ISSN 1644-0692
www.acta.media.pl

ACTA *Acta Sci. Pol. Hortorum Cultus, 15(3) 2016, 3-16*

THE EFFECT OF IODINE BIOFORTIFICATION ON SELECTED BIOLOGICAL QUALITY PARAMETERS OF LETTUCE AND RADISH SEEDLINGS

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Abstract. Iodine deficiency disorders are one of the serious worldwide public health problem in the world. The need to search for alternative methods of iodine supplementation results from the recommendation of the World Heath Organization and aims to significantly reduce iodine malnutrition in humans diet. Iodine is not included among essentials nutrients for plants, but the plants are able to accumulate it. Seedlings biofortified with iodine can become an alternative source of this element for humans. The aim of the study was to attempt to obtain iodine-fortified lettuce and radish seedlings and to determine the effect of the level of iodine applied in the form of potassium iodide on their biological quality. The following levels of KI were used: 0 (control), 0.075, 0.15, 0.0375, 0.75 and 1.5 mg per Petri dishes. The effect of potassium iodide on the selected parameters of their biological quality varied depending on the KI doses and species of plant. The seedlings grown in the presence of KI had a higher iodine content. The results showed that the most appropriate biofortification application rates were 0.075 and 0.15 mg because the enriched seedlings had biological quality parameters similar to the control. Statistically significant differences in the parameters characterizing seedling quality were noted most often in the case of the highest amounts of KI (0.375–1.5 mg). These KI concentrations reduced seedling's lenght in radish and lettuce seedling but increased dry weight only in lettuce. A significant increase in ascorbic acid concentration only in the lettuce seedlings was obtained. In comparison with the control, no significant differences in the content of biomass and chlorophyll content were noted in the biofortified seedlings. Thiol group content was decreased in both radish and lettuce, but the antioxidant activity measured by DPPH method only in lettuce seedling extracts.

Key words: iodine – enriched vegetables, potassium iodide, sprouts

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INTRODUCTION

Iodine is one of the trace elements essential for the normal functioning of the human body. It is supplied to the body mainly via food, table salt and drinking water. Iodine deficiency remaines a serious public health problem in the developing world, and thus is at risk of thyroid disease [Zimmermann 2011]. Iodine deficiency is also a problem in certain areas of Europe [Szybiński 2012]. For this reason, many countries practice iodine prophylaxis involving the introduction of iodine into the diet via iodized salt [Untoro et al. 2010]. The effectiveness of this method is mainly due to excess consumption of salt, which has led to a global increase in cardiovascular disease, including hypertension and certain cancers. Iodine deficiency in the human diet and the need to seek new sources for fortifying foods with this nutrient provide basis for undertaking research aimed at identifying the most suitable species for biofortification with iodine [Allen et al. 2006]. Iodine supplementation may cause problems during cooking, processing and food storage. Therefore, enhancing iodine content in raw eaten plants is a good way to control its deficiency, as this limits the loss of iodine and thus iodine in vegetables is readily bioassimilated. In scientific publications the conviction predominates that iodine fertilization is not essential for the development of plants. Land plants can take up iodine from the soil through their roots [Strzetelski 2010] or directly from the atmosphere, e.g. through the leaves [Fuge 2013]. Since vegetables are able to accumulate iodine, increasing iodine application to the soil or water results in enhancing its accumulation in plants [Zhu et al. 2003]. Several methods of iodine plant enrichment have been proposed, and the positive results were obtained particularly in hydroponic culture [Zhu et al. 2003, Dai et al. 2004, Blasco et al. 2008, Smoleń et al. 2011b]. However achievement of iodine accumulation levels may lead to loss of biological quality of seeds. The biofortification of seedlings with iodine has therefore been proposed as a strategy to improve human nutrition. The seeds of various plants are germinated for consumption and after a few days of growth the plants are consumed in the early stage of development, when they are known as sprouts. Due to the large quantities of easily assimilated nutrients produced during the germination process (including proteins, fats, vitamins, and mineral compounds), sprouts are a valuable element of the diet [Gajewski et al. 2008]. Owing to the diversity of seeds and the sprouts obtained from them, they are a varied source of taste sensations and of health-promoting compounds. Seedlings a few days after germination are a natural source of nutrients, dietary fiber, vitamins and micronutrients [Dueñas et al. 2009]. High-molecular-weight reserve substances present in seeds (proteins, carbohydrates and fats) are broken down during the germination process into simple compounds which are easily assimilated by the human body. Seedlings contain more nutrients than seeds or adult plants. The literature reports that most sprouts contain more vitamin C, polyphenols and B vitamins and exhibit higher antioxidant activity than mature vegetables [Zieliński et al. 2007]. Regular consumption of sprouts provides protection against many serious illnesses, activates the immune system, corrects vitamin and mineral deficiencies, and most importantly, has anticarcinogenic effects [Sasikala and Kannan 2014].

