# **PRELIMINARY EVALUATION OF THE INFLUENCE OF SOIL FERTILIZATION AND FOLIAR NUTRITION WITH IODINE ON THE EFFICIENCY OF IODINE BIOFORTIFICATION AND CHEMICAL COMPOSITION OF LETTUCE\***

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#### Abstract

According to the World Health Organization, consumption of table salt (being a major carrier of iodine in human diet) should be reduced of 50%. Vegetables biofortified with iodine can become an alternative source of this element for humans. Agronomic recommendations with reference to biofortification have to be developed, including the evaluation of side-effects associated with iodine application to plants. Iodine is not an essential element for plants and hence its effect on crops has not yet been diagnosed. The aim of the study has been to assess the influence of soil fertilization with KI and foliar application of  $KIO_3$  on the success of iodine biofortification as well as the mineral composition of lettuce. Lettuce cv. Melodion  $F_1$  was cultivated in a field experiment in 2008-2009. Combinations with different soil fertilization and foliar nutrition with iodine were distinguished in the research including: control (without iodine application), three combinations with presowing soil fertilization of iodine (in the form of KI) in doses of 0.5, 1.0 and 2.0 kg I ha<sup>-1</sup> as well three combinations with four applications of foliar nutrition with iodine solution

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(as  $KIO_2$ ) in the concentration of: 0.0005%, 0.005% and 0.05% after using 1000 dm<sup>3</sup> of working solution per 1 ha so that the following amounts of iodine were applied: 0.02, 0.2 and 2.0 kg I ha<sup>-1</sup>, respectively. In lettuce heads, the iodine content as well as the content of: P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb were determined using the ICP-OES technique, while N-total was assayed by Kjeldahl method. In comparison to the control, only foliar nutrition with 0.05% solution of iodine significantly improved accumulation of this element in lettuce. At the same time, a lower level of nitrogen nutrition was observed in plants from this combination. A significant increase in the N-total content was found only in lettuce plants fed with 2.0 kg I ha<sup>-1</sup> dose of KI. In reference to the control, both foliar and soil application of iodine contributed to a higher content of K, Mg, Ca, Mn and Cd as well as a decreased level of P, Cu and Zn in lettuce. Doses, forms and application methods of iodine were found to have produced diverse effects on the content of S, Na, B, Fe, Mo, Al and Pb in lettuce plants.

Key words: biofortification, iodine, mineral composition, lettuce.

### WSTEPNA OCENA WPŁYWU NAWOŻENIA I ODŻYWIANIA DOLISTNEGO JODEM NA EFEKTYWNOŚĆ BIOFORTYFIKACJI SAŁATY W JOD ORAZ JEJ SKŁAD CHEMICZNY

#### Abstrakt

Zgodnie z zaleceniem WHO spożycie soli kuchennej (głównego nośnika jodu w diecie) musi zostać ograniczone o 50%. Warzywa biofortyfikowane w jod mogą stać się głównym źródłem jodu dla człowieka. Zalecenia agrotechniczne dotyczące sposobów biofortyfikacji muszą być opracowane na postawie badań określających uboczne oddziaływanie jodu na rośliny. Wynika to z tego, że jod nie jest składnikiem pokarmowym roślin, a jego oddziaływanie na rośliny nie zostało dostatecznie zdiagnozowane. Celem badań było określenie wpływu doglebowego nawożenia jodem (w formie KI) i odżywiania dolistnego tym pierwiastkiem (w formie KIO<sub>3</sub>) na efektywność biofortyfikacji w jod oraz na zawartość składników pokarmowych i balastowych w sałacie.

