Iodine biofortification of sweet basil and lettuce grown in two hydroponic systems

Puccinelli M.*, Landi M., Maggini R., Pardossi A., Incrocci L.

Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

*Corresponding author: Martina Puccinelli

E-mail: martina.puccinelli@agr.unipi.it

Phone +39 3885822149

Postal Address: Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

Abstract

Two hydroponic techniques (floating system and aeroponics) were assessed for iodine (I) biofortification of sweet basil (*Ocimum basilicum* L.) and baby-leaf lettuce (*Lactuca sativa* L.). Iodine was supplemented by adding KI into the nutrient solution to achieve a final I concentration of 10 µM. Shoot biomass production and leaf concentration of I, nitrates, total phenols and pigments were measured on the occasion of two successive cuts, 14 and 28 days after transplanting. In both the hydroponic systems, the supplementation of KI represented an effective method for the biofortification of basil as it did not affect the plant growth, while it moderately reduced the biomass production in lettuce. Leaf I accumulation occurred to a greater extent in aeroponics than the floating system in both species. In KI-treated basil plants, leaf I content ranged between 9.76 and 23.58 mg kg^{-1} FW. Consequently, 6 g of fresh basil leaves, which is contained in a portion of

Italian pesto sauce (12 g), could satisfy 40% to 94% of the Recommended Daily Intake (RDI) of iodine for healthy adults (150 µg day^{-1}). In lettuce, leaf I content of first-cut leaves were 1.55 and 3.60 mg kg^{-1} FW, in the floating system and aeroponics, respectively. Therefore, a serving size of 26 g of lettuce containing I from 1.55 (floating culture) to 3.60 (aeroponics) mg kg^{-1} FW could satisfy 27 % to 62% of the RDI of iodine.

Lettuce grew much less in the floating system than aeroponics, probably because of the lower dissolved oxygen level in the nutrient solution as compared to aeroponics. Basil was less sensitive to the oxygen availability in the root zone, since no differences were detected between the two hydroponic systems in terms of fresh and dry biomass.

Keywords: aeroponics; floating system; dietary supplement; iodine supplementation; leafy vegetables; potassium iodide; soilless culture

1. Introduction

Iodine (I) deficiency is one of the most common micronutrient deficiencies worldwide and is responsible for a series of 'iodine deficiency disorders' (IDDs), due to insufficient secretion of thyroid hormones (Zimmermann et al., 2008). Seafood is the main source of I and the Recommended Daily Intake (RDI) ranges from 90 to 250 µg day^{-1} (EFSA, 2014). Several countries have successfully implemented the "universal salt iodization" for dietary I supplementation. However, the use of iodized salt is still inadequate due to the loss of I during storage, transportation and cooking (Aburto et al., 2014). Moreover, many countries have implemented policies aimed at reducing salt intake to prevent hypertension and cardiovascular diseases. To contrast IDDs, I biofortification of vegetables is a valid alternative to salt iodization (Gonzali et al., 2017). Biofortification is the process by which the concentration of essential elements is increased in plant tissues, in order to improve the nutritional quality of plant-based food during the plant growth rather than during post-harvest processing (Díaz-Gómez et al., 2016).

52 The production of biofortified vegetables is facilitated by the application of closed-loop hydroponic
53 (or soilless) cultivation, which results in higher crop yield, better product quality, lower
54 consumption of water and fertilisers, and reduced release of agro-chemicals to the environment
55 (Pardossi et al., 2006; Rouphael et al., 2018).
56 Among closed-loop hydroponic techniques, the floating system is widely used for the cultivation of
57 leafy vegetables with short cycle and grown at high plant density, such as many baby-leaf
58 vegetables and herbs including sweet basil (Kiferle et al., 2019; Landi et al., 2013) and lettuce
59 (Blasco et al., 2008).
60 Aeroponics is a soilless system in which the plant roots are suspended in the air in the dark and
61 frequently sprayed with a fine mist of nutrient solution (Pardossi et al., 2006). This system has been
62 successfully used for the cultivation of several vegetables, such as basil (Salachas et al., 2015),
63 lettuce (Jie and Kong, 1998), tomato (Dannehl et al., 2017), potatoes (Ritter et al., 2001) and
64 cucumber (Park et al., 1997).
65 Sweet basil (*Ocimum basilicum* L.) is a popular herb mostly used for food preparation (Makri and
66 Kintzios, 2008); for example, it is the main ingredient of the Italian green sauce 'pesto'. Lettuce
67 (*Lactuca sativa* L.) is widely cultivated in the open field or under a greenhouse for fresh
68 consumption of mature heads or leaves (in non-heading genotypes), or immature (baby) leaves as
69 ready-to-eat vegetables. The market for baby-leaf vegetables is rapidly growing in many countries
70 as it offers healthy and convenient foods to consumers (Saini et al., 2017).
71 This study was conducted on sweet basil and baby-leaf lettuce to investigate the effect of two
72 hydroponic techniques (floating system and aeroponics) and the addition of potassium iodide (KI)
73 into the nutrient solution in terms of plant growth and leaf I accumulation. To the best of our
74 knowledge, for the first time, the cultivation of basil and lettuce in floating system and aeroponics
75 were compared and in the latter hydroponic system, I biofortification of leafy vegetables was tested.
76 The I level in the KI-enriched nutrient solution was 10 µM. This concentration was chosen as
77 previous works with I concentration in the nutrient solution ranging from 0.4 to 240 µM