The aim of the study was to attempt to obtain iodine-fortified lettuce and radish seedlings and to determine the effect of iodine on biological quality of seedlings.

MATERIALS AND METHODS

Plant material. The material for the study was radish (*Raphanus sativus* L.) cv. Krasa and iceberg lettuce (*Lactuca sativa* L.) cv. Great Lakes 118. The seeds were sown on Petri dishes (Ø 150 mm) lined with filter paper. Seeds were weighed out in the amount of 0.5 g per dish (on average 59 Krasa radish seeds and 452 Great Lakes 118 lettuce seeds). KI solution in the amount of 10 cm^3 was applied to the Petri dishes at concentrations of 7.5, 15, 37.5, 75 and 150 $mg \cdot dm^{-3}$, so that the amounts of KI applied to the dish were 0.075 mg, 0.15 mg, 0.375 mg, 0.75 mg and 1.5 mg. Hereafter 'dose of KI' will refer to the amount of potassium iodide applied to one Petri dish. A control sample was prepared in which the seeds received only distilled water. The Petri dishes were placed under a light with a 12/12 hour light cycle at 22–24°C. The seedlings were watered on the second and fourth days. The germination rate of the lettuce seeds, tested each time according to ISTA recommendations [ISTA 2011], was 90%, and that of the radish seeds was 93%. Seedlings for further testing were collected on the sixth day.

Laboratory analysis procedure. Iodine content in the material was determined following incubation with TMAH (tetramethylammonium hydroxide) by ICP-MS according to standard PN-EN 15111:2008P using a Varian 820 MS mass spectrometer. Method agreed with PN-EN 15111:2008P norm [Smoleń et al. 2011a]. The analysis was performed by the Central Laboratory of Agroecology the University of Life Sciences in Lublin. CLA was accredited by the Polish Centre for Accreditation; nr AB 1375.

The seedlings' length was measured precisely to 1 mm. All seedlings growing on the Petri dishes were weighed on an analytical scale to determine their biomass (i.e. fresh weight). The dry weight of the seedlings was determined by the oven-dry method at 105°C.

The content of chlorophyll in the acetone extracts from the seedlings was determined by spectrophotometry. The sprouts (1 g) were homogenized in a mortar with 5 cm^3 of 80% acetone. The procedure of acetone extraction was repeated a couple of times. Absorbance was measured at 663 nm and 645 nm [Ni et al. 2009]. Chlorophyll content was expressed in $mg \cdot g^{-1}$ FW. The chlorophyll concentration was calculated as follows:

$$\text{chlorophyll a} = [12.7A_{663} - 2.69A_{645}]\ V/1000\ W;$$

$$\text{chlorophyll b} = [22.9A_{645} - 4.86A_{663}]\ V/1000\ W;$$

$$\text{chlorophyll a + b} = (8.02 \cdot A_{663} + 20.20 \cdot A_{645}) \cdot V/1000\ W;$$

where V = volume of the extract; W = weight of fresh seedlings (g FW).

The antioxidant activity of the seedling extracts was determined by the method using the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl), which react with antioxidant compounds present in the extract and change color during the reaction [Brand-Williams et al. 1995]. The changes in the absorbance of the solutions are proportional to the quantity of antioxidants in the solution. Total antioxidant capacity measured by the DPPH method indicates the antioxidant activity of the seedling extracts. The stock solution was prepared by dissolving 24 mg DPPH with 100 cm^3 methanol and then stored at -20°C until needed. The working solution was obtained by dissolving DPPH stock solution with methanol to obtain an absorbance of 1.0 ±0.02 units at 515 nm using the spectrophotometer. Methanol extract was prepared using the method of Thaiponga et al. [2006] with some modifications: 10 grams of seedlings were mixed with 100 cm^3 of methanol and homogenized. The homogenates were kept at 4°C for 12 h and then centrifuged. The supernatants were recovered and stored at -20°C until analysis. Seedlings methanol extracts (0.150 cm^3) were allowed to react with 2.850 cm^3 of the DPPH solution for 30 minutes in the dark. The absorbance was measured at $\lambda = 515$ nm. The total antioxidant activity was expressed as trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2--carboxylic acid) equivalent per g of FW. The standard curve was linear between 20 and 850 µM of trolox. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

Thiol group content was determined using Ellman’s reagent, containing 5,5’-dithiobis-(2-nitrobenzoic acid), according to Rice-Evans [Rice-Evans 1996]. The reaction solutions contained 1.470 cm^3 of 0.1 mM DTNB solution and 0.05 cm^3 of the water seedlings extract (10 g seedlings were homogenized with 100 cm^3 cold water and centrifuged). The absorbance of the solution was measured with a spectrophotometer at 412 nm. Thiol group concentration in the sample was expressed as GSH equivalent per g of FW.

