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ORIGINAL PAPERS

EFFECT OF IODINE BIOFORTIFICATION OF LETTUCE SEEDLINGS ON THEIR MINERAL COMPOSITION AND BIOLOGICAL QUALITY

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Abstract

Iodine deficiencies in the human diet have created the need to search for new sources of food enriched with this element. The aim of biofortification of plants with iodine is to increase the iodine content in plants while ensuring that food products are safe for health. Seedlings enriched with iodine may be an alternative source of this element. Iodine is not an essential nutrient for plants, and its role and effects on plants are not fully known, particularly in the early stages of development. The aim of the study was to determine the effect of applying KI on the iodine biofortification of lettuce seedlings of the variety Michalina. The seedlings were grown on Petri dishes lined with filter paper. The doses of KI used for biofortification were 0 (control), 0.5, 1, 2.5 and 5 μM dm^{-3}. Iodine concentration was determined in the seedlings by ICP-MS, while K, Na, Mg, Ca, Fe, Zn, Mn and Cu were assayed by atomic absorption spectrometry. In respect of the biological quality of the iodine-enriched seedlings, we determined their length, biomass yield and chlorophyll content by spectrophotometry following extraction with acetone. A significant increase in the iodine content in seedlings was obtained after each dose of KI and we can claim that even the lowest dose, 0.5 μM dm^{-3} KI, is sufficient for biofortification. In comparison with the control, KI reduced the length of seedlings but did not affect their biomass or the chlorophyll content. Seedlings growing in the presence of 1, 2.5 and 5 μM dm^{-3} KI contained more potassium and less sodium and manganese than the control seedlings. The magnesium concentration was lower than in the control seedlings following the application of 2.5 or 5 μM dm^{-3} KI. No significant differences in the content of calcium, zinc, iron or copper were noted in the biofortified seedlings.

Keywords: biofortification, iodine, mineral composition, seedlings, lettuce.

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INTRODUCTION

Vitamin and mineral deficiencies are a global problem. According to the WHO, over two billion people worldwide have dietary deficiencies of iodine, iron, zinc, folic acid, calcium, selenium, fluorine, vitamins A, C and D, as well as B-group vitamins (Allen et al. 2006). Vitamin and mineral deficiencies are the cause of specific illnesses, but they also exacerbate the effects of infectious agents, increase morbidity and mortality rates, and negatively affect the quality of life. Inadequate consumption of micronutrients affects both industrialized and developing regions of the world. Intensive agricultural production does not always ensure a suitable quality of crops, especially in terms of their mineral content. Biofortification of plants makes it possible to improve the quality of foods of plant origin, but is mainly used to correct deficiencies in crop plants of micronutrients such as magnesium, calcium, iron and zinc (Garcia-Banuelos et al. 2014).

Research on iodine enrichment of foods of plant origin is less advanced, but is gaining importance because the WHO recommends reducing the intake of table salt, which is the main source of iodine in the human diet. Iodine is essential for the proper functioning of human metabolism, particularly the synthesis of thyroid hormones. Iodine deficiencies are the main cause of developmental delays, mental retardation, endemic goitre and other health problems termed iodine deficiency disorders (Luo et al. 2014).

Biofortification of plants with iodine may be one strategy for improving the human diet. Studies have been conducted on fertilization of mature plants with iodine salts in order to increase the content of this nutrient, e.g. in lettuce (Blasco et al. 2008, Smoleń et al. 2011b), tomatoes and potatoes (Caffagni et al. 2011), spinach, cabbage, cucumbers, aubergines and bell pepper (Weng et al. 2013). Leaf vegetables accumulate greater amounts of iodine than root vegetables. The same problem appeared in the publications cited above, that is how to dose iodine so as to obtain the right concentrations of this element in individual plant organs and to ensure good quality crops. In the present study, lettuce seedlings were chosen as the subject of biofortification research. Lettuce seedlings can be consumed fresh at all times of year, they come in a variety of flavours and are a good source of antioxidants, such as phenolic acids, flavonoids, trace elements and vitamins. During germination of seeds, metabolic processes occur that increase their nutritional value in comparison to dormant seeds (Pająk et al. 2014). A few days old seedlings contain more bioactive compounds than mature vegetables (Moriyama, Oba 2004).

