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RESEARCH PAPER

Effect of iodine concentration on growth, iodine uptake and antioxidant activity of lettuce (*Lactuca sativa* L.) biofortified with iodine in nutrient film technique hydroponic system

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Abstract

According to the National Iodine Deficiency Disorder (IDD), 48.2% of primary school children aged 8 to 10 years have deficiencies in iodine. In fact, a shortage of iodine is one of the causes of goitre problems in 2.1% of youngsters. Furthermore, pregnant women and school pupils have lower than ideal iodine levels. The iodine biofortification of vegetables provides an excellent opportunity to boost human iodine intake. The study conducted at the Plant Factory, Horticulture Research Centre, MARDI aimed to investigate the effect of potassium iodide (KI) on the growth, iodine uptake and antioxidant activity of butterhead lettuce grown in a nutrient film technique (NFT) hydroponic system. The study used different doses of KI (0, 1, 5, 7, 13 and 26 mg/L) for the biofortification of lettuce with iodine. The results of the study showed that the application of 5 mg/L of KI significantly increased the biomass, leaf area, phenolic content, and antioxidant capacity of butterhead lettuce. Additionally, application of KI less than 7 mg/L increased iodine content without toxicity effects on plants. This indicates that the biofortification of lettuce with iodine is easily applicable in a hydroponic growing system and can significantly improve the nutritional quality of the crop. One of the major implications of this study is that the iodine biofortification of vegetables, such as lettuce, can provide an excellent opportunity to boost human iodine intake. The study found that 1 g of iodine-biofortified lettuce would provide 60%, 46%, and 30% of the recommended daily allowance of iodine for children, pregnant women, and adolescents, respectively.

1. Introduction

Iodine (I) deficiency is among the most frequent micronutrient deficiencies globally which leads to several types of 'iodine deficiency disorders' (IDDs) due to insufficient thyroid hormone production (Zimmermann *et al*., 2008). In Malaysia, the overall national prevalence of IDD with UI<100 µg L^{-1} among children was 48.2% whereby higher in rural areas than in urban areas (Selamat *et al*., 2010). A variety of strategies and activities have been designed and implemented to address the problem of IDD in Peninsular Malaysia including the Universal Salt Iodisation (USI) Programme, the most popular iodine deficiency prophylactic. Additionally, providing health counseling and nutrition education to help people improve their nutritional condition has contributed to the eradication of this issue.

Biofortification of plants with iodine might be an approach for improving the human diet. Biofortification is the technique of increasing the concentration of important components in plant tissues in order to improve the nutritional content of plant-based foods during plant growth rather than post-harvest processing (Díaz-Gómez *et al*., 2016). Studies have been conducted on the fertilization of plants with iodine in order to increase the content of this nutrients, e.g. in spinach (Zhu *et al*., 2003), tomato (Caffagni *et al*., 2012), cabbage (Weng *et al*., 2008). However, food preparation such as cooking using high-temperature oil will result in the volatilization of iodine. Hence, a leafy vegetable crop, consumed as a fresh salad is the preferable crop in an iodine biofortification strategy.

The optimum dose of iodine varies for different crops. Studies carried out on the iodine uptake by lettuce

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plants grown in water culture at different concentrations ranging from 1.3 to 30.5 mg iodine L^{-1} resulted in a significant reduction of biomass at iodine application rates higher than 5.1 mg iodine L^{-1} (Blasco *et al*., 2008). Meanwhile, some studies showed that increases in iodine concentration with a dose rate of 13 to 129 µg L^{-1} significantly enhanced iodine content in plants without affecting plants biomass (Voogt *et al*., 2010). Hence, the goals of the present study are to investigate the effect of different dose rates of iodine sources in nutrient solutions on lettuce biomass production and plant quality.

