Agronomic biofortification with iodine in lettuce plants cultivated in floating hydroponic system[1]

Mairon Neves de Figueiredo[2]*, *Hermínia Emilia Prieto Martinez*[3], *Edimar Aparecida Filomeno Fontes*[4],
Carla do Carmo Milagres[3], *José Maria da Silva*[3]

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ABSTRACT

Iodine is essential to human metabolism, being fundamental in the production of the thyroid hormones. The consumption of biofortified foods can contribute to the increase of its intake. The objective of this study was to increase the iodine concentration in lettuce, also evaluating its effects on plant growth and production. The experiment was conducted in a greenhouse, in a hydroponic system of aerated static solution. A completely randomized design was used, in a 2 x 5 factorial scheme (variety x iodine dose), with three repetitions. Two lettuce varieties were used, the iceberg lettuce and the crispy leaf lettuce, submitted to five doses of iodine (0, 10, 20, 30 and 40 µmol L^{-1}), having as source potassium iodide. At 40 days after transplantation, both cultivars were collected and evaluated for the weight of fresh and dry matter, iodine content and levels in the leaves and the root volume. With increased doses, the plants showed symptoms of phytotoxicity, resulting in lower productivity. However, all doses promoted elevations in the total iodine levels of plants. Therefore, it is possible to increase the iodine content in lettuce leaves, being necessary to adjust the doses to be used.

Keywords: iceberg lettuce; bioaccumulation; crispy leaf lettuce; iodide; *Lactuca sativa* L.

INTRODUCTION

Iodine (I) is an essential element to humans and is directly involved in the synthesis of thyroid hormones (Zimmermann *et al*., 2008). Its main sources are related to seafood (fish, shellfish, algae, etc.), while foods from plants grown in most of the continent's soils have low levels (Haldimann *et al.*, 2005). This is a reflection of the geochemical cycle of I, which due to its high mobility can easily be lost from soils, either by leaching or evaporation (Fuge & Johnson, 2015).

The World Health Organization (WHO) in 2005 estimated that 2 billion people, about 35.2% of the world's population, had symptoms of iodine deficiency, which is still considered a serious public health problem. In 2019, estimates indicated that 25 countries, including Russia, Ukraine and Italy, still have iodine consumption below the recommended (Iodine Global Network, 2019). The main consequences of iodine deficiency in daily human needs relate to insufficient secretion of thyroid hormones. Weakened thyroid activity can result in iodine deficiency disorders, causing damage mainly in the early stages of life. Spontaneous abortions, infant mortality, cognitive deficit and neuropsychological problems are some examples. In more severe cases, the deficiency also presents the classic symptom, goiter, a significant increase in the thyroid gland, capable of reaching all age groups, and can cause lifelong sequelae (Zimmermann *et al*., 2008). The daily recommendation intake of iodine in the diet varies between 90-250 µg, according to the age group, which is more demanded in pregnant and lactating women (Andersson *et al*., 2012).

The supplementation of iodine in the diet is widely practiced, being the most common and successful way via enrichment of the kitchen salt with iodized forms *(*Andersson *et al*., 2012). However, the use of iodized salt in food still presents serious problems in the implementa-

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[2] Universidade Federal de Viçosa, Departamento Solos, Viçosa, Minas Gerais, Brasil. mairon.figueiredo@ufv.br
[3] Universidade Federal de Viçosa, Departamento de Agronomia, Viçosa, Minas Gerais, Brasil. hermínia@ufv.br; carlacmilagres@gmail.com; jose.m.maria@ufv.br
[4] Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, Viçosa, Minas Gerais, Brasil. eaffontes@ufv.br
*Corresponding author: mairon.figueiredo@ufv.br

tion of public health policy. Volatilization during food processing, transportation and storage dramatically reduces iodine utilization (Winger *et al*., 2008). In addition, faced with the risks related to high salt consumption, public campaigns aimed at reducing its intake, by turning it into the big villain, generating more and more obstacles to this prophylaxis (WHO, 2006).

