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THE EFFECT OF DIVERSE IODINE FERTILIZATION ON NITRATE ACCUMULATION AND CONTENT OF SELECTED COMPOUNDS IN RADISH PLANTS (*Raphanus sativus* L.)

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Abstract. Plant fertilization with iodine may be an alternative source of this element in human diet. Iodine influence on nitrogen metabolism in plants has not yet been thoroughly described. Thus, there is an urgent need to determine the effect of iodine application on nitrate(V) accumulation in plants. The aim of the study was to determine the influence of soil and foliar application of iodine forms (I^-, IO_3^-) on nitrate accumulation and concentration of selected compounds in radish plants. The following treatments were applied in the experiment: 1 – control (without application of iodine), 2 – foliar application in KI form, 3 – foliar application in KIO_3 form, 4 – soil fertilization in KI form, 5 – soil fertilization in KIO_3 form, 6 – soil fertilization in KI form + foliar application in KI form, 7 – soil fertilization in KIO_3 form + foliar application in KIO_3 form. Soil fertilization with iodine was carried out before radish sowing to the level of 15 mg $I \cdot dm^{-3}$ soil. Foliar application of this element was performed twice using iodine solution in a concentration per pure element of 0.2%, in dose of 0.4 $dm^3 \cdot m^{-2}$. In all tested combinations with iodine treatment an increase of ammonium ion content in radish roots was observed in comparison to the control. Both, foliar nutrition with KI as well as nitrogen fertilization with KIO_3 (combination 2 and 6, respectively) resulted in a significant increase of free amino acids concentration in radish roots. No significant influence of tested factors was noted for the root and leaf content of: dry mass, nitrates(V), nitrates(III) as well as root level of total soluble sugars and leaf concentration of photosynthetic pigments and ammonium ions in radish.

Key words: iodine, I^-, IO_3^-, radish, nitrate, amino acids, biofortification

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INTRODUCTION

At present, the most common, simple as well as economical way of iodine supplementation in human diet is iodizing salt. Still, iodized salt poses also serious hazards to human health. Salt is by far the biggest source of sodium in the human diet and has long been known to increase blood pressure [Jacobson 2009]. For that reason, according to WHO recommendations [2007], there is a urgency to significantly reduce (nearly doubly) sodium consumption by humans. As a consequence, it would contribute to decreased iodine intake by population. In that case, in the last few years a vital necessity has arisen to develop alternative methods of introducing iodine to the diet. It appears that biofortification of vegetables with this element could play an important role in supplementing iodine deficiency. Iodine included in plant tissues can be relatively easily absorbed by humans as its bioavailability can reach up to 99% [Strzetelski 2005].

Plant biofortification with iodine can be performed through biotechnological techniques as well as traditional agricultural methods i.e. soil fertilization or foliar application of this element [Hong et al. 2008, Ledwożyw et al. 2009, Strzetelski et al. 2009]. This way of increasing iodine content in plants can cause certain difficulties resulting, among others, from the fact that this element does not play any particular physiological function in plants [Dai et al. 2004, Dai et al. 2006, Kabata-Pendias and Mukherjee 2007]. Iodine application in small doses can though improve plant growth and development [Borst-Pauwels 1961]. On the other hand, excessive iodine fertilization can induce plant damages manifested as initially marginal chlorosis mainly of older leaves accompanied with necrosis. Symptoms of iodine surplus in younger leaves include dark-green pigmentation and in extreme cases whole-plant death occurs [Maćkowiak and Grossl 1999, Maćkowiak et al. 2005].