2. Materials and methods

2.1 Trial set-up and growing conditions

A trial was conducted in the Plant Factory at Horticulture Research Centre, MARDI. Rex RZ Butterhead was sown on span before transplanting to the NFT hydroponic system. The standard nutrient solution for lettuce in water culture was used (Table 1). All containers were filled with this standard nutrient solution with an electrical conductivity (EC) of 2.0 µS. The pH of water was measured using a pH meter and maintained within a range of 5.5 to 6.5. Then, each individual container was supplemented with the required quantity of potassium iodide (KI).

Table 1. The standard nutrient solution for lettuce in water culture, standardized at an EC value of 1.8 µS, as used in the experiment.

Nutrient	Concentration
Macronutrients (mmol L^{-1})	
N	1.3
P	0.5
K	2.0
Ca	1.0
Mg	0.5
Su	0.6
Micronutrients (mmol L^{-1})	
Fe	50.1
Mn	14.5
Zn	4.5
B	64.7
Cu	0.7
Mo	0.5

2.2 Treatment

The trial consisted of KI with doses T2: 1 mg/L, T3: 5 mg/L, T4: 7 mg/L, T5: 13 mg/L, T6: 26 mg/L. A zero treatment without any iodine application was included as a control (T1). Iodine was added to the containers only once at transplanting.

2.3 Postharvest quality

The evaluation included chemical and phytochemicals (ascorbic acid content, total phenolic content, DPPH radical-scavenging activity, FRAP and FIC) characteristics. Ascorbic acid content was determined by extracting 10 g of the sample with 100 mL of 3% metaphosphoric acid. Then, 10 mL of extraction was titrated immediately with a standard dye solution to the first permanent pink endpoint. Total phenolic content was determined using the Folin-Ciocalteu method which is based on a colourimetric oxidation and reduction reaction with some modification (Sunita and Dhananjay, 2010). Antioxidant activity for lettuce was studied through the evaluation of the free radical scavenging effect on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Yen and Hsieh, 1997). The antioxidant activity was reported as the percentage of radical scavenging as follows: % radical scavenging = (1-A sample/A control) × 100 where A sample is the absorbance of the mixture of the sample extract and DPPH, A control is the absorbance of the mixture of DPPH and acidified methanol. For the Ferric-Reducing Ability of Plasma (FRAP) Assay, the FRAC of the samples was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986). The FRAC of a sample is estimated in terms of Trolox equivalent antioxidant capacity (TEAC) in millimoles per litre Trolox. Each assay was carried out in triplicate. The Ferrous Ion Chelating (FIC) Ability was carried out according to the method of Singh and Rajini (2004) with some modifications. The inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated by using the formula chelating effect (%) = [(1-AS) / AB] × 100 where AB = absorbance of the control sample (the control contains $FeCl_2$ and ferrozine) and AS = absorbance of a tested sample. Each assay was carried out in triplicate.

2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using the SAS software version 9.4. Data were processed by two-way analysis of variance and mean separations were performed through the least significant difference (LSD) test at the 5% level of significance.

3. Results and discussion

3.1 Plant biomass and crop quality

After 14 days of iodine biofortification, toxicity effects were observed on lettuce plants regarding biomass and growth. A significant reduction in head fresh and dry weight was observed in plants treated with more than 7 mg/L of KI. The same pattern was observed for the total leaf area, with iodine concentrations greater than 7 mg/L reducing leaf area as a likely consequence of the strong iodine phytotoxicity under the NFT hydroponic system (Figure 1). According to Voogt *et al.*, 2010, the iodide form is more effective in biofortification processes, but it has a negative impact on plant growth, owing to its excessive accumulation in plant tissues, as it is directly taken up by plant roots. Nevertheless, plant growth parameters such as plant height, leaf number, plant canopy, and chlorophyll index were not influenced by iodine treatment weekly (Figures 2 and 3).