Ascorbic acid content in the seedling extracts was determined using Folin-Ciocalteu’s phenol reagent which was adapted from Bajaj and Kaur [1981] with some modifications [Biliński and Bartosz 2006]. Extracts for ascorbic acid analysis were obtained by homogenizing 10 g of seedlings in 100 cm^3 cold solution of 10% TCA. The homogenates were centrifuged at 4°C for 10 min. The supernatants were recovered and ascorbic acid immediately measured. Sprout extracts (2.8 cm^3) were allowed to react with 0.2 cm^3 of the Folin-Ciocalteu's phenol reagent for 10 minutes in the dark condition. Readings of the colored product were then taken at 750 nm. Ascorbic acid content was calculated by the standard curve method.

Statistical analysis. All analyses were carried out in three independent replications. For each KI concentration the number of samples in the statistical calculations was at least $n = 9$ (i.e. 3 parallel replications of each series × 3 replications of the experiment in time), and $n = 80$ for determination of the length of the seedlings. Statistical analysis of the results was performed using one-way analysis of variance (ANOVA) in Statistica 10.0 PL at a significance level of $P < 0.05$. Homogenous groups were determined using Tukey’s test.

RESULTS AND DISCUSSION

A review of the literature provides a great deal of information about biofortification of plants with iodine. Specific agricultural practices have been developed in order to increase iodine content in several fruits, vegetables and crops: as tomato [Kiferle et al. 2013, Landini et al. 2011], carrot [Smoleń et al. 2009], barley and wheat [Caffagni et al. 2012], cabbage, long cowpea, cucumber, coriander and eggplant [Weng et al. 2013]. However, the subject of most of this research is adult plants. The experiments presented here were conducted on seedlings of radish and lettuce seedlings, which according to literature data have considerable health-promoting properties and good flavour. During seed germination intense biochemical processes take place and a number of compounds are synthesized *de novo*, so that it will be possible to ascertain the effect of iodine on these processes.

THE IODINE ACCUMULATION IN THE RADISH AND LETTUCE SEEDLINGS

After 6 days the iodine concentration was determined in collected seedlings. In the control samples the iodine concentration was higher in the radish seedlings (tab. 2) than in the lettuce seedlings (tab. 1). The content of nutrients and minerals in the seeds depends on their genetic traits, including size and weight, the predisposition of the plant to accumulate iodine, and the environmental conditions of its growth. To date the literature has lacked data on iodine content in the seeds of these plant species. It could be suggested that the reason for the differences in the iodine content noted in the control seedlings of lettuce and radish may be genetic and environmental factors. One of these could be the weight of the seeds. The weight of the radish seeds was about 7.5 times greater than that of the lettuce seeds. We used commercial seeds in the study, and the plants that produced them may have grown on soils with different levels of iodine and could thus have accumulated it in different amounts. Another possible explanation is the use of iodine solution to prevent seed-borne diseases (at a minimum of 500 ppm for at least one hour) [EP 1018883 A1].

Table 1. The effect of the KI doses on selected biological quality parameters of the lettuce seed -lings

KI doses (mg per Petri dish)	0	0.075	0.15	0.375	0.75	1.5
Iodine content (mg·kg^{-1} FW of seedlings)	0.41a	12.22b	24.53c	33.18d	107.18e	175.73f
Iodine content per FW of seedlings from one Petri dish (mg) *	0.0020a	0.0529b*	0.1111c*	0.1743d*	0.5094e*	0.8459f*
In parentheses the percentage of the iodine dose applied to Petri dish (%)	–	(92.3)	(96.9)	(60.7)	(88.9)	(73.7)
Iodine content per seedling (µg)	0.005	0.131	0.274	0.430	1.258	2.089
Biomasse (g FW per Petri dish)	4.910a	4.495a	4.610a	5.315a	4.772a	4.825a
Dry matter (% FW)	4.45a	4.52a	4.47a	4.68a	4.96b	4.87b
Length of seedlings (mm)	62.82a	61.93a	60.67a	53.43b.c	48.13c	48.15c
Chlorophyll *a* (mg·g^{-1} FW)	0.22a	0.22a	0.23a	0.21a	0.25a	0.24a
Chlorophyll *b* (mg·g^{-1} FW)	0.092a	0.094a	0.089a	0.091a	0.102a	0.114a
Chlorophyll *a* + *b* (mg·g^{-1} FW)	0.321a	0.326a	0.314a	0.328a	0.369a	0.331a
Total antioxidant capacity (mM Trolox·g^{-1} FW)	0.44a	0.42a	0.43a	0.35b	0.38b	0.36b
Thiol group content (µmol GSH·g^{-1} FW)	0.60a	0.59a	0.59a	0.55b	0.55b	0.54b
Ascorbic acid content (mg·100 g^{-1} FW)	10.78a	11.41a	11.84a	13.49b	16.98c	16.73c