Sałatę odm. Melodion  $F_1$  uprawiano w doświadczeniu polowym w latach 2008-2009. W badaniach uwzględniono kombinacje ze zróżnicowanym nawożeniem doglebowym i odżywianiem dolistnym jodem. Wyróżniono kontrolę (nienawożoną i nieodżywianą dolistnie jodem), kombinacje z przedsiewnym nawożeniem doglebowym jodem w dawkach 0,5, 1,0 i 2,0 kg I ha<sup>-1</sup> oraz 4-krotne dolistne odżywianie roślin jodem w stężeniach 0,0005%, 0,005% i 0,05% - sumarycznie po zastosowaniu 1000  $dm^3$  cieczy roboczej na 1 ha zaaplikowano roślinom odpowiednio 0,02, 0,2 i 2,0 kg I ha<sup>-1</sup>. W główkach sałaty oznaczono: zawartość jodu oraz P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd i Pb technika ICP-OES oraz zawartość azotu metodą Kiejdahla.

W porównaniu z kontrolą, jedynie odżywianie dolistne jodem w stężeniu 0,05% wpłyneło na statystycznie istotne zwiększenie zawartości tego pierwiastka w sałacie. Jednocześnie stwierdzono pogorszenie stopnia odżywienia roślin w azot w tej kombinacji. Istotny wzrost zawartości azotu w sałacie uzyskano wyłącznie w wyniku nawożenia jodem w dawce 2,0 kg I ha<sup>-1</sup>. W odniesieniu do obiektu kontrolnego, zarówno dolistna, jak i doglebowa aplikacja jodu wpłynęła na zwiększenie zawartości K, Mg, Ca, Mn i Cd oraz na zmniejszenie zawartości P, Cu i Zn w sałacie. Stwierdzono zróżnicowane oddziaływanie dawki, formy i sposobu aplikacji jodu na zawartość S, Na, B, Fe, Mo, Al i Pb w sałacie.

Słowa kluczowe: biofortyfikacja, jod, skład mineralny, sałata.

### **INTRODUCTION**

Plant biofortification is defined as such an increase in the content of mineral element or biologically active substance in an edible part of crop plant that a notable improvement of human health can be achieved (WHITE, BROADLEY 2005, 2009, CAMPOS-BOWERS, WITTENMYER 2007).

The need to undertake research on possible increase of the iodine content in vegetables results from the recommendation of the World Health Organization to significantly reduce (even by half) salt uptake by humans. Excessive consumption of table salt is considered as one of the major factors contributing to the development of numerous civilization diseases such as cardiovascular events or osteoporosis. At the same time, table salt is a major carrier of iodine in human diet (SZYBIŃSKI et al. 2010). Reduced salt uptake can therefore pose a threat to the effective iodine prophylaxis in Poland as well an in other countries, resulting in increased incidence of iodine deficiency disorders (IDD). Consequently, search for alternative methods of iodine supplementation has been carried out in the last few years, indicating iodine biofortification of crop plants as the most promising direction (NESTEL et al. 2006, WHITE, BROADLEY 2009).

Current studies on iodine biofortification focus mainly on the effectiveness of increasing its content in edible parts of plants as well as on the influence of a dose, form and way of iodine application on biomass and yield (MAÆKOWIAK, GROSSL 1999, ZHU et al. 2003, DAI et al. 2004). It has been demonstrated that plants preferably uptake iodine by roots in the form of  $I^-$ (rather than  $IO_3^-$ ) although excessive doses of this iodine form can negatively affect biomass (MAĆKOWIAK, GROSSL 1999, ZHU et al. 2003). What can influence the effectiveness of iodine biofortification is the method of its application. In the research conducted by ALTINOK et al. (2003), increased accumulation of iodine in alfalfa plants was observed as a result of foliar nutrition with potassium iodide in comparison to soil introduction of this compound. In just a few studies, evaluation of the interaction between increased content of iodine and biological value of crop plants has been undertaken (BLASCO et al. 2008, WENG et al. 2008). No information, however, is available on the influence of iodine foliar or soil application on the uptake and accumulation of mineral elements in plants.