In Brazil, even if the current legislation establishes criteria for the use of iodized salt, with doses between 15 to 45 mg of iodine per kilogram of salt (Brasil, 2013), iodine deficiency is still a reality to be faced. Several studies at the local level, such as in the cities of Ouro Preto (Nimer *et al*., 2002), Novo Cruzeiro (Macedo *et al*., 2014) in Minas Gerais and Ribeirão Preto (Alves *et al*., 2010), as well as others in the state of São Paulo (Duarte *et al*., 2004); and even studies in the American continent, carried out by the WHO (Pretell *et al*., 2004), reveal iodine deficiency as a chronic process in the population.

Enabling the supply of iodine in a more diverse way, combined with vegetables and fruits can be a good alternative to increase daily intake. Thus, plant enrichment with iodine through biofortification already becomes a reality and may represent an effective alternative to control the deficiency of this element (White & Broadley, 2009).

The biofortification of food consists in raising the levels of a certain nutrient in the agricultural crop in question. This way the population has access to an agricultural product of greater nutritional value. Usually, biofortification can be performed in two ways, genetics and agronomic. Genetic biofortification is the selection of plants capable of extracting and accumulating higher nutrient contents. However, its process has limited scope, considering that bioaccumulation is directly related to the presence and availability of the nutrient in the culture medium. Agronomic biofortification becomes more desirable, since it consists in supplying the nutrient by means of fertilizers. Thus, fertilizers increase the concentration of the element in the medium, increasing its availability, which stimulates its absorption and accumulation (Lyons *et al*., 2004).

Iodine is not classified as an essential element to vegetables. Its presence may present toxic effects depending on its sources and concentrations (Umaly & Poel, 1970). Its use in biofortification seeks to increase its concentrations in plants reducing to a minimum its damage to the crop. In this sense, several studies have been produced aiming to complement the supply of iodine in food. For most olericultural cultures, there is still a lack of specific studies in the field, even though the importance and viability of this technique being known (Gonzali *et al*., 2017).

Lettuce is a vegetable of great consumption and acceptance throughout the world (Blasco *et al*., 2008). Lettuce is a vegetable whose leaves are consumed preferably in the form of fresh salad, which reduces the risk of iodine loss, as it occurs in cooking processes. In addition, in the cultivation of lettuce on a commercial scale, the use of hydroponic systems is already a reality. These cultivation systems provide the necessary conditions for plants to develop without the use of soil, becoming nutritionally dependent on the solution employed.

Aiming at the biofortification of food, the closed hydroponic systems, allow greater control and management of the culture, facilitating the study and analysis of the absorption of the element. In this case, iodine has its effects more easily controlled and its interactions can be better evaluated (Voogt & Sonneveld, 1997).

Thus, the objective was to increase the iodine content in lettuce through agronomic biofortification and evaluate the effects of this technique on hydroponic lettuce production.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the plant mineral nutrition laboratory of the Departamento de Agronomia da Universidade Federal de Viçosa-MG (Department of Agronomy at the Federal University of Viçosa). The experiment was installed in a completely randomized design (CRD) in a 2 x 5 factorial scheme, two varieties of lettuce, Iceberg (A) and Crispy leaf (B), and five doses of iodide (0,10, 20, 30 and 40 μmol L^{-1}), with 3 repetitions.

The varieties of iceberg lettuce (Yasmin) and crispy leaf lettuce (Filó), both of the company Feltrin Sementes, were cultivated. The seeding was done in phenolic foam, which received irrigation with water until the appearance of cotyledonous leaves. After this phase, the seedlings began to receive irrigation every three days, with nutrient solution at ¼ of the strength, until they presented two definitive leaves. Then, the uniform seedlings were transplanted into a floating hydroponic system (aerated static solution), containing ½ strength nutrient solution, with composition based on the work of Blom-Zandstra & Lampe (1983). After 15 days of transplantation, the nutrient solution was adjusted, reaching 100% of strength. Thus, the adjusted nutrient solution contained macronutrients, all in mmol L^{-1}, nitrogen (13.6), phosphorus (1.0), potassium (5.94), calcium (4.48), magnesium (2.5), sulfur (2.5). For the micronutrients used in μmol L^{-1}, iron (45), boron (46), manganese (32), zinc (1.5), copper (0.9) and molybdenum (0.2).

The plants were grown in rectangular polyethylene boxes (0.5 x 0.18 x 0.18 m) containing 16 liters of nutrient solution. The roots were completely submerged in the nutrient solution, and a compressed air pumping system was used to provide the necessary oxygenation. The plants were anchored in polystyrene plates (styrofoam), coated with foil, with holes, to support them in the vertical position. In each cultivation box were placed two plants, one of each variety, with spacing of 25 cm between plants.