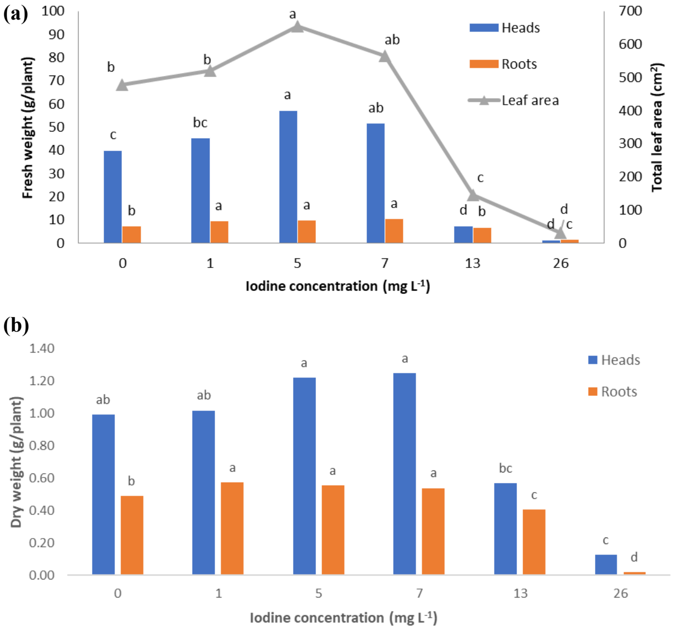

Figure 1. Effect of KI application on biomass [(a) fresh and (b) dry weight] and leaves area of butterhead lettuce. Bars with different notations are statistically significantly different according to the LSD test at p≤0.05.

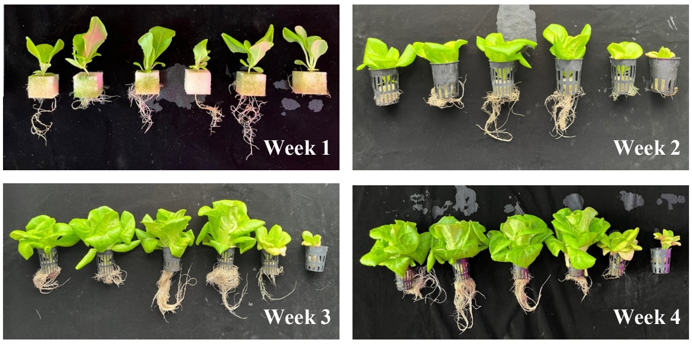

Figure 2. Plant growth development weekly for plants treated with iodine.

3.2 Iodine uptake and nitrate content

Butterhead lettuce proved to be a good plant for biofortification with iodine. It was discovered that the accumulation of iodine in the fortified plants was found to depend on the amount of KI applied. Butterhead lettuce showed a capacity to accumulate iodine in large amounts, with a higher iodine concentration (Figure 4). However, the control butterhead plants had low iodine content. The highest level of KI application caused a very high increase in iodine content by nearly 6 times as compared to the plant without iodine. The nitrate concentrations found in this study are low. The average nitrate content measured in butterhead lettuce was in the range of 91 to 115 mg/kg FW (Figure 5). These might be due to iodide decreasing both the concentration of nitrate and the nitrate reductase activity in plants (Blasco *et al.*, 2008).