The aim of the study has been to determine the effect of iodine nutrition in reference to its form  $(KI \text{ and } KIO_3)$ , dose and method of introduction (foliar application or soil fertilization) on the effectiveness of iodine biofortification as well as chemical composition of lettuce heads.

### **MATERIAL AND METHODS**

In 2008-2009, field cultivation of lettuce cv. Melodion  $F_1$  was conducted in a crop rotation system on single soil complex at the Experimental Field Station of Agricultural University in Kraków (Poland). Lettuce was cultivated on silt loam soil (35% sand, 28% silt and 37% clay) with the content of organic matter in the 0-30 cm soil layer: 3.41% and 3.76% (in 2008 and 2009, respectively), and the following content of the available forms of nutrients soluble in 0.03 M acetic acid (in the two consecutive years): N  $(NO_3-N+NH_4-N)$  – 3.9-1.3, P – 27.8-46.1, K – 142.6-124.1, Mg – 118.3-113.3 and Ca – 1469.0- 1062.5 mg dm<sup>-3</sup> of soil. In 2008 and in 2009, the soil's pH<sub>(H<sub>2O)</sub> was 6.99-</sub> 6.73, while the salinity of soil (EC) was 0.13-0.12 mS cm<sup>-1</sup>, respectively. Lettuce seeds were sown on 11 and 15 March (in 2008 and 2009, respectively). Seedlings were planted into soil in rows 30 cm apart with 30 cm plant spacing on 11 and 15 April of the following year. Nitrogen as ammonium nitrate in a dose of 100 mg N  $dm<sup>-3</sup>$  soil as well as iodine soil fertilization in the form of KI were applied directly prior to lettuce planting.

Different iodine fertilization (in the form of KI) and foliar nutrition (as  $KIO<sub>3</sub>$ ) were applied in the experiment including: 1 – control (without soil fertilization and foliar nutrition with iodine); three combinations with presowing soil fertilization with iodine :  $2 - 0.5$  kg I ha<sup>-1</sup>,  $3 - 1.0$  kg I ha<sup>-1</sup> and  $4 - 2.0$  kg I ha<sup>-1</sup> as well as three combinations with four treatments of foliar application of iodine in the following concentrations:  $5 - 0.0005\%$  (total 0.02 kg I ha<sup>-1</sup>),  $6 - 0.005\%$  (0.2 kg I ha<sup>-1</sup>) and  $7 - 0.05\%$  (2.0 kg I ha<sup>-1</sup>). Foliar nutrition was performed using  $1000 \text{ dm}^3$  of working solution per hectare on the following dates:  $1^{st}$  – 28 and 29 April,  $2^{nd}$  – 05 and 13 May, 3<sup>rd</sup> – 12 and 20 May,  $4^{\text{th}}$  – 23 and 27 May (in 2008 and 2009, respectively)

The experiment was arranged in a split-plot design with four replications. Each experimental treatment was randomized in four repetitions on  $5 \text{ m} \times 1.5 \text{ m}$  (7.5 m<sup>2</sup>) plots. The total area used for experiment was 270 m<sup>2</sup>.

Lettuce heads were harvested on 2 and 1 June (in 2008 and 2009, respectively). Soil samples from the 0-30 cm layer were collected during lettuce harvesting using a soil drill.

In lettuce samples, the iodine content was assessed after incubation with 25% TMAH according to the standard method (prEN 15111- R2-P5-F01). The total nitrogen was determined by Kjeldahl method (PERSSON, WENNERHOLM 1999). The content of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after mineralization of plant samples in 65% super pure  $HNO<sub>3</sub>$  (Merck no. 100443.2500) using a CEM MARS-5 Xpress microwave oven (PAS£AWSKI, MIGASZEWSKI 2006).

In soil samples, pH was determined by a potentiometer and the content of I, N-NH<sub>4</sub>, N-NO<sub>3</sub>, P, K, Mg, Ca, S and Na was determined after extraction of the soil with 0.03 M acetic acid (NOWOSIELSKI 1988). The content of B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after extraction with 1 M HCl (GORLACH et al. 1999).