A separate issue concerning plant fertilization with iodine includes insufficient information available on iodine metabolism in plants [Kabata-Pendias i Mukherjee 2007]. The range of iodine side-effects on biochemical and physiological reactions in plants has not yet been recognized [Xia et al. 2003]. It is thus important to determine the influence of iodine forms (I^-, IO_3^- ions) and methods of its application not only on the processes mentioned before but also on biological quality of yield. What needs a particular attention is the interaction between iodine and metabolic pathway of nitrogen, especially in the step of nitrate(V) reduction [Laurens and Commaneay 1970]. Even though Waite and Truesdale [2003] showed that iodate reduction to iodide in *Isochrysis galbana* (microalgae) does not require nitrate reductase (NR) activity, later study conducted by Hung et al. [2005] indicated that there could be a potential possibility of reducing IO_3^- to I^- by this enzyme. In that case a hypothesis can be stated that greater availability of IO_3^- forms for plants could result in decreased nitrate(V) reduction to nitrate(III) by NR. As a consequence, higher accumulation of nitrates(V) and decreased quality of plant yield (e.g. vegetables) can occur. Results of preliminary studies conducted by Ledwożyw et al. [2010] confirmed the potential connection between NO_3^- and IO_3^- reduction. Investigating the effect of foliar application of iodine in the form of IO_3^- on nitrate reductase activity in lettuce leaves (*Lactuca sativa* L.) a significant decrease in NR activity as well as an increase in nitrate(V) content were observed. Still, plants biofortufied with iodine (irrespective of its dose: 2 kg I · ha^{-1} or 5 kg I · ha^{-1}) generally accumulated less nitrate(V) ions in leaves than control plants.

The aim of the present study was to determine the influence of two iodine forms (I^- and IO_3^-) applied into soil or foliarly on nitrate(V) accumulation as well as concentration of selected compounds in radish roots.

MATERIAL AND METHODS

Small radish (*Raphanus sativus* L. var. *radicula* Pers.) 'Opolanka' c.v. was cultivated in 2006–2007 in open-work containers sized 60 × 40 × 20 cm placed in the open field under a shade-providing fabric. The containers were filled with silt loam (35% sand, 28% silt and 37% clay) with mean content of organic matter 2.52 % and the following content of available (in 0.03 M CH_3COOH) nutrient forms: N (N-NO_3+N-NH_4) 14.9 mg, P 73.8 mg, K 92.4 mg, Mg 159.8 mg and Ca 1299.0 mg in 1 dm^{-3} soil. Soil reaction $pH_{(H_2O)}$ was 6.88 and $pH_{(KCl)}$ 6.20, while total concentration of salt in soil (EC) was 0.15 mS·cm^{-1}.

The following treatments with soil and foliar application of iodine were applied: 1 – control (without application of iodine), 2 – foliar application of KI form, 3 – foliar application of KIO_3 form, 4 – soil fertilization with KI form, 5 – soil fertilization with KIO_3 form, 6 – soil fertilization with KI form + foliar application of KI form, 7 – soil fertilization with KIO_3 form + foliar application of KIO_3 form. Soil fertilization with KI and KIO_3 to the level of 15 mg I·dm^{-3} soil was carried out before sowing radish seeds. Foliar application of KI and KIO_3 was performed twice using iodine solution in 0.2% concentration of pure element, in level of 0.4 dm^3·m^{-2}. First foliar application of iodine was performed at the two-leaf stage i.e. on 24 and 15 May (in 2006 and 2007, respectively), while the second one on 1 June and 18 May in the subsequent years.

Seed sowings were performed on 04.05.2006 and 11.04.2007 in 5 rows per 1 container with 25 seeds in each row. Studies were carried out in three replicates. After germination plants were thinned to 12 seedlings per row. The content of available nitrogen and potassium forms in soil was supplemented before cultivation to the level of 100 mg·dm^{-3} of soil with the use of solid fertilizers of ammonium nitrate and potassium sulphate. The soil level of other macro elements (K, Mg, Ca) did not need supplementation. In periods of insufficient rainfall, the radish was watered with municipal water. Radish harvest combined with taking leaf, root and soil samples were performed on 08.06.2006 and 22.05.2007, respectively. Radish roots suitable for consumption (of weight: 3.0–7.6 g) were used in further analyses.