Means designated with the same letters for a given species, in the same row, do not differ significantly at $P < 0.05$

* – the iodine content in seedlings growing in the presence of KI was reduced by the content of iodine in the control seedlings

Table 2. The effect of the KI doses on selected biological quality parameters of the radish seed -lings

KI doses (mg per Petri dish)	0	0.075	0.15	0.375	0.75	1.5
Iodine content ($mg \cdot kg^{-1}$ FW of seedlings)	1.04a	14.71b	22.89c	42.97d	105.94e	199.02f
Iodine content per FW of seedlings from one Petri dish (mg)	0.0044a	0.0553b*	0.0953c*	0.1810d*	0.4334e*	0.7990f*
In parentheses the percentage of iodine dose applied to Petri dish (%)	–	(96.5)	(83.2)	(63.1)	(75.6)	(69.7)
Iodine content per one seedling (µg)	0.080	1.005	1.734	3.291	7.880	14.527
Length of seedlings (mm)	78.00a	75.73a	68.93a.b	68.79a,b	61.73b	60.21b
Biomasse (g FW per Petri dish)	4.223a	4.056a	4.357a	4.314a	4.133a	4.037a
Dry matter (% FW)	7.21a	7.25a	7.51a	7.41a	7.57a	7.38a
Chlorophyll *a* ($mg \cdot g^{-1}$ FW)	0.201a	0.19a	0.184a	0.171a	0.185a	0.181a
Chlorophyll *b* ($mg \cdot g^{-1}$ FW)	0.011a	0.093a	0.089a	0.092a	0.087a	0.095a
Chlorophyll *a* + *b* ($mg \cdot g^{-1}$ FW)	0.305a	0.278a	0.274a	0.284a	0.271a	0.292a
Total antioxidant capacity (mM $Trolox \cdot g^{-1}$ FW)	1.46a	1.44a	1.46a	1.54a	1.58a	1.52a
Thiol group content (µmol $GSH \cdot g^{-1}$ FW)	1.27a	1.19a	1.13a	0.99b	0.84b	0.83b
Ascorbic acid content ($mg \cdot 100\ g^{-1}$ FW)	77.84a	78.66a	78.96a	79.18a	82.42a	82.52a

Means designated with the same letters for a given species. in the same row. do not differ significantly at $P < 0.05$
* – the iodine content in seedlings growing in the presence of KI was reduced by the content of iodine in the control seedlings

Accumulation of iodine in the seedlings increased with the level of the salt applied. In comparison with the control, a higher iodine increase was noted in the lettuce seedlings (tab. 1) than in the radish seedlings (tab. 2). In seedlings produced from seed samples of the same weight (0.5 g) in the presence of KI applied to the Petri dish in the amounts of 0.075 and 0.15 mg, the iodine concentration ($mg \cdot kg^{-1}$ FW) in the lettuce seedlings was 2.980 and 59.383% respectively, of the control value, and in the radish seedlings 1.414 and 2.201% of the control value. Calculations showed that the lettuce seedlings growing in the presence of 0.075 mg KI took up 92.3% of the iodine applied to the Petri dish (tab. 1), while the radish seedlings took up 96.5% (tab. 2). At higher doses of KI the percentage of iodine taken up by the seedlings decreased, with the exception of iodine uptake by the lettuce seedlings growing in the presence of 0.15 mg KI. It should be emphasized that although the biomass of the lettuce seedlings was only slightly greater than that of the radish seedlings from the same sample size of 0.5 g (tabs 1 and 2), the number of lettuce seedlings formed and the biomass of a single seedling was over 7 times greater than in the case of the radish. According to our calculations, the mean amount of iodine per control seedling was 16 times greater in the radish seedlings than in the lettuce seedlings, and single biofortified radish seedlings contained about 6.2–7.6 times more iodine than single lettuce seedlings (tabs 1 and 2). Radish seeds are larger than lettuce seeds, which may affect the ability of the seedlings to accumulate iodine after germination. After the short experiment was completed (6 days) the amount of iodine remaining in the environment where the seedlings had grown was not determined, and therefore we cannot definitively state whether the differences in iodine content noted in the seedlings of the two species were the result of different predispositions to accumulate iodine or to differing capacity to emit it into the atmosphere in the form of CH_3I.