The aim of the study was to enrich seedlings with iodine and to determine the effect of the applied KI on the content of selected macro- (K, Na, Ca, Mg) and microelements (Fe, Mn, Zn, Cu) and also iodine in cv. Michalina lettuce seedlings. Another objective was to evaluate the influence of KI on selected parameters characterizing the biological quality of seedlings: length, biomass and chlorophyll content.

MATERIAL AND METHODS

Batches of cv. Michalina lettuce seeds weighing 0.5 g each were prepared. The seeds' germination rate was tested according to the ISTA recommendation (Ista 2011) and found to range from 91.5% to 93.5%. The seeds (0.5 g) were placed in Petri dishes 150 mm in diameter, lined with filter paper, and then either 10 cm^3 potassium iodide solution (KI in the doses of 0.5, 1, 2.5 and 5 μM dm^{-3}) or water (the control) was added. The seedlings grew for six days in a 12h/12h photoperiod under a Sylvania grow light. The temperature was 25°C/22°C for day and night, respectively. From day 2 to 6, the seedlings were watered with Hoagland's solution diluted four times (relative to full-strength Hoagland medium) (Hoagland, Arnon 1951). Plant material for analysis was collected after 144 h of KI treatment.

The 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 (Smoleń et al. 2011*b*).

The content of K, Na, Ca, Mg, Zn, Mn, Fe and Cu was determined according to standard PN-EN 14084:2004. Samples of plant material were mineralized in 65% super pure HNO_3 using a CEM MARS-5 Xpress microwave oven, and the content of nutrients was determined by atomic absorption spectrometry using a Varian AA 280FS Atomic Absorption Spectrometer (Pasławski, Migaszewski 2006).

Seedling length was measured with a ruler. Biomass was determined by weighing all the seedlings growing in a Petri dish on an analytical scale and expressed as g g^{-1} of seeds sown.

The chlorophyll content in the seedlings was determined by spectrophotometry following extraction with 80% acetone (Ni et al. 2009). Absorbance was measured at 645 nm and 663 nm. The chlorophyll content in a sample was calculated according to the following equation: Chlorophyll $a = (12.7A_{663} - 2.69A_{645})V/1000W$; Chlorophyll $b = (22.9A_{645} - 4.86A_{663})V/1000W$; Chlorophyll $a+b = (8.02A_{663} + 20.20A_{645})V/1000W$; where A – absorbance measured at the given wavelength, V – volume of the extract (cm^3); W – weight of fresh sprout (g). The chlorophyll content was expressed in mg g^{-1} FW.

The experiments were carried out in at least three independent replications.

The results were analysed statistically by one-way analysis of variance (ANOVA) in Statistica 10.0 software, with a level of significance of $p < 0.05$. Homogeneous groups were determined by the Tukey's test.

RESULTS AND DISCUSSION

The content of iodine in lettuce seedlings

Iodine content in plants grown on iodine-poor soils may be only 0.01 mg kg^{-1} DW, but when plants are grown in areas rich in this nutrient they may contain even 1 mg I kg^{-1} DW (Leung et al. 2012). Most iodine in fresh edible parts is found in broccoli – 0.15 mg kg^{-1} FW. Spinach (0.12 mg kg^{-1} FW) and dry peas (0.14 mg kg^{-1} FW) are also rich in iodine. The control seedlings of cv. Michalina lettuce contained more iodine than these vegetables (Table 1).