The pH of the solution was monitored daily and maintained between 5.5 and 6.0 and adjusted with the addition of HCl or NaOH both to 1 mol L^{-1}. Periodic evaluations of the electrical conductivity (EC) of the nutrient solution were performed, and this was readjusted to 1.5 dS m^{-1} whenever there was a 30% reduction in its initial value. After 20 days of conducting the experiment, the solution was renewed.

Soon after transplanting the seedlings, doses of 0, 10, 20, 30 and 40 μmol L^{-1}, of iodide were provided, initially at ½ strength and after 15 days at one strength, in the same way as the other nutrients. The source of iodine used was potassium iodide (76.45% iodide).

At 40 days after transplantation, the plants were collected, sectioned in the region of the collection and its parts properly weighed to obtain the mass of fresh matter of part area and root. The root volume was determined by the displacement of the liquid in a graduated tube and measured in cm^3.

In order to minimize iodine losses, a sub-sample of the leaves was subjected to immediate freezing in ultrafreezer at -80°C. Then this fraction of the sample was subjected to lyophilization, its dry mass value being counted throughout the process.

The remaining samples were subjected to drying in a greenhouse at 65 °C until reaching a constant weight, to determine the mass of dry matter. The percentage of dry matter was calculated based on the values obtained before and after drying.

The iodine content in 100 grams of leaf fresh matter (Imf) was calculated, following the formula below:

$$Imf = \frac{100 * (Ms * I)}{Mfp}$$

Imf: iodine content in 100 g.

Ms: leaf dry matter (g).

I: iodine content (μg g^{-1}).

Mfp: leaf fresh matter (g).

The iodine per portion of lettuce ingested “in natura” was obtained by dividing the iodine present in 100 grams of leaf fresh matter (Imf) divided by 10 grams corresponding to a portion (IBGE, 2011). Thus, it was possible to obtain the amount of iodine to be ingested in the diet, if the intake of the treated plants was made.

The iodine determination methodology adopted was proposed by Sveikina (1975), adapted by Moxon & Dixon (1980), verified and widely validated for iodine analysis in food and plant samples, with adaptations of Perring (2001).

The data were submitted to the Shapiro-Wilk normality test after the regression analysis of variance (Anava) was performed. Linear and nonlinear models were adjusted to unfold doses within varieties according to the Anava assumptions. The models were chosen based on biological logic, significance of regression coefficients, using the T test at 1 and 5% probability. The F test at 5% probability was also performed in the appropriate situations to test the equality between the means.

The data of mass production of fresh and dry foliar matter and root volume as variables dependent on iodine concentration in nutrient solution were adjusted to nonlinear regression models. On the other hand, the percentage of leaf dry matter, iodine content in plants and iodine content in fresh matter were adjusted to linear regressions. The R software was used to perform the analyses.

RESULTS AND DISCUSSION

The addition of iodide in nutrient solution significantly influenced ($p \leq 0.01$) the production performance of the lettuce, as well as the concentration, and total iodine content in leaves (Table 1).

Biomass Production

As the doses of iodide added to the nutrient solution were increased, the leaf fresh matter mass decreased in both lettuce varieties (Figure 1). Iodine is not classified as an essential element in plants. Its presence in nutrient solution may represent toxicity, according to its concentration and available form (Umaly & Poel, 1970). When added in aqueous medium, potassium iodide (KI) is rapidly dissociated forming K^+ and $I^{''}$. These iodide ($I^{''}$) anions can react quickly due to their reducing role, giving rise to aqueous I_2. In hydroponic systems, studies demonstrate that the roots absorb $I^{''}$ at a higher rate than iodate (IO_3^-). The process of iodine absorption is not yet well defined. Its behavior is quite variable according to the type of production system and means of supply (Kato et al., 2013).

The toxic character to plants of I^- may be related to its role as a reducing agent, its availability and easy absorption by plants. Thus, I^- has negative effects on crop production. Being the form of cultivation and conditions in which plants were subjected fundamental in the expression of the toxic character of iodine (Blasco *et al.*, 2008).