demonstrated that I supplementation reduced the growth in many hydroponically-grown vegetables only at concentrations higher than 10-12 µM (Zhu et al., 2003; Voogt et al., 2010; Gonnella et al., 2019; Kiferle et al., 2019). Besides, Blasco et al. (2008) detected the highest I translocation factor at the concentration of 10 µM KI; thus, this concentration resulted as the most efficient for biofortification.

The experiment was performed during the spring-summer period in order to evaluate the possible advantages of the aeroponic system as compared to floating system, under conditions of high air temperature (>40 °C), typically experienced by the Mediterranean crops grown under greenhouse conditions. One of the major drawbacks of floating system is the risk of the occurrence of root hypoxia stress due to the depletion of oxygen at the root-water interface as a consequence of the stagnant nutrient solution (Soffer and Burger, 1988). This risk is aggravated under high-temperature conditions, which decreases the oxygen solubility while increasing the respiratory demand for oxygen in root tissues (approximately, it doubles for each 10°C rise in temperature, up to about 30 °C) (Jitsuyama, 2013). In contrast, in aeroponics frequent irrigation and the creation of mist results in nutrient solution saturated with dissolved oxygen (Alshrouf, 2017; Gopinath et al., 2017).

Quality attributes of leafy vegetables include greenness, low level of nitrates, and high antioxidant content and capacity. Therefore, we also investigated the effect of I supplementation and hydroponic system on leaf content of nitrates, pigments, and total phenols and the leaf antioxidant capacity.

2. Materials and methods

2.1. Plant material and growing conditions

The experiment were conducted in a glasshouse at the University of Pisa, Italy (lat. 42'42"48N, long. 10°24'52"92 E), between late spring and early summer 2019.

Basil (*Ocimum basilicum* L. “Tigullio”) seeds were purchased from Franchi Sementi (Bergamo, Italy) and lettuce (*Lactuca sati*va var. crispa L. “Salad Bowl”) seeds from Gargini sementi (Lucca,

104 Italy). Seeds were sown in 240-cell plug-trays filled with rockwool and vermiculite; the trays were
105 placed in a growth chamber at 25 °C for 5 days. Basil and lettuce seedlings were then planted in
106 aeroponics and floating systems, 20 and 10 days after sowing, respectively.
107 The aeroponics was made of two separate systems consisting of two plastic chambers with a total
108 volume of 220 L m^{-2} and height of 22 cm. Each chamber was closed at the top with eight
109 polystyrene panels, each hosting 20 plants. In total, 80 plants of each species were placed in each
110 system. The mixing tank of each system contained 200 L of nutrient solution which was sprayed for
111 20 s every 10 min during the day and every 40 min during the night. The total volume of nutrient
112 solution delivered to the system at each irrigation event was approximately 6.4 L.
113 The floating system was made up of six separate 50-L plastic tanks (water depth 25 cm) with a
114 polystyrene tray hosting 8 plants of basil and 8 plants of lettuce (16 plants in total).
115 In both the hydroponic systems, crop density was 100 plants m^{-2} (on a ground area basis).
116 All the plants were fed with a nutrient solution containing: $N\text{-}NO_3^-$ 14.0 mM, $N\text{-}NH_4^+$ 2.0 mM, P
117 2.0 mM, K 10.0 mM, Ca 4.5 mM, Mg 2.0 mM, $S\text{-}SO_4$ 5.0 mM, Fe 40.0 µM, B 40.0 µM, Cu 3.0
118 µM, Zn 10.0 µM, Mn 10.0 µM, Mo 1.0 µM. For the preparation of the nutrient solution, the
119 following salts were used: $5[Ca(NO_3)_2 *2H_2O]NH_4NO_3$, NH_4NO_3, KH_2PO_4, $MgSO_4 *7H_2O$, KNO_3,
120 K_2SO_4, Fe EDDHA, H_3BO_3, Cu EDTA, Zn EDTA, Mn EDTA, $Na_2MoO_4 *2H_2O$.
121 The pH and electrical conductivity (EC) values were 5.6 and 2.32 dS m^{-1}, respectively. They were
122 checked every day and remained within 10% of the values measured in the newly prepared nutrient
123 solution. EC value close to 2.32 dS m^{-1} is commonly used for the hydroponic cultivation of basil
124 and lettuce (Voogt et al., 2010; Incrocci et la., 2019).
125 In the floating system, the nutrient solution was continuously aerated and the oxygen content ranged
126 between 4.9 and 5.4 g m^{-3} with an average of 5.2 mg L^{-1}.
127 In the aeroponics system, the oxygen content of the nutrient solution in the mixing tank was higher
128 than 7.0 mg L^{-1} throughout the entire experiment.