Table 1

Effect of KI application on the content of iodine and selected characteristic of cv. Michalina lettuce seedlings

Selected characteristic	Unit	Doses KI (μM dm^{-3})				
		0	0.5	1	2.5	5
Iodine content	(mg I kg^{-1} FW)	0.621*a*	9.532*b*	24.61*c*	66.43*d*	126.4*e*
Seedling length	(mm)	53.03*a*	48.23*b,c*	45.57*b,c*	43.60*c*	41.24*d*
Biomass	(g FW g^{-1} seeds sown)	15.66*a*	15.90*a*	15.46*a*	14.89*a*	14.85*a*
Chlorophyll *a*	(mg g^{-1} FW)	0.112*a*	0.123*a*	0.119*a*	0.125*a*	0.122*a*
Chlorophyll *b*		0.061*a*	0.063*a*	0.058*a*	0.059*a*	0.064*a*
Chlorophyll *a* + *b*		0.171*a*	0.168*a*	0.166*a*	0.173*a*	0.165*a*
Na	(mg kg^{-1} FW)	130.5*a*	133.1*a*	119.9*b*	117.8*b*	118.6*b*
K		681.5*a*	692.4*a*	730.7*b*	724.5*b*	740.3*b*
Mg		161.4*a*	160.5*a*	158.4*a*	137.5*b*	140.1*b*
Ca		250.6*a*	254.9*a*	248.1*a*	253.6*a*	264.9*a*
Zn		10.55*a*	9.512*a*	9.982*a*	10.11*a*	10.51*a*
Mn		2.051*a*	1.889*a*	1.693*b*	1.587*b*	1.703*b*
Fe		7.611*a*	7.774*a*	7.724*a*	7.807*a*	7.645*a*
Cu		2.684*a*	2.882*a*	2.613*a*	2.533*a*	2.699*a*

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

The content of iodine in plants depends on its availability to plants and on the plant genotype (Strzetelski 2005). According to this author, the most iodine is accumulated by leafy vegetables, which include lettuce. It is still difficult to conclude whether the high content of iodine in the control seedlings is a characteristic of the lettuce cultivar Michalina. There are no reliable data on the iodine content of the seedlings of other varieties of lettuce. 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). The experiment was conducted on commercial cv. Michalina lettuce seeds, but no data are available on procedures using iodine compounds during the seed production process.

In the biofortified lettuce seedlings, the concentration of iodine increased about 16-210 times depending on a dose of KI applied (Table 1). Even the smallest dose of potassium iodide (0.5 μM dm^{-3}) caused an approximately 16-fold increase in the iodine content in lettuce seedlings. Keeping in mind that the WHO recommends a daily iodine intake of 150 μg by adults (Allen et al. 2006), and taking into account only iodine from the control seedlings, it would be necessary to consume about 242 g of lettuce seedlings. As the iodine concentration in the biofortified lettuce seedlings is higher, seedling portions can be much smaller; even a portion of 12.30 g grown in the presence of 0.5 μM dm^{-3} KI would meet the daily iodine requirement. These results suggest that the lowest of the potassium iodide doses applied is sufficient for a biofortification programme. The literature to date lacks data on attempts to enrich edible seedlings with iodine. Other researchers have attempted to enrich seedlings with other micronutrients, such as selenium (Arscott, Goldman 2012), iron (Zielińska-Dawidziak, Siger 2012) and zinc (Zou et al. 2014). Research on iodine biofortification of mature plants has been carried out using much higher concentrations of KI. Landini et al. (2011) added 20 mM dm^{-3} of KI to a hydroponic solution for a tomato culture, carrying out three treatments (once a week). They found a very high iodine concentration in the leaves and stems (about 9,000 mg kg^{-1} FW), but the concentration in the fruits was lower, maximally 30 mg kg^{-1} FW. In field conditions, the heads of lettuce plants fertilized through foliar application of iodine salts at a concentration of 0.05% had an increased content of this element (Smoleń et al. 2011*b*). Attempts at biofortification of carrot have not been successful (Smoleń et al. 2009).