The physiology of iodine in plants is poorly characterized. The aerial organs of plants of different species may emit methyl iodide into the atmosphere. Specific enzymes possessing S-adenosyl-L-methionine-dependent methyltransferase activity are responsible for this iodine volatilization. Daikon radish (*Raphanus sativus*) is characterized by high activity of this enzyme, particularly in the leaves of young seedlings (3–5 days), while activity is lower in young roots and was not detected in the roots of mature plants. High activity of this enzyme has also been noted in young cabbage seedlings – higher in the leaves than in the stems and roots [Attieh et al. 2000]. In vivo emission of CH_3I is correlated with activity of this enzyme. Emission of CH_3I from the sprouting leaves of *R. sativus*, *T. aestivum* and *O. sativa* grown hydroponically increased with the concentration of supplied iodide [Itoh et al. 2009]. In seeking suitable plants for biofortification it is worth considering the intensity of CH_3I emission and the activity in its organs of the enzyme S-adenosylmethionine: halide ion methyltransferase, because it may influence the iodine content in the plants.

Biofortification of plants with selected minerals begins with their uptake by the roots (minerals present in the soil solution) or leaves (foliar fertilization). They are then redistributed in the plant and accumulated in edible tissues in the form of non-toxic compounds [Weng et al. 2013]. The ability to accumulate minerals is a species-specific trait, but transport to target tissues also depends on the mobility of trace elements in the plant. Se and Mg, for example, are well transported through the phloem, while transport of iron, zinc, copper and iodine is limited [White and Broadley 2005]. For this reason tissues such as fruits, seeds and tubers supplied with nutrients via transport through the phloem are often low in Fe, Zn Cu, I and Ca, while leafy vegetables are a rich source of these trace elements [White and Broadley 2005].

In our experiments both species accumulated iodine well in the form of potassium iodide (tabs 1 and 2). In other researches both iodide and iodate are used to fortify plants with iodine, and both forms are taken up well by plants. The iodate form is less harmful for plants than the iodide form, but many authors emphasize that that plants are able to take up iodide better [Smoleń et al. 2011b]. A pot experiment compared the ability of six vegetables to accumulate iodine, including leafy vegetables: pakchoi (*Brassica chinensis* L.), spinach (*Spinacia oleracea* L.), tuber vegetables: onion (*Allium cepa* L.), shoot vegetables: water spinach (*Ipomoea aquatica* Forsk), celery (*Apium graveolens* L.) and root vegetables: carrot (*Daucus carota* L. var. *sativa* DC.). Spinach (a leafy vegetable) was found to be the best vegetable for biofortification with iodine [Dai et al. 2004]. In a hydroponic culture of spinach [Zhu et al. 2003] a direct correlation was observed between the iodine I^- levels applied (0, 1, 10, 50 and 100 μM) and iodine accumulation in the plant tissues. The highest accumulation of iodine was found in leaves of spinach fertilized with 2 mg I and 1 g sucrose dm^{-3} of soil [Smoleń and Sady 2011]. In a pot culture of carrot, iodine at a concentration of 1 mg I dm^{-3} of soil applied to the soil in the form of KI did not cause an iodine biofortification effect in the storage roots or leaves, while fertilization with KIO_3 increased iodine concentration in the leaves [Smoleń et al. 2009]. In a hydroponic culture of tomato fertilized with iodine salts, a very high concentration of this mineral was noted in the leaves and stems of plants treated with 20 mM of sodium iodide (about 9.000 mg · kg^{-1} FW) [Landini et al. 2011]. The tomato fruits had a lower concentration of iodine, attaining a maximum concentration of 30 mg · kg^{-1} FW in plants treated with 20 mM KI. Tomato fruits in a pot culture fertilized with iodine salts contained from a few mg to up to 10 mg of iodine per kg of fresh weight [Kiferle et al. 2013].

World Health Organization recommends daily iodine intake of 150 µg for adults [Allen et al. 2006]. The results obtained in this work suggest that the lowest concentration of the potassium iodide is sufficient for a biofortification programme. The iodine concentration in the biofortified seedlings is elevated. The daily iodine requirement would meet 10.2 g portion of radish or 12 g of lettuce seedling growing in the presence of 0.075 mg KI per Petri dish.