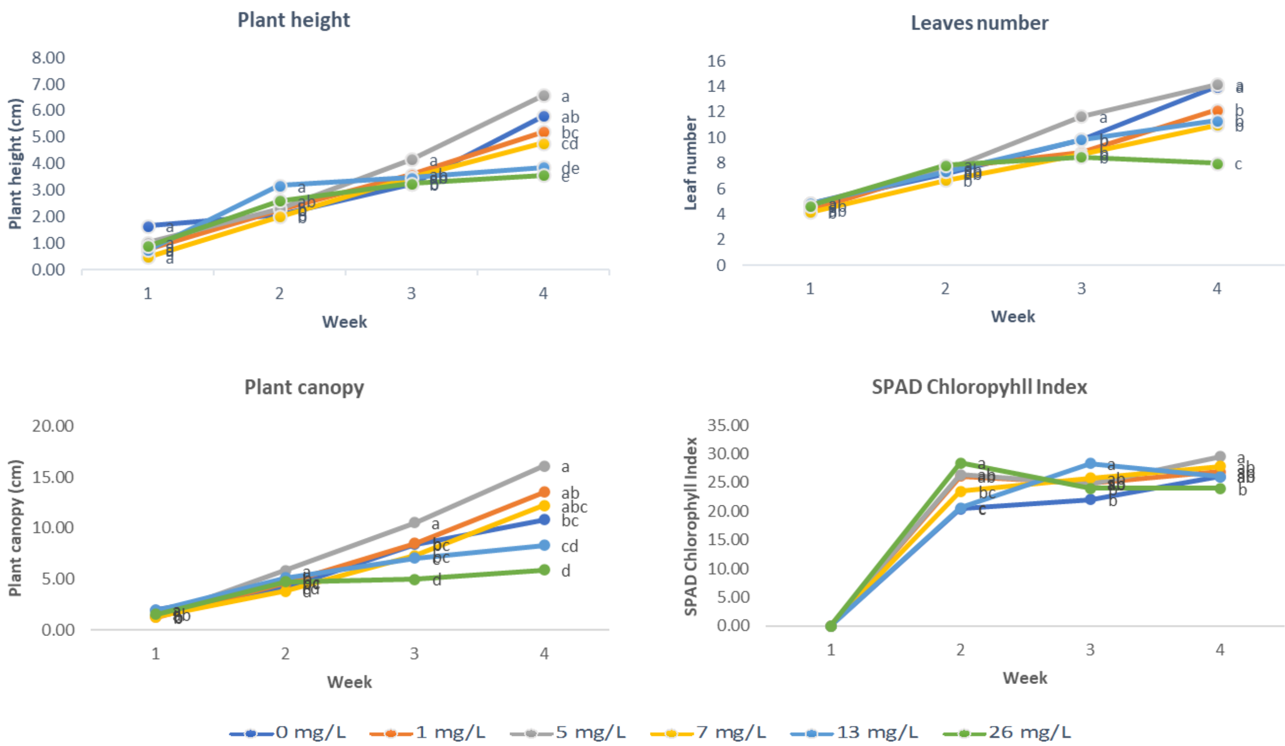

Figure 3. Effect of KI application on the growth of butterhead lettuce weekly. Means followed by different letters are significantly different according to the LSD test at p≤0.05.

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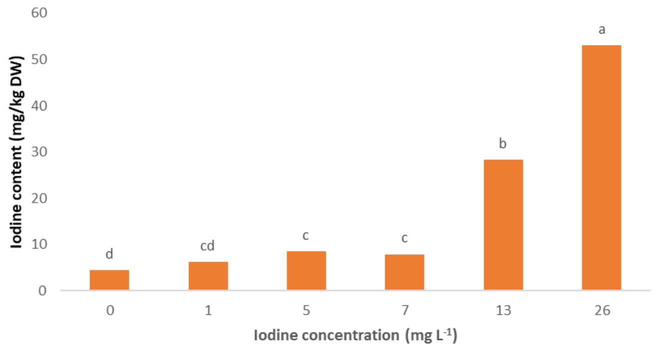

Figure 4. Effect of KI application on the iodine contents of butterhead lettuce. Bars with different notations are statistically significantly different according to the LSD test at p≤0.05.

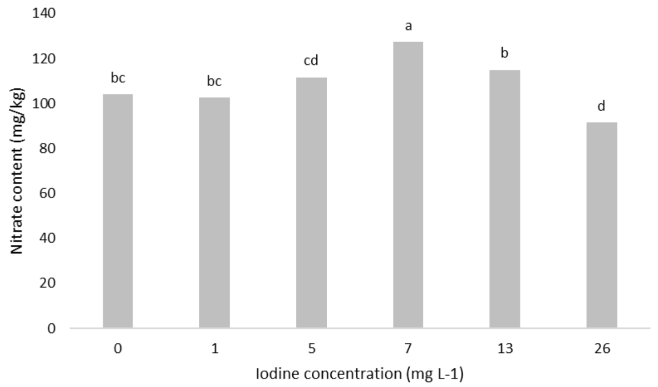

Figure 5. Effect of KI application on the nitrate contents of butterhead lettuce. Bars with different notations are statistically significantly different according to the LSD test at p≤0.05.