During the experiment, the nutrient solution was completely replaced every week in order to minimize the changes in the ion concentration of the nutrient solution.

Basil and lettuce plants were cut twice, 14 and 28 days after transplanting, at 2 cm above the first node or above the collar level, since these species are re-cut one or more times in a season as new leaves grow.

Climatic conditions were continuously monitored by a weather station located inside the greenhouse. The climatic and cultivation parameters are shown in Table 1.

2.2. Experimental designs

The treatments were defined by a combination of three factors: I concentration in the nutrient solution, the hydroponic system, and the time of cutting. The treatments were arranged in a totally randomized design. The I treatments were differentiated one week after transplanting in the hydroponic system by adding potassium iodide (KI) into the nutrient solution. The control nutrient solution contained approximately 0.07 µM KI, which was the constitutive level of raw water.

2.3. Determinations

2.3.1. Biomass production

At each sampling, leaf area, fresh weight (FW), and dry weight (DW) (after drying in a ventilated oven at 60 °C till constant weight) of basil shoots and lettuce leaves were determined on three individual plants selected randomly from each replicate.

2.3.2. Leaf gas exchange and chlorophyll *a* fluorescence parameters

Leaf gas exchanges and fluorescence were measured in nearly fully-expanded leaves.

153 The rate of leaf photosynthesis (Pn; μmol CO_2 m^{-2} s^{-1}) and transpiration (E; mmol H_2O m^{-2} s^{-1}) were
154 measured using a portable gas exchange system with a broad-leaf chamber, lamp and infrared
155 temperature sensor (CIRAS-2, PPSystems, Haverhill, MA). The measurements were performed the
156 day before each cut in light-saturated conditions (1000 μmol photons m^{-2} s^{-1} PAR) and at ambient
157 air CO_2 concentration (400 ± 5 μmol CO_2 mol air^{-1}) and temperature.
158 Modulated chlorophyll *a* fluorescence parameters were measured with a fluorometer (PAM-2000,
159 Walz, Effeltrich, Germany) in dark-adapted leaves (30 min) before (F_0) and after (F_m) a saturating
160 pulse (8000 μmol m^{-2} s^{-1} for 1 s). The maximal photosystem II (PSII) photochemical efficiency
161 [$F_v/F_m = (F_m - F_0)/F_m$] was calculated according to Genty et al. (1989).
162
163 **2.3.3. Leaf iodine and nitrate content**
164 Inorganic iodine content was determined in oven-dried ground samples after extraction with
165 deionized hot water (60 °C) for 60 min. When cooled at room temperature, the extract was filtered
166 and analyzed as reported by Perring et al. (2001). Briefly, 1 mL of potassium thiocyanate (KSCN)
167 (0.023% m/v), 2 mL of $NH_4Fe(SO_4)_2$ (7.7% m/v) in 2.4 M HNO_3 and 2 mL of $NaNO_2$ (0.02% m/v)
168 were added to the extracts and to the KI standard solutions, mixed and maintained at 60 °C for 1
169 hour. After the incubation, the reaction was stopped by placing the solutions in a water-ice mixture
170 for 10 min and the absorbance was read at 454 nm (Perring et al., 2001).
171 The above method was previously checked through parallelism tests, using standard solutions (Std)
172 with or without the addition of leaf extracts. In the former case, 50 μL of extracts from either
173 control or KI-treated leaves were added to 1.950 mL of standards at different concentrations (Sam
174 CT + Std and Sam TRAT + Std, respectively). The agreement between the measured and predicted
175 concentrations were excellent (Figure S2); as expected, the regression lines for both Std and Sam
176 CT + Std overlapped with the identity line, having a slope and intercept statistically equal to 1 and
177 0, respectively (Figure S2).Furthermore, a parallel regression line was obtained for Sam TRAT +
178 Std, with slope statistically equal to 1 and intercept significantly higher than 0.