EFFECT OF IODINE ON RATE OF SEEDLING GROWTH

Fortification of plants with iodine by means of fertilization at high rates entails the risk of damage to the plants by the toxic effects of iodine. Excessive levels of iodine cause a decrease in biomass. To evaluate the effect of KI on the growth rate of the seedlings their length, biomass and dry weight were determined. The radish and lettuce seedlings grew rapidly and the presence of KI in the growth environment significantly affected the length of the lettuce seedlings (tab. 1), which in the case of the highest level, 0.375–1.5 mg KI, were 15–23% shorter than the control. The radish seedlings growing in the presence of doses of 0.375 to 1.5 mg KI were about 12–23% smaller than in the control, and the differences were statistically significant (tab. 2). The iodine added to the radish seedling culture did not significant change the content of the dry matter and biomass in the plants (tab. 2). In the case of the lettuce seedlings there was even a statistically significant increase in dry matter – about 12 and 9% in the case of the plants growing in the presence of 0.75 and 1.5 mg KI respectively. The biomass (fresh weight) of the lettuce biofortified seedlings did not differ significantly from the control (tab. 1).

In a pot culture of lettuce a substantial decrease in biomass was noted when the plants were fertilized with potassium iodide at a level higher than 40 µM, while fertilization with potassium iodate had no negative effect on biomass [Blasco et al. 2008]. In a culture of rice fertilized with iodide salt at levels of 10 µM and higher, lower biomass was noted in the rice plants [Mackowiak et al 2005]. Similarly, a decrease in biomass was observed during a pot culture of carrot fertilized via soil application of nitrogen and different forms of iodine (iodide and iodate) [Smoleń et al. 2009]. High levels of iodide, 10 and 100 µM, has also been found to decrease the biomass of spinach [Zhu et al. 2003].

EFFECT OF IODINE ON CHLOROPHYLL CONTENT IN THE SEEDLINGS

Another indicator of the biological quality of plants is content of chlorophyll, a pigment vital for the photosynthesis process. In our experiment, the addition of KI had no effect on chlorophyll (a, b, a + b) in lettuce and radish seedlings (tabs 1 and 2). During the seedling culture in the presence of KI no clear signs of toxic effects of iodine were observed. A small but not statistically significant increase was noted in the content of chlorophyll a, b and a + b in the lettuce seedlings growing in the presence of 0.75 mg·dm^{-3} and 1.5 mg KI (tab. 1). There was no statistically significant decrease in all types of chlorophyll concentration for all levels of KI applied in the radish samples (tab. 2).

The toxicity of potassium iodide for beans (*Phaseolus vulgaris*) is greater in the light than in the dark. Subjecting leaves to KI in the dark or in the light decreases photosynthesis activity equally, but the loss of chlorophyll is less pronounced in the dark. Iodine behaves in a similar manner, but is effective at lower concentrations. Iodide does not inhibit chlorophyll synthesis. Iodine is equally effective as iodide at inhibiting the Hill reaction in isolated chloroplasts. These results are consistent with the hypothesis that iodine toxicity is the result of intracellular oxidation of iodine. The degree of iodine toxicity depends on the form of iodine occurring in the soil solution. Iodide ions (I^-) are more toxic than iodate ions (IO_3^-). In plant tissues I^- may oxidize to I_2, which iodinates photosystem II components [Mackowiak et al. 2005].

Iodine is not an essential nutrient for plants, so there is little data on the effect of iodine on plant metabolism. A study of the effect of different levels – 20, 40 and 80 μM – and forms – iodate and iodide – of iodine on photosynthesis and carbohydrate metabolism in lettuce plants found that none of the concentrations applied substantially affected saccharose metabolism. However, the highest level of iodide, 80 μM, reduced the photosynthetic rate and photosynthetic parameters (stomatical conductance and transpiration). The use of iodate, in contrast, increased photosynthetic rate, stomatical conductance, and transpiration with respect to the control [Blasco et al. 2011].

EFFECT OF IODINE ON ANTIOXIDANT ACTIVITY

Radish sprouts have high content of phenolic compounds and high antioxidant properties, which decrease as growth continues [Sasikala and Kannan 2014]. The chemical composition of seeds is reflected in the content of active substances in sprouts. Sprouts of various plants have different composition and concentration of antioxidant compounds. This is confirmed by the results of our study. The higher total antioxidant capacity (TAC) was observed in the control radish plants (tab. 2) than in the control lettuce seedlings (tab. 1).