The application of iodide (I^-) in nutrient solution limited the growth and development of plants (Figure 1). Both varieties showed a large reduction in the mass production of fresh matter from the dose of 10 μmol L^{-1}. Higher doses of iodide significantly limited plant development. In both varieties, the plants submitted to treatments of 20, 30 and

40 µmol L^{-1}, showed stagnation in growth, producing less than 5 grams of leaf fresh matter mass. While plants with 0 µmol L^{-1} I^- dose provided fresh matter production within the expected for each variety.

Mass production of leaf dry matter mass was also impaired in both varieties with increased doses of iodide ($p \leq 0.01$). In this case, the doses used were able to reduce by up to ten times the mass production of dry matter. Thus, there is a gradual decrease in dry matter production with increasing doses (Figure 2).

During cultivation, it was observed that the plants submitted to iodine treatments were less turgid. The dry matter percentage results corroborate with the observed, since both varieties, although there is no interaction between them (Table 1), had similar changes with the addition of iodide. Through this result, it was found that with the increase in iodide doses, there was increase in the percentage of dry matter in the leaves (Figure 3). Thus, iodine, when absorbed and transported through the potted plants, was able to stimulate water loss. After absorption by the plant, the iodine is translocated to the tissues of the aerial part until it is volatilized. Iodine enters the stomata being volatilized as I_2 or can take gaseous form as methyl iodide (CH_3I). During the process, stomatal activity increases, raising leaf sweating (Caffagni *et al.*, 2012). The rapid xylematic translocation and subsequent evaporation as methyl iodide may have stimulated the stomatal opening in a prolonged manner, the data indicate that the treatments with iodide caused a reduction in plant moisture. In this case, even though variety A has a higher percentage of dry matter, when subjected to treatments, varieties A and B presented similar results in this matter (Table 1). Thus, it can be observed that within the doses of iodide worked, the dose elevation, provides a higher percentage of dry matter in both varieties (Table 2).

According to the increase in iodide doses in the nutrient solution, the root volume decreased in both varieties of lettuce (Figure 3). The plants submitted to treatments containing I had compromised root performance. The presence of iodide acted as an inhibitor of root development in both varieties. From 10 µmol L^{-1} the roots showed progressive reduction in their total volume. Thus, the root volume in the iceberg variety increased from 21.33 cm^3 at zero dose to 0.83 cm^3 in the treatment

Table 1: Analysis of variance (Anava) of biometric growth data of two varieties of lettuce subjected to doses of iodine in nutrient solution content and iodine in leaves dry matter and iodine content in 100 g of fresh matter.

Factor	GL	QM					
		Vr	**Mff**	**Ms**	**% Ms**	**I**	**Imf**
Iodine	4	690.95**	46674.5**	101.71**	18.97**	269767.75**	23364168**
Res (Iodine)	10	0.48	13	0.14	0.69	1477.30	353554.4
Portion	14						
Variety	1	93.63**	59^{ns}	0.08 ^{ns}	6.54**	3185^{ns}	116748^{ns}
Var X Iodine	4	33.45**	367**	0.96**	0.85 ^{ns}	6700.75**	654303.75*
Res (Var)	10	2.62	75.8	0.07	0.39	1029.2	52589.4
CV (%)							
A		8.40	7.84	14.38	10.72	13.10	23.33
B		19.76	18.95	10.85	8.10	10.94	9.00
Total	29						

Iodide dose in ìmol/l; Root volume (Vr) expressed in cm^3; Leaf fresh matter mass (Mff) expressed in grams; Leaf dry matter mass (Ms) expressed in grams; Percentage of dry matter (%Ms); Iodine (I) content in ìg g^{-1} in dry matter; Iodine content (µg) in one hundred grams of fresh matter (Imf). Significance values were represented as $p > 0.05$, non-significant (^{ns}); $p \leq 0.05$ (*); $p \leq 0.01$ (**).