The method presented in this paper for enriching seedlings with iodine is an innovative approach enabling precise dosing of iodine salts and presumably, owing to their small size, obtaining a uniform iodine content in seedlings. Because seedlings are mainly consumed raw, there is no micronutrient loss during cooking or processing, and a cupful is sufficient to meet the daily iodine requirement.

The effect of potassium iodide application on parameters characterizing the intensity of seedling growth

After six days of growth, the control seedlings were about 53 mm long (Table 1). No visible signs of necrosis or morphological changes were observed on the lettuce seedlings growing in the presence of KI. Following the application of a dose of 1 μM KI dm^{-3} and higher, a statistically significant decrease was noted in their length, but there was no statistically significant change in the amount of biomass obtained.

Enrichment of plants with iodine, however, creates the risk of reducing their biological quality. A decrease in yield of cv. Melodion lettuce grown in a hydroponic system was noted following foliar enrichment with iodine (Ledwożyw-Smoleń et al. 2011). Iodine salt solutions used to watering plants led to some inhibition of their growth: maize and tobacco responded with the greatest reduction in produced biomass, while the growth of barley was inhibited the least (Caffagni et al. 2011).

The effect of potassium iodide application on the chlorophyll content

By determining the chlorophyll content we can evaluate the state of the photosynthetic apparatus of leaves. In horticultural practice, this helps to diagnose biotic and abiotic stresses to which plants are subjected (Gorbe, Calatayud 2012). Moreover, chlorophyll derivatives exhibit biological activity that may be valuable in terms of human health. Chlorophyll is synthesized *de novo* in growing seedlings; no visible signs of chlorosis were observed in our study on the plants growing in the presence of KI. In cv. Michalina lettuce seedlings, the KI doses applied caused no significant changes in the chlorophyll content in comparison with the control (Table 1). Similarly, no significant changes were observed in the content of photosynthetic pigments in radish leaves following foliar biofortification with various forms of iodine (Strzetelski et al. 2010). Iodine can have a phytotoxic effect on photosynthesis in plants. Fertilization of lettuce with potassium iodide in the amount of 80 μM dm^{-3} (added to the nutrition solution and maintained for 21 days) was found to reduce photosynthetic efficiency (Blasco et al. 2011*b*). Following fertilization of tomatoes with iodine in the form of iodate and iodide, a decrease in the content of chlorophyll *a+b* was observed in the oldest leaves (Smoleń et al. 2011*a*). Foliar fertilization of cinnamon basil with iodine in the form of iodide salt decreases the efficiency of photosystem II in leaves, but does not affect the content of photosynthetic pigments (Wojciechowska et al. 2013).

Content of selected elements in the biofortified seedlings

The seedlings growing in the presence of potassium iodide had more potassium and less sodium than the control seedlings. However, potassium accumulation was not as high as that of iodine; the potassium concentration increased with a dose of KI applied by 6 - 9% in comparison with the control (Table 1). The sodium concentration decreased by about 3 - 23% in response to the increasing doses of KI. The dynamics of potassium accumulation by a plant depends on its stage of growth, irrespective of a genotype (Grzebisz 2008). In the initial phase of plant development, potassium accumulation is relatively low, which was also noted in the experiments conducted on cv. Michalina lettuce. The critical phase of potassium uptake corresponds to the linear phase of growth of a plant, during which the biomass of organs, particularly the aerial ones, rapidly increases. The lettuce plants were not tested during this or subsequent stages. The results show that lettuce seedlings growing in a medium with KI doses 1 μM dm^{-3} and higher contained significantly more potassium than the control seedlings and the seedlings exposed to the lowest level of KI, i.e. 0.5 μM dm^{-3} (Table 1). According to Shabala and

Pottosin (2014), mild salt treatments often result in an increased rate of K^+ uptake and higher K^+ tissue content.