The analysis of plant samples was performed in the fresh plant material immediately after harvesting. In radish leaves the concentration of assimilation pigments was measured spectrometrically after extraction with 80% acetone [Arnon 1949]. In both leaf and root samples the content of dry weight was determined in 105 °C. The concentration of: nitrate(V) (NO_3^-), nitrate(III) (NO_2^-) and ammonium ions (NH_4^+) was determined by FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)] in extracts prepared using 2% CH_3COOH [Nowosielski 1988]. In radish roots the concentration of free amino acids was measured in the reaction with ninhydrin [Korenman 1973] and total soluble sugars by anthronic method [Yemm and Wills 1954].

In soil samples collected after the radish harvest the content of: $N\text{-}NH_4$, $N\text{-}NO_3$, P, K, Mg, Ca, Na and S was determined after extraction with 0.03 M CH_3COOH [Nowosielski 1988]. The concentration of nitrogen forms: $N\text{-}NH_4$, $N\text{-}NO_3$ was determined by FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)] while P, K, Mg, Ca, Na and S were determined with the ICP-OES technique using a Prodigy Teledyne Leeman Labs USA spectrometer.

Obtained results were statistically verified by ANOVA module of Statistica 8.0 PL programme for significance level $P < 0.05$. Significance of changes was assessed with the use of variance analysis. In case of significant changes homogenous groups were determined on the basis of Duncan test.

RESULTS AND DISCUSSION

The results of yield, iodine content in radish plants and soil as well as antioxidant properties of radish were presented in previous work [Strzetelski et al. 2009].

Tested combinations of soil fertilization and foliar application of iodine demonstrated statistically significant interaction only in the case of the content of ammonium ions and free amino acids in radish roots (tab. 1). Increased concentration of NH_4^+ ions (in comparison to control plants) was observed in all surveyed combinations. The highest level of NH_4^+ was noted for radish roots after soil fertilization and foliar application of iodine in the form of KIO_3 (combination no. 7). Similarly, in pot experiments with carrot cultivation Smoleń et al. [2009b] observed an increase in ammonium ion content in carrot roots after soil fertilization with KIO_3 but only in combination with soil nitrogen fertilization in the form of ammonium sulphate. In carrot plants fertilized with this N form, KIO_3 application (when compared to KI) contributed to higher level of free amino acids in storage roots. Adversely, in the present work iodide form of iodine (I^-) applied foliarly as well as through soil and foliar fertilization (combination no. 2 i no. 6 – tab. 1) led to a significant increase of free amino acid level in radish roots. Statistically greater accumulation of these compounds was also found in roots of plants fertilized in both ways with IO_3^- form when compared to the control and combinations: 3 and 4. No significant influence of tested factors was found in reference to the content of: dry matter, nitrates(V), nitrates(III) in leaves and roots, leaf level of photosynthetic pigments and root concentration of ammonium ions and total soluble sugars (tab. 1).

The method of iodine application as well as a chemical form of this element (I^-, IO_3^-) did not significantly affect the accumulation of nitrates(V) and nitrates(III) in both leaves and roots of radish. A clear tendency was though observed that plants fertilized with IO_3^- irrespective of the application method accumulated the highest amount of nitrate(V) ions. This dependency was particularly strong for leaves of plants from combinations: 3 and 5 (tab. 1).

In study conducted by Smoleń et al. [2009a, 2009b], diversified fertilization with iodine and nitrogen had no significant influence on root content of total soluble sugars as well as total N in carrot leaves. On the other hand, these authors revealed a significant

Table 1. Results of leaf and root analysis in radish (means from 2006–2007)
Tabela 1. Wyniki analiz laboratoryjnych liści i zgrubień rzodkiewki (średnie z lat 2006–2007)