The content of I as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in soil and plant samples was determined with the ICP-OES technique with a Prodigy Teledyne Leeman Labs USA spectrometer. The content of nitrogen forms in soil samples  $(N-NH_A, N-NO_3)$  was determined by the FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)].

The results were statistically verified using an ANOVA module of Statistica 9.0 PL programme at the significance level *P*<0.05. Significance of changes was assessed with the use of variance analysis. In the case of significant changes, homogenous groups were determined on the basis of Duncan's test.

# **RESULTS AND DISCUSSION**

RThe rsearch conducted by DAI et al. (2006) revealed that root application of oxidized form of iodine  $({\rm IO}_3^-)$  contributed to significantly higher (even by ten-fold) accumulation of this element in spinach leaves. However, some others studies with iodine applied to soil or solution culture indicate that iodine application in  $I^-$  form (when compared to  $IO_3^-$ ) gives better results with regard to the uptake and bioaccumulation of this element in plants. What is more, increasing iodine doses improve the effectiveness of biofortification (MURAMATSU et al. 1983, ZHU et al. 2003, BLASCO et al. 2008, WENG et al. 2008). In the present work, relatively small differentiation of the iodine content in lettuce plants from particular combinations was found (Table 1). Statistically higher accumulation of iodine (in comparison to the control) was noted only for the foliar application of  $KIO<sub>3</sub>$  in the dose of 2 kg I ha<sup>-1</sup>. It is worth noticing that significant build-up of the iodine content was observed owing to the application of a higher  $KIO_3$  concentration used for foliar nutrition. Soil fertilization with KI did not result in iodine biofortification of lettuce when compared to the control plants not fertilized with this element (Table 1). Considering the total values of iodine, it could be concluded that application of higher doses of KI (1.0 and 2.0 kg I ha<sup>-1</sup>) raised to a certain degree the iodine accumulation in lettuce plants. It should also be noted that the iodine content in soil after lettuce cultivation from individual combinations of the study (Table 2) did not correlate with the detected content of this element in lettuce heads.

 Varied influence of soil fertilization and foliar nutrition with iodine was found in reference to the content of N, P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in lettuce (Table 1). Slight changes were noted in the N-total content in lettuce heads resulting from the tested iodine treatments. Statistically significant increase in the N-total content, when compared to the control, was observed only in the case of soil fertilization with the high ${\rm Table}~1$ 





\*\*\*in combinations 5-7 the total dose of iodine applied in four foliar nutrition treatments was given;<br>\*\*\*results for Mo only from 2009

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Chemical properties of soil after lettuce cultivation  $-$  means for the 0-30 cm soil laver from 2008-2009



Test  $F\cdot$  "means are significantly different, n.s. – not significant;  $^{***}$  For Mo results only from 2008

est dose of KI  $(2.0 \text{ kg I ha}^{-1})$ , whereas foliar nutrition with the same dose but in the form of  $\rm{KIO}_3$  depressed this element. It should be mentioned that despite observed changes in the N-total content, no differences in lettuce yield obtained from individual combinations were observed – detailed data not presented.

As a result of iodine nutrition, irrespective of its dose, form and method of application, an increase in the content of K, Mg, Ca as well as Mn and Cd in lettuce plants was found in comparison to the control – with the exception of K in combination no 6 (Table 1). The highest content of K was recorded for soil fertilization with KI in a dose of 2 kg I ha<sup>-1</sup> and for foliar nutrition with  $KIO_3$  in the lowest dose (0.02 kg I ha<sup>-1</sup>). Soil application of higher doses of KI  $(1.0 \text{ and } 2.0 \text{ kg I ha}^{-1})$  contributed to a gradual decrease in the concentraions of Ca, Mg, Mn and Cd in lettuce in comparison to the lowest KI dose  $(0.5 \text{ kg I ha}^{-1})$  although they remained higher than their values detected for the control. It should be noted that introduction of the lowest amount of KI  $(0.5 \text{ kg I ha}^{-1})$  to soil intensified most profoundly (in the comparison to the other combinations) plant accumulation of Ca, Mg, Mn, as well as boron. This dependence can indirectly support the hypothesis of the positive influence of low iodine doses on plants (KABATA-PENDIAS, MUKHERJEE 2007). Increasing KI doses applied to soil reduced the content of Mn in lettuce heads (Table 1) along with increasing the soil content of this element to a level comparable with the control (Table 2). This observation suggests an antagonistic effect of higher doses of iodine applied as KI on the uptake of manganese by lettuce roots.