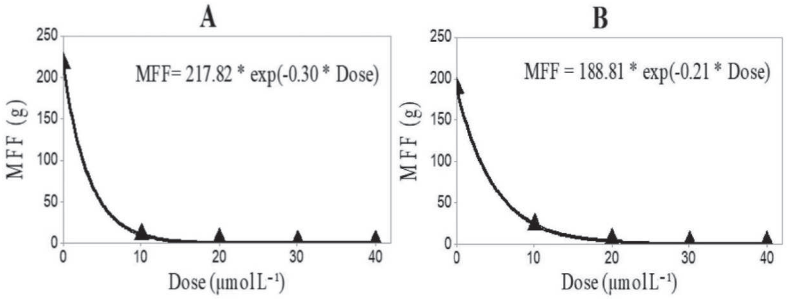

Figure 01: Variation of leaf fresh matter mass (MFF) in grams of two lettuce varieties: iceberg (A) and crispy leaf (B), submitted to doses of iodide in nutritive solution. *Significant values for $p \leq 0.01\%$.

with 40 μmol L^{-1} of I . In the same way, in the crispy leaf variety, the plants went from 31.66 cm^3 to 0.8 cm^3 of root volume. The toxic effects of iodine on roots had already been reported in studies carried out in nutrient solution or even in soil (Mackowiak et al., 2005).

Iodine in plants

In treatments in which plants were grown without the addition of iodide, iodine levels were very low. The plants of the iceberg variety in control treatment presented 2.99 μg g^{-1} of iodine, while for the crispy variety, the same treatment presented 3.57 μg g^{-1} (Table 3). With the increase in doses, both varieties of lettuce showed an increase in iodine content. The application of 10 μmol L^{-1} of I$^-$ was able to raise the iodine content substantially. For this treatment, iceberg (A) and crispy leaves (B) levels of iodine in dry matter increased more than 50 times.

In the iceberg variety for doses of 20 and 30 μmol L^{-1} of I$^-$ were found 353.40 and 345.76 μg g^{-1} of iodine. Treatment with a dose of 40 μmol L^{-1} presented 511.99 μg g^{-1} of iodine for this variety. The iodine content in dry matter increased with higher doses (Figure 4). The doses of 20, 30 and 40 μmol L^{-1} of I in the crispy leaf variety presented respectively 311.53 μg g^{-1} of iodine, 373.35 and 642.76 μg g^{-1}. Thus, the accumulation of iodine for both varieties was gradual with the elevation of I$^-$ in the nutrient solution ($p < 0.01$).

The difference between iodine bioaccumulation in the varieties used in the present study had not yet been reported. Following the proposed model (Table 3), even if they presented correlated behavior, the varieties have significant differences regarding the iodine content at the dose 40 μmol L^{-1}. In this dose, the crispy leaf lettuce showed a significant increase in iodine content, compared to the other variety. It is likely that in the treatments with 30 and 40 μmol L^{-1}, the iceberg variety (A) was already saturated with iodine, this presenting extremely toxic concentrations that inhibited its development. To a large extent, in the productive questions (Figures 1, 2 and 3), this variety has already shown itself to be more sensitive to the toxic character of iodine even at lower concentrations.

When converting the iodine content in the dry matter mass to the iodine content in 100 grams of leaf fresh matter

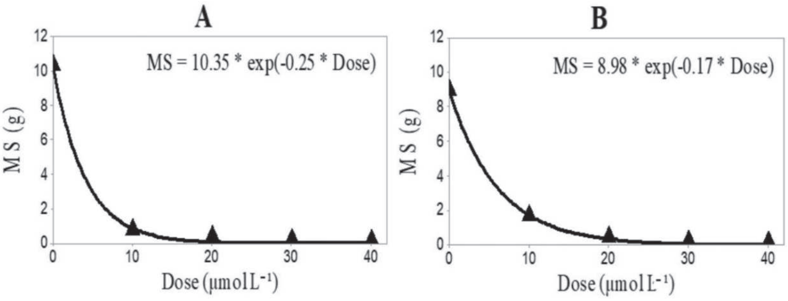

Figure 02: Variation of the leaf dry matter mass (MS) in grams, of two varieties of lettuce: iceberg (A) and crispy leaf (B), subjected to doses of iodide in nutrient solution. *Significant values for p d $\leq$ 0.01%.

Table 2: Observed means of dry matter percentage in the iceberg (A) and crispy leaf (B) varieties compared by F test at 5% probability and observed means in different doses for varieties adjusted by regression analysis (*significant by F test).

Varieties	Dry matter	Regression equation	R^2
A	8.21 a	$y = 4.98 + 0.25{*}Dose - 0.004{*} Dose^2$	0.97
B	7.28 b		

Means followed by equal letters in the column do not differ from each other by the F test ($p \geq 0.05$).