Combinations Kombinacje		Dry matter % d.m. Sucha masa % s.m.	Nitrate(V) – Azotany(V) (NO_3^-)	Nitrate(III) – Azotany(III) (NO_2^-)	Ammonium ions Jony amonowe (NH_4^+)	Chlorophyll a Chlorofil a	Chlorophyll b Chlorofil b	Chlorophyll a+b Chlorofil a+b	Carotenoids Karotenoidy
			$mg \cdot kg^{-1}$ f.w. – $mg \cdot kg^{-1}$ św.m.			$mg \cdot g^{-1}$ f.w. – $mg \cdot g^{-1}$ św.m.			
Lesves Liście	1.	8.96 a	2947 a	1.10 a	6.5 a	0.79 a	0.30 a	1.09 a	0.42 a
	2.	9.95 a	2431 a	0.77 a	9.3 a	0.77 a	0.28 a	1.05 a	0.40 a
	3.	9.12 a	3355 a	0.37 a	8.4 a	0.77 a	0.28 a	1.05 a	0.39 a
	4.	8.77 a	2905 a	0.85 a	7.0 a	0.77 a	0.27 a	1.04 a	0.39 a
	5.	8.75 a	3580 a	0.74 a	8.3 a	0.75 a	0.27 a	1.01 a	0.39 a
	6.	9.59 a	3161 a	1.19 a	9.1 a	0.78 a	0.27 a	1.05 a	0.40 a
	7.	9.17 a	3032 a	1.33 a	9.3 a	0.77 a	0.27 a	1.03 a	0.39 a
Test *F* – Test *F*		-	-	-	-	-	-	-	-
Combinations Kombinacje		dry matter % d.m. sucha masa % s.m.	$mg \cdot kg^{-1}$ f.w. – $mg \cdot kg^{-1}$ św.m. nitrate(V) azotany(V) (NO_3^-)	nitrate(III) azotany(III) (NO_2^-)	ammonium ions jony amonowe (NH_4^+)	free amino acids $N_2 \cdot 100\ g^{-1}$ f.w. aminokwasy $N_2 \cdot 100\ g^{-1}$ św.m.		total soluble sugars $mg \cdot 100\ g^{-1}$ f.w. cukry rozpuszczalne $mg \cdot 100\ g^{-1}$ św.m.	
Roots Zgrubienia	1.	4.52 a	2065 a	0.94 a	26.1 a	44.0 a		1756.8 a	
	2.	4.61 a	2109 a	0.34 a	33.9 ab	69.2 d		1620.5 a	
	3.	4.35 a	2260 a	0.43 a	28.9 ab	41.9 a		1574.8 a	
	4.	4.42 a	2116 a	0.18 a	28.5 ab	44.7 a		1719.5 a	
	5.	4.57 a	2276 a	0.49 a	31.7 ab	48.5 ab		1668.4 a	
	6.	4.60 a	2234 a	0.61 a	32.3 ab	64.9 cd		1541.6 a	
	7.	4.63 a	2310 a	0.33 a	36.6 b	56.0 bc		1619.1 a	
Test *F* / Test *F*		-	-	-	-	*		-	

Where: 1. Control, 2. Foliar nutrition KI, 3. Foliar nutrition KIO_3, 4. Soil fertilization KI, 5. Soil fertilization KIO_3, 6. Soil fertilization + Foliar nutrition KI, 7. Soil fertilization + Foliar nutrition KIO_3.

Gdzie: 1. Kontrola, 2. Dokarmianie dolistne KI, 3. Dokarmianie dolistne KIO_3, 4. Nawożenie doglebowe KI, 5. Nawożenie doglebowe KIO_3, 6. Nawożenie doglebowe + Dokarmianie dolistne KI, 7. Nawożenie doglebowe + Dokarmianie dolistne KIO_3.

Test *F* for cooperation: combination × year of study – Test *F* dla współdziałania: kombinacja × rok badań.