The seedling extracts had high concentrations of antioxidant compounds. The total antioxidant capacity in the extracts from the control lettuce seedlings (tab. 1) was about 70% lower than in the control radish seedlings (tab. 2) as measured by the DPPH test. The study found varied effect of KI on antioxidant content in the seedling extracts. The applied method confirmed that the presence of KI in the growth environment caused an decrease in total antioxidant capacity in the lettuce seedlings (tab. 1). In the case of the radish seedlings, KI did not cause statistically significant changes in TAC (tab. 2).

Measurements of antioxidant properties using FRAP or DPPH tests have shown that extracts from radish sprouts have less beneficial properties than extracts from sprouts of lettuce [Samotyja et al. 2007]. Blasco et al. [2008] found substantial differences in the antioxidant properties of lettuce grown in a pot experiment with different forms and concentrations of iodine. The total antioxidant capacity of the extract, measured using FRAP, TEAC, and Fe^{3+}-TPTZ Reducting Power, was higher for plants growing in the presence of I^- at levels from 20 μM up to 240 μM. Fertilization with iodate also increased the antioxidant properties of the extract, but to a much lesser degree.

EFFECT OF IODINE ON THIOL GROUP CONTENT

Thiol groups are present in cysteine, methionine, glutathione, homocysteine, coenzyme Q, coenzyme A and proteins which are building material for many cell structures. The glutathione thiol group, due to its reactivity, takes part in reactions detoxifying electrophilic compounds and neutralizing reactive oxygen species [Gruhlke and Slusarenko 2012].

The concentration of the thiol group in the control radish seedlings (tab. 2) was about two times higher than in the lettuce seedlings (tab. 1). In the lettuce and radish seedlings enriched with KI at levels of 0.375 $mg \cdot dm^{-3}$ and higher was observed a statistically significant decrease in the concentration of these compounds (tabs 1 and 2). It is worth noting the high content of thiol compounds in the radish seedlings (tab. 2). Radish contains a high concentration of active sulphur compounds with antineoplastic properties [Aghajanzadeh et al. 2014]. Cruciferous vegetables are unique in that they are rich sources of glucosinolates, sulphur-containing compounds that are responsible for their pungent aromas and spicy (some say bitter) taste [Higdon et al. 2007].

The enzyme S-adenosylmethionine: halide ion methyltransferase can transfer a methyl group from S-adenosyl-L-methionine to a halide ion. This same enzyme catalyses the formation of methanethiol (CH_3SH) and methyl thiocyanate (CH_3SCN) in the presence of the bisulphide ion (SH^-) or thiocyanate ion $(SCN)^-$. In this reaction S-adenosyl-L-methionine reacts with a thiol, thereby generating S-adenosyl-L-homocysteine and a thioether. The thioether is emitted into the atmosphere. *In vivo* production of CH_3SH and dimethyl sulphide was observed from *R. sativus* when iodide was supplied. In the organs of *R. sativus* CH_3SH is generated on a variety of metabolic pathways. It is additionally produced from methionine, in a reaction catalysed by the enzyme methionine γ-lyase [Itoh et al. 2009].

The main compound containing thiol groups is glutathione, a key antioxidant involved in processes maintaining redox homeostasis in the cell [Anjum et al. 2012]. In the present study the applied KI levels caused a decrease in the content of compounds containing thiol groups (tabs 1 and 2). In cells glutathione mainly occurs in its reduced form, and changes in its concentration may indicate a response of the plant to a stress factor appearing in its environment.

Effect of iodine on ascorbic acid content. Vitamin C is one of the antioxidants found in abundance in plants. Human beings must take it in with food, and plants are a good source. The extracts from the control radish seedlings contained about 7 times more vitamin C (tab. 2) than the lettuce seedling extracts (tab. 1). In the lettuce seedlings enriched with KI at levels of 0.375, 0.75 and 1.5 mg, significantly higher ascorbic acid content was noted than in the control. In the case of the radish seedlings, a slight but not statistically significant increase in ascorbic acid content was shown following application of KI (tab. 2).

Blasco et al. [2008] reported a substantial increase in vitamin C concentration in lettuce biofortified with iodine at a level of 80 µM. Smoleń et al. [2011b] found that iodine in the form of I^- and iodate caused a significant decrease in lycopene content and an increase in ascorbic acid content in tomato fruits from the third cluster. The form KIO_3 exerted a more beneficial effect on the biological quality of the tomato fruits by increasing the total acidity of the extract and total soluble sugars (including glucose and fructose), both in comparison to the control and to plants fertilized with iodide. There was no significant effect of iodine, in either the form of KI or KIO_3, on content of β-carotene, phenolic compounds, phenylpropanoids, flavonols or anthocyanins in the tomato fruits.