3.3 The postharvest quality

Iodine biofortification treatments high significantly affected all the antioxidant capacities except for the ascorbic acid content of butterhead lettuce (Table 1). T3 with an Iodine concentration of 5 mg/L showed a positive effect on antioxidant capacity compared to control and other concentrations. Table 1 shows the effective concentrations of Iodine (5 mg/L) required to scavenge DPPH radical and the scavenging values as inhibition percentages. It can be seen that concentration exhibited various degrees of scavenging ability and showed the strongest ($p < 0.05$) radical-scavenging effect (52.81%) compared to other concentrations, respectively. Thus, the measurement of the DPPH assay of Butterhead lettuce samples increased the antioxidant activity with the optimum concentrations of iodine content. Besides that, Table 2 shows the ferric-reducing capacity obtained using the FRAC assay. A concentration-dependent ferric -reducing capacity was found for all of the Iodine biofortification studied. The treatment with 5 mg/L of iodine concentration (T3), presented the FRAP values increased in biofortified Butterhead lettuce, indicating enhanced antioxidant capacity. Followed by the FIC values also higher in the sample (Table 2). The iodine concentration of 5 mg/L showed the highest iron-chelating ability in vegetables, as assessed by the FIC assay and contributes to improved antioxidant capacity (Table 2). According to Blasco *et al*. (2008), the treated lettuces show a significant increase in antioxidant compounds after the application of iodine. The total phenolic in lettuce plants was increased after biofortified with an iodine dosage ranging from 0 to 240 µM. A similar finding by Sabatino *et al.* (2021), stated that the plants biofortified with the highest dosage of iodine displayed the highest total phenolics concentration, followed by those biofortified with 250 mg/L. The lowest total phenolic concentration was observed in control plants.

4. Conclusion

Biofortification of lettuce with iodine is applicable in an NFT hydroponic system. A recommended I dose of 5 mg/L increased biomass, plant growth, iodine content and antioxidant activity without toxicity effects on plants. The study found that one gram of iodine-biofortified lettuce would provide 60%, 46%, and 30% of the recommended daily allowance of iodine for children, pregnant women, and adolescents, respectively.

Table 2. The effect of different iodine concentrations on the phytochemical content (ascorbic acid content, total phenolic content, and antioxidant capacity) of quality butterhead lettuce.

Factor	Ascorbic acid content (mg/100g FW)	Total phenolic content (mg/GAE 100 g FW)	Antioxidant capacity		
			DPPH (% inhibition)	FRAP (mg $FeSO_4$ Eq/100 g)	FIC (% inhibition)
Iodine Concentration (mg/L)					
T1 – 0 (control)	4.18±0.10	22.65±0.69^b	15.46±3.60^c	91.88±2.71^c	26.49±0.98^b
T2 – 1	3.88±0.36	17.00±1.63^c	16.65±0.53^c	88.92±2.79^c	25.15±1.57^b
T3 – 5	3.36±0.21	32.62±2.51^a	52.81±1.39^a	136.04±1.09^a	29.01±0.30^a
T4 – 7	3.89±0.78	15.89±0.66^c	44.59±2.02^b	104.34±3.03^b	17.70±1.32^c
F-Test significant	ns	**	**	**	**

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different by DMRT test at p≤0.05. ns: not significantly different, *significantly different at p≤0.05, **highly significantly different at p≤0.05.

Conflict of interest

The authors declare no conflict of interest.

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