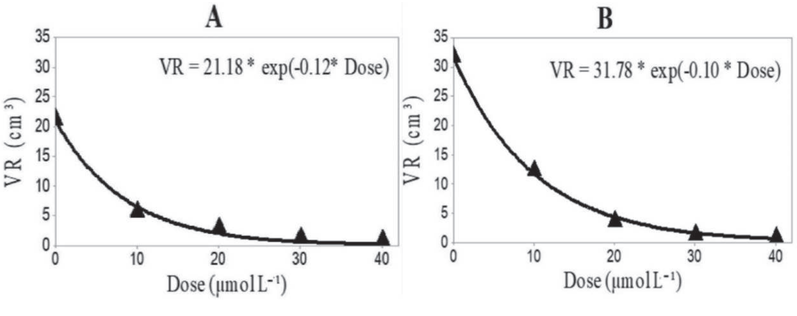

Figure 03: Variation of root volume (VR) in cm^3 of two lettuce varieties: iceberg (A) and crispy leaf (B), submitted to doses of iodide in nutrient solution. * Significant values for $p \leq 0.01$%.

mass (Imf), significant iodine accumulation values were obtained ($p \leq 0.01$). With the increase in iodide doses, the increase in the percentage of dry matter resulting favored the greater accumulation of iodine in its fresh matter.

The iceberg variety presented in its treatment with zero dose 14.24 µg of iodine in 100 g of fresh matter. The same treatment in the crispy leaf variety presented 17.04 µg, demonstrating the presence of iodine in small amounts even in the lettuce varieties studied where there was no addition via I treatment.

In treatments with doses of 10 µmol L^{-1} of I^-, the iodine content found in plants showed a marked increase. The iceberg lettuce variety presented for this treatment 1605.59 µg of iodine per 100 grams of fresh matter. For the crispy leaf variety, it was possible to observe the content of 1309.89 µg of iodine for the same treatment.

In the treatment of 20 µmol L^{-1} iodide, the result revealed greater accumulation of the element in the iceberg variety, while for the treatment with 40 µmol L^{-1}, the crispy leaf variety showed greater accumulation of iodine. However, with the increase in iodide doses in treatments, there was respectively an increase in iodine content in leaf fresh matter (Figure 4).

The treatments with the addition of iodide resulted in the increase of the iodine content in the crispy leaf and iceberg varieties. Thus, even if there are small differences in the accumulation of the element, in both varieties the plants were able to absorb and accumulate the iodine present in the nutrient solution.

Biofortified foods

Lettuce is part of human food as raw food, being little processed and not requiring cooking processes. Its daily consumption varies from half a portion to a portion, about 10 g, approximately an average leaf (IBGE, 2011). In this case, the values obtained with the estimated iodine content in leaf fresh matter (Figure 5) were adjusted in order to estimate the value ingested in a portion of 10 g.

While the lettuce not treated with iodide provided only 1.42 µg for the iceberg variety and 1.70 µg for the crispy leaf variety, the results were much higher in the other doses tested. As presented in Table 3, the behavior of the lettuce varieties studied differs in this characteristic. For treatment with 10 µmol L^{-1} of iodide, the iceberg variety presented 160.55 µg of iodine per serving, while the crispy variety presented 130.98 µg of iodine per serving. For the treatments with 40 µmol L^{-1}, the iceberg variety resulted in 489.97 µg of iodine, while the crispy presented 569.88 µg of iodine per same portion.

For adults, the minimum daily intake recommended of iodine is around 150 µg. For pregnant and lactating women, the minimum daily dose recommended is 250 µg (Andersson et al., 2012). Its excess (values above 1 mg per day) can be easily eliminated, with a good

Table 3: Mean values for the iodine content (µg g^{-1}) present in the leaf dry matter and mean values of the iodine content (µg) in 100 grams of leaf fresh matter (Imf) in the varieties of the iceberg (A) and crispy leaf (B) lettuce subjected to iodide doses in nutrient solution.

Varieties	**Dose (µmol L^{-1})**				
	0	**10**	**20**	**30**	**40**
			Iodine content (µg g^{-1})		
A	2.99 a	200.44 a	353.41 a	345.77 a	512.00 b
B	3.57 a	181.71 a	311.56 a	373.36 a	642.77 a
			Imf		
A	14.24 A	1605.59 A	3308.54 A	3217.06 A	4899.73 B
B	17.07 A	1309.90 A	2268.25 B	3127.23 A	5698.90 A

Means followed by equal letters in the column do not differ from each other by the F test ($p \geq 0.05$).

