sałaty oraz zawartości sacharozy stwierdzono, że skrajnie różny przebieg warunków klimatycznych w latach 2012–2014 w istotny sposób modyfikował wpływ nawożenia I i Se na gospodarkę mineralną azotem oraz jakość biologiczną sałaty. Nawożenie Na_2SeO_4, KI + Na_2SeO_4 oraz KIO_3 + Na_2SeO_4 (ale nie nawożenie samym Na_2SeO_4) było czynnikiem stresowym, zmniejszało masę główek sałaty i zawartość w niej cukrów oraz równocześnie zwiększającym zawartość związków fenolowych, fenolopropanoidów i flawonoli oraz aktywność antyoksydacyjną.

Słowa kluczowe: biofortyfikacja, jakość biologiczna, azotany, związki fenolowe

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