Enrichment of seedlings with iodine did not cause a significant change in the content of Ca, Zn, Fe and Cu. A significant decrease in the concentration of magnesium was observed for 2.5 and 5 μM dm^{-3} KI, and of manganese for 1, 2.5 and 4.5 μM dm^{-3} KI (Table 1).

In regard of the other elements, studies on the effect of iodine on the mineral metabolism in plants are conducted on a small scale, and there are few publications available. The reduction in the content of minerals noted in this study may be the result of dilution of these nutrients due to the seedlings' increased biomass. Blasco et al. (2011*a*) postulate that application of iodine (80 μM dm^{-3} IO_3^- added to the hydroponic solution during the 21 days of growth) may cause oxidative stress, manifested as changes in the activity of the antioxidant enzymes catalase and superoxide dismutase and in the concentration of ascorbic acid and glutathione. Another well-known response of plants to stress is the disturbance of K^+ and Na^+ homeostasis. Experiments conducted on mature vegetables suggest that changes in mineral composition vary depending on the amount and form of iodine applied, the type of cultivation, and the species or even the part of a plant analyzed. In a field crop of lettuce fertilized with iodine in different ways, both foliar and soil application of iodine increased the content of K, Mg, Ca, Mn and Cd and decreased the content of P, Cu and Zn (Smoleń et al. 2011*b*). Blasco et al. (2012) also attempted to determine whether application of different forms of iodine (iodide and iodate) affects the content of minerals in lettuce. Three weeks of application of a hydroponic solution with 80 μM dm^{-3} of iodide decreased the content of nitrogen, phosphorus and potassium to values below the optimal levels for the crop. In contrast, optimal contents of these nutrients were obtained following application of iodate. Smoleń et al. (2011*c*) studied the effect of iodine fertilization on the content of nutrients in the leaves and fruits of tomato plants grown in a hydroponic system. Application of iodine in the form of KI or KIO_3 significantly increased content of Na, Mg, B and Mn and reduced that of K, Cu, Fe, S, Mo and Zn in the tomato leaves over the third cluster. Iodine (in the form of KI and KIO_3) had no significant effect on content of Ca and P in this part of the plant.

In a study on soil application of iodine and nitrogen, a small but significant effect was noted on content of Ca, K, Mg, Na, P and S in the storage roots of carrot (Smoleń et al. 2009). Fertigation of spinach plants with KIO_3 solution caused an increase in K and Fe and a decrease in P, Ca, Na, Mo and Zn in leaves, while the content of Mg, S, Mn and B did not differ from the control (Smoleń, Ledwożyw-Smoleń 2011). Spinach leaves from a pot culture fertilized with iodine in the form of KI at a rate of 2 mg I dm^{-3} of soil contained more of Na, Fe, Zn and Al and less P, S, Cu and Ba than after a dose of 1 mg I dm^{-3} (Smoleń, Sady 2011). Differences between the relationships obtained in our study and the literature data in terms of the effect of potassium iodide on minerals may also be due to the form, dosage and means of iodine application, and to the development stage of the plants analyzed and species -specific differences. The effect of iodine on the content of macro- and micronutrients may also depend on cultivation conditions.

CONCLUSIONS

1. The proposed method for biofortification of seedlings of cv. Michalina lettuce with iodine is simple as iodine can be easily dosed and controlled, and the seedlings are enriched with a high content of iodine. The lowest dose applied, 0.5 μM KI dm^{-3}, is sufficient to enrich seedlings with iodine without affecting the biological quality of the yield.

2. The doses of KI cause no significant changes to the biomass or chlorophyll content of the seedlings, but decreased their length.

3. Application of iodine had varied impact on the content of selected minerals. The content of Potassium increased with the doses of KI applied. The Ca, Zn, Fe and Cu content did not significantly change and the biofortification with iodine generally decreased the Na, Mg and Mn content.

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