Means followed by the same letters are not significantly different for $P < 0.05$. – Średnie oznaczone tymi samymi literami nie różnią się istotnie dla $P < 0,05$.

diverse interaction of soil application of iodine in KI and KIO_3 forms and nitrogen fertilization in reference to the content of: nitrates(V), ammonium ions and free amino acids. It should be pointed out, that in the carrot study iodine was applied in much lower doses (1 mg $I \cdot dm^{-3}$ in soil) when compared with the present research (15 mg $I \cdot dm^{-3}$ soil).

Interestingly, with considerably small differences of chemical composition between tested combinations, particularly in respect of NO_3^- accumulation, a very high diversity was observed in reference to $N\text{-}NO_3$ concentration in soil after radish cultivation (tab. 2). What should be noted is the fact that soil after cultivation of plants fertilized with KI in both ways of application (combination no 2, 4 and 6) contained the highest level of mineral nitrogen, particularly $N\text{-}NO_3$. High concentration of mineral nitrogen in plants from these three combinations can indicate that iodine application in the form of I^- possibly limits nitrate(V) uptake from soil by radish plants. A comparable relation was found in carrot study conducted by Smoleń et al. [2009b].

Results presented in table 2 indicate that method of iodine application as well as its chemical form did not cause any significant changes in residual P, Mg, Ca, Na and S concentration in soil. Tested variants of experiments contributed to significant changes of final content of potassium in soil. Interestingly, the lowest K level remained in soil after cultivation of plants with foliar application of IO_3^- form of iodine (combination no. 3).

Table 2. Concentration ($mg \cdot dm^{-3}$ soil) of $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NH_4$+$N\text{-}NO_3$, P, K, Mg, Ca, Na and S in soil after radish cultivation (means from 2006–2007)

Tabela 2. Zawartość ($mg \cdot dm^{-3}$ gleby) $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NH_4$+$N\text{-}NO_3$, P, K, Mg, Ca, Na i S w glebie po uprawie rzodkiewki (średnie z lat 2006–2007)

Combinations[1] Kombinacje	$N\text{-}NH_4$	$N\text{-}NO_3$	$N\text{-}NH_4$+ $N\text{-}NO_3$	P	K	Mg	Ca	Na	S
1.	1.7 a	32.6 a	34.2 a	23.4 b	56.9 d	160.0 a	1178.6 a	13.7 b	62.9 a
2.	1.2 a	70.8 e	71.9 d	20.7 a	48.4 bc	143.3 a	1111.9 a	9.3 a	58.4 a
3.	1.4 a	40.7 b	42.1 b	20.7 a	40.1 a	147.4 a	1116.2 a	8.6 a	59.2 a
4.	4.6 b	50.5 c	55.1 c	22.5 ab	62.5 e	151.4 a	1137.7 a	9.8 a	53.2 a
5.	5.5 b	34.6 ab	40.1 ab	20.8 a	46.6 b	138.1 a	1094.1 a	10.1 a	66.4 a
6.	9.2 c	61.1 d	70.2 d	23.2 b	55.7 d	137.9 a	1082.4 a	10.5 a	62.1 a
7.	5.1 b	33.9 ab	39.0 ab	20.5 a	51.3 c	148.3 a	1156.6 a	9.4 a	66.2 a

Where: 1. Control, 2. Foliar nutrition KI, 3. Foliar nutrition KIO_3, 4. Soil fertilization KI, 5. Soil fertilization KIO_3, 6. Soil fertilization + Foliar nutrition KI, 7. Soil fertilization + Foliar nutrition KIO_3.
Gdzie: 1. Kontrola, 2. Dokarmianie dolistne KI, 3. Dokarmianie dolistne KIO_3, 4. Nawożenie doglebowe KI, 5. Nawożenie doglebowe KIO_3, 6. Nawożenie doglebowe + Dokarmianie dolistne KI, 7. Nawożenie doglebowe + Dokarmianie dolistne KIO_3.