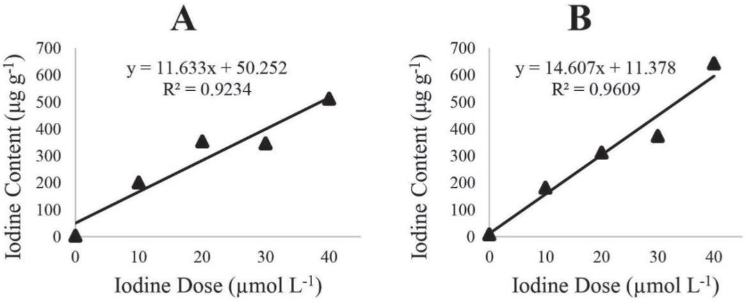

Figure 04: Variation in iodine content in leaf dry matter for lettuce varieties: iceberg (A) and crispy leaf (B), subjected to doses of iodide in nutrient solution. *Significant values for $p \leq 0.01\%$.

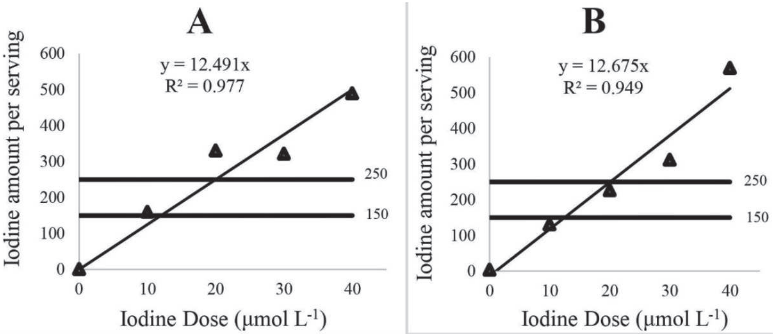

Figure 05: Variation amount of iodine in a 10 gram serving of lettuce for iceberg (A) and crispy leaf (B) varieties as a function of the dose of iodide applied in nutrient solution. *Significant values for $p \leq 0.01\%$.

tolerance due to its easy excretion. Therefore, in food, it is well tolerated and can hardly come to harm (Lopes et al., 2012).

The two varieties lettuce treated with doses of 10 µmol L^{-1} of iodide presented high amounts of this element (Figure 5), sufficient values to complement and to meet the demand for the iodine daily intake. However, the decrease in production is indisputable (Figures 1, 2, 3), so lower doses of iodide – less than 10 µmol L^{-1} – should be tested to allow to combine productive performance with the increase of iodine in the diet.

On the other hand, the plants treated with more than 20 µmol L^{-1} of iodide extrapolated the recommendations of daily consumption. The two varieties treated with doses of 40 µmol L^{-1} had levels above 500 µg of iodine per serving, representing more than twice the recommended for daily intake.

In groups of hypertensive patients, the use of biofortified lettuce may represent a source capable of meeting daily iodine demands, considerably reducing the need to consume other sources such as iodized table salt. Similarly, in groups of pregnant and lactating women, the use of biofortified foods with iodine may complement the diet, being an alternative to traditional supplements. Both varieties show themselves potential ways to supplement iodine in the diet. However, it is necessary to carry out more studies aimed at reconciling the increase in iodine levels, without drastic reductions in productivity.

CONCLUSIONS

Doses of iodide between 10 and 40 µmol L^{-1} added to the nutrient solution promoted a large increase in the content and levels of iodine in plants. However, the addition of iodine by means of iodide, at the concentrations studied, showed to be toxic to plants.

The treated plants were able to store sufficient amounts of iodine to supply and even extrapolate the values corresponding to daily human demands. The results validate the agronomic biofortification technique as an efficient means of supplementing the supply of iodine to the population, provided that doses are adjusted to minimize the toxic effects on plants.

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ERRATUM

The paper “Agronomic biofortification with iodine in lettuce plants cultivated in floating hydroponic system”, with DOI: 10.1590/0034-737X202269020012, published by Revista Ceres, 69(2): 210-217, starting from page 210:

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