Nutrition with iodine generally depressed the accumulation of P, Cu and Zn in lettuce (except Zn in combination 6, cf. Table 1). Higher doses of iodine applied foliarly as  $KIO_3 (0.2 \text{ and } 2.0 \text{ kg I ha}^{-1})$  raised the content of P in comparison to combination 5. In respect to soil fertilization with KI (irrespective of its dose), an increased amount of easily soluble forms of phosphorus was noted in soil (Table 2) along with a reduced content of this element in lettuce leaves. The effect could have been caused by the inhibition of P uptake by lettuce roots, which may have resulted from the introduction of the I– form of iodine to soil. A similar observation was reported for Cu, but then an increasing KI dose was accompanied by a significant decrease in the uptake and accumulation of copper in lettuce plants.

A variable effect of the iodine form, dose and method of application was found in respect of S, Na, B, Fe, Mo, Al and Pb content in lettuce heads (Table 1). In comparison to the control, using higher concentrations of  $KIO<sub>3</sub>$ solution for foliar nutrition (combinations 6 and 7) raised the accumulation of sodium in lettuce leaves. Soil fertilization with iodine as KI (irrespective of its dose) reduced Fe but slightly increased the Mo and Pb content in lettuce in comparison to the control. No influence of soil application of iodine was observed on the Al content in plants. The highest accumulation of S was noted in plants fertilized with the biggest dose of KI  $(2.0 \text{ kg I ha}^{-1})$ .

Foliar nutrition with  $KIO<sub>3</sub>$  slightly decreased the S content in lettuce but the iodine dose had no significant influence on the observed changes. Application of different iodine forms and doses contributed to small but statistically significant variation in the boron concentration in lettuce leaves.

It is particularly difficult to discuss our results on the influence of iodine introduction (through soil or foliarly) on the chemical composition of lettuce due to a very limited number of available publications referring to this issue. The results obtained by SMOLEÑ et al. (2010) in carrot cultivation carried out according to an analogous experimental design (iodine dose, form and method of application) revealed different relations than shown in the present study, which could have been caused by species-specific differences.

## **CONCLUSIONS**

1. A statistically significant effect of iodine biofortification of lettuce was obtained only through foliar application of  $KIO<sub>3</sub>$  in a dose of 2.0 kg I ha<sup>-1</sup>.

2. Iodine accumulation in lettuce heads tended to increase as a result of application of higher doses of KI through soil fertilization (1.0 kg and 2.0 kg I ha<sup> $-1$ </sup>).

3. An increased N-total content in lettuce heads (in comparison to the control) was noted in the case of soil fertilization with the highest dose of KI (2.0 kg I ha<sup>-1</sup>), while a reduced content of this macronutrient occurred as a result of foliar application of the same dose but in the form of  $KIO<sub>3</sub>$ .

4. Significantly higher content of K, Mg, Ca, Mn and Cd in lettuce was found after iodine introduction irrespective of its dose, form and method of application.

5. Nutrition with iodine (regardless of its dose, form and method of application) generally decreased the P, Cu and Zn accumulation in lettuce heads.

6. Varied influence of iodine application was observed in reference to the S, Na, B, Fe, Mo, Al and Pb content in lettuce heads.

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