An in-depth discussion of obtained results seems difficult to undertake as relatively small number of studies has been published regarding iodine effect on mineral nutrition of plants including metabolic pathways of nitrogen. An unequivocal answer for the question whether iodine application in the form of IO_3^- stimulates plant nitrate(V) accumulation is impossible to give. The possibility of mentioned relation could be supported by results obtained by Hung et al. [2005] as well as preliminary study conducted by Ledwożyw et al. [2010]. It can be thus hypothesized that IO_3^- influence on nitrogen metabolism in plants, including nitrate(V) accumulation, is dependent on the following factors: iodine dose, plant species, weather conditions throughout cultivation as well as type and concentration of other anions in soil. Anion ions, e.g. SO_4^{2-}, after its simultaneous uptake with IO_3^- by plants can interfere in mineral nutrition of nitrogen, inter alia, through inhibiting nitrate(V) reduction what results in greater accumulation of these compounds in plants [Smoleń et al. 2009a, 2009b]. Explanation of these relations requires further studies.

CONCLUSIONS

In all tested variants of experiments, application of iodine, irrespective of its dose and method of fertilization, resulted in increased content of ammonium ions in radish roots, in comparison to the control.

Foliar nutrition with KI as well as soil fertilization followed by foliar application of KI, and to lesser extent soil and foliar application of KIO_3, considerably increased free amino acid concentration in radish roots.

Iodine application in radish, regardless of its dose and application method, had no significant effect on the content of: dry matter, nitrates(V), nitrates(III) in leaves and roots as well as on the level of photosynthetic pigments in leaves and total soluble sugar concentration in radish roots.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA JODEM NA AKUMULACJĘ AZOTANÓW ORAZ NA ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW W ROŚLINACH RZODKIEWKI (*Raphanus sativus* L.)

Streszczenie. Nawożenie roślin jodem może być alternatywnym źródłem tego pierwiastka stosowanym w diecie człowieka. Wpływ jodu na gospodarkę azotową roślin jest zagadnieniem, które jak do tej pory nie zostało jeszcze rozpoznane. Dlatego też istnieje pilna potrzeba określenia wpływu aplikowanego jodu roślinom na akumulację azotanów. Celem badań określenie wpływu form jodu (I^- i IO_3^-) aplikowanego doglebowo i dolistnie na akumulację azotanów oraz zawartość wybranych składników w roślinach rzodkiewki.

W badaniach wyróżniono następujące warianty dolistnej i doglebowej aplikacji jodu: 1 – kontrola (bez aplikacji jodu), 2 – aplikacja dolistna w formie KI, 3 – aplikacja dolistna w formie KIO_3, 4 – nawożenie doglebowe w formie KI, 5 – nawożenie doglebowe w formie KIO_3, 6 – nawożenie doglebowe + dolistna aplikacja w formie KI, 7 – nawożenie doglebowe + dolistna aplikacja w formie KIO_3. Nawożenie doglebowe jodem zastosowano przedsiewnie w dawce 15 mg $I \cdot dm^{-3}$ gleby. Dokarmianie dolistne tym pierwiastkiem zastosowano dwukrotnie, stosując roztwór jodu w stężeniu czystego składnika 0,2% w dawce cieczy roboczej 0,4 $dm^3 \cdot m^{-2}$. We wszystkich obiektach traktowanych jodem stwierdzono zwiększenie zawartości jonów amonowych w zgrubieniach rzodkiewki w porównaniu z kontrolą. W zgrubieniach roślin z kombinacji nr 2 i 6, jak również w mniejszym stopniu z kombinacji nr 7, stwierdzono znaczące zwiększenie zawartości wolnych aminokwasów. Nie stwierdzono istotnego wpływu badanych kombinacji na zawartość suchej masy, azotanów(V), azotanów(III) w liściach i zgrubieniach oraz na zawartość barwników asymilacyjnych i jonów amonowych w liściach, jak i na zawartość cukrów rozpuszczalnych w zgrubieniach rzodkiewki.

Słowa kluczowe: jod, I^-, IO_3^-, rzodkiewka, azotany, aminokwasy, biofortyfikacja

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