CONCLUSIONS

The effect of potassium iodide on the iodine content in the seedlings and their biological quality varied depending on the doses applied and species of plant. All applied doses of KI resulted in the biofortification of our seedlings. Seedlings can be effectively fortified with iodine even with the use of the lowest level of applied KI in the study (0.075 and 0.15 mg per Petri dish); iodine content increased in seedlings fortified with this amount of KI, and the enriched seedlings had biological quality parameters similar to the control. Statistically significant differences in the parameters characterizing seedling quality were noted most often in the case of the highest amounts of KI (0.375, 0.75 and/or 1.5 mg per Petri dish). The amounts of applied KI did not cause statistically significant changes in the dry matter only in radish seedlings however the seedlings were smaller. Although the iodine content per kg fresh weight of biofortified seedlings of the two species differed only slightly, the content of this element in a single radish seedling was over 6 times greater than in a single lettuce seedling. Applied doses of KI were exerted varied influence on the content of the examined antioxidants properties: total antioxidant capacity, ascorbic acid and thiol group content in the seedlings. Thiol group content in the biofortified seedlings decreases, but ascorbic acid content increases only in lettuce seedlings as the amount of KI applied increases. A reduction in total antioxidant capacity was noted only in biofortified lettuce seedlings. In the seedlings of the species tested KI did not cause statistically significant changes in chlorophyll concentration.

The proposed method of fortifying seedlings may have practical significance. The authors believe that when a method has been developed for enriching seedlings with iodine, they can become a functional food with broad applications. They can be eaten raw, added to processed foods, lyophilized, or dried and added in this form to dietary supplements. Other advantages of the method proposed will be its repeatability, the possibility of obtaining a beneficial iodine concentration in seedlings, and ease of cultivation. Future research can be conducted on iodine enrichment of seeds grown for sprouts so that consumers will be able to grow seedlings biofortified with iodine at their own convenience.

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WPŁYW BIOFORTYFIKACJI W JOD NA WYBRANE PARAMETRY BIOLOGICZNEJ JAKOŚCI SIEWEK SAŁATY I RZODKIEWKI

Streszczenie. Zaburzenia spowodowane niedoborem jodu są ważnym problem zdrowotnym na świecie. Poszukiwanie alternatywnych metod suplementacji żywności w jod wynika z zaleceń Światowej Organizacji Zdrowia i ma na celu zmniejszenie niedoborów jodu w żywieniu człowieka. Jod nie jest zaliczany do podstawowych składników odżywczych dla roślin, ale rośliny mogą go akumulować. Siewki biofortikowane w jod mogą stać się dla ludzi źródła tego składnika. Celem przeprowadzonych badań była próba uzyskania siewek sałaty i rzodkiewki wzbogaconych w jod i ustalenie wpływu dawki jodu stosowanego w formie jodku potasu na ich jakość biologiczną. Zastosowano następujące dawki KI: 0; 0,075; 0,15; 0,75 i 1,5 mg na szalkę Petriego. Wpływ jodku potasu na oznaczane parametry jakości biologicznej zależał od dawki KI i gatunku testowanej rośliny. Siewki rosnące w obecności KI charakteryzowały się zwiększoną zawartością jodu. W warunkach doświadczenia najbardziej odpowiednie do biofortyfikacji dawki to 0,075 i 0,15 mg, przy których parametry jakości biologicznej siewek są zbliżone do kontroli. Stwierdzono, że statystycznie istotne różnice wartości parametrów charakteryzujące jakość biologiczną siewek obu gatunków występują najczęściej w przypadku najwyższych dawek KI (0.375–1.5 mg). Zastosowanie KI w tych dawkach spowodowało zmniejszenie długości siewek sałaty i rzodkiewki, ale tylko w przypadku siewek sałaty zwiększenie suchej masy. Tylko w siewkach sałaty stwierdzono znaczący wzrost zawartości kwasu askorbinowego. W porównaniu z kontrolą nie odnotowano znaczących różnic biomasy i zawartości chlorofilu w biofortyfikowanych siewkach. Stężenie grup tiolowych uległo zmniejszeniu zarówno w siewkach rzodkiewki, jak i sałaty, ale całkowita zdolność antyoksydacyjna mierzona metodą DPPH zmniejszyła się tylko w siewkach sałaty.

Słowa kluczowe: warzywa wzbogacone w jod, jodek potasu, siewki

Accepted for print: 26.01.2016

For citation: Krzepiłko, A., Zych-Wężyk, I., Molas, J., Skwaryło-Bednarz, B., Święciło, A., Skowrońska, M. (2016). The effect of iodine biofortification on selected biological quality parameters of lettuce and radish seedlings. Acta Sci. Pol. Hortorum Cultus, 15(3), 3–16.