The nitrate content was measured spectrophotometrically in dry leaf samples extracted with distilled water (100 mg DW in 20 mL) at room temperature for 2 h using the salicylic-sulfuric acid method (Cataldo et al., 1975).

2.3.4. Photosynthetic pigment content, total phenolic content and total antioxidant capacity

Leaf chlorophyll and carotenoid content were measured in fresh samples according to Lichtenthaler (1987). An aliquot of 100 mg of each sample was extracted with 5 mL methanol 99% (v/v), and the concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids were spectrophotometrically determined using the equation reported by Welburn and Lichtenthaler (1984).

Total phenol content was determined in the same methanol extracts using the Folin–Ciocalteau reagent according to Kang and Saltveit (2002). The total phenol content was calculated using the calibration curve containing 0, 50, 100, 150 and 250 mg gallic acid L^{-1}; values were expressed as mg of gallic acid (GAE) g^{-1} FW.

According to Huang et al. (2005), the total antioxidant capacity was measured with two methods: the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams et al., 1995), using an aliquot of methanol extract, used for the determination of pigments and phenol content. The methanol extract was added to 0.9 mL of FRAP reagent, which consisted of 1 mM 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mM ferric chloride in 250 mM sodium acetate (pH 3.6). It was then mixed and kept at 20 °C for four min, and the absorbance was read at 593 nm. The results were expressed as µmol Fe(II) mg^{-1} FW. The DPPH reagent was added to the methanol extract and, after 30 min of incubation in the dark at room temperature, the absorbance at 515 nm was read. The percentage of inhibition was calculated comparing the absorbance of samples with the absorbance of a solution where methanol was added instead of the extract. The results were expressed as the concentration of Trolox equivalents (µmol TE g^{-1} FW).

2.3.5. Iodine Transference factor

The iodine transference factor (TF) was calculated using both the I concentration (IC) of the fresh leaves and the IC of the solution (Zhu et al., 2003). Thus, the TF was calculated in the following way:

$$ITF = \frac{IC\ leaves}{IC\ solution}$$

2.3.6. Statistical analysis

Data were tested for homogeneity of variances using Levene's test and were subjected to three-way ANOVA with the hydroponic system, KI concentration, and the time of cut as variables. Mean values were separated by the Duncan's post-hoc test ($P < 0.05$). Statistical analysis was performed using R Statistical Software.

For the sake of simplicity, tables and figures show only the mean values of each treatment and the significance of different sources of variability and their interaction effects. The mean values for each source of variability and interactions are reported in the supplementary material.

3. Results

3.1. Sweet basil

Biomass production and shoot growth were significantly influenced by the cut time without significant effects of the hydroponic system used, KI concentration and the interactions among the three factors (Table 2). On average, shoot FW, DW and leaf area were 34.2%, 48.3% and 343% respectively, greater in the second-cut (C2) (Table S1). The DW/FW ratio was greater in the first-cut (C1) leaves as compared to the C2 ones (+10.4%). A similar trend was found in the floating system when compared to aeroponics (+6.5%) (Table 2).

Leaf Pn and E did not differ significantly in all the treatments (Table 2).

229 Plants treated with I showed slightly reduced values of F_v/F_m only in C2 leaves and in the floating
230 system. On average, F_v/F_m and F_m were lower in C2 leaves by 3.6% and 14.3%, respectively (Table
231 S2).
232 The supplementation of KI resulted in a large I accumulation in leaf tissues in both hydroponic
233 systems and to a larger extent in the C2 leaves (Figure 1). Leaf I content ranged from 9.76 to 23.58
234 mg kg^{-1} FW in KI-treated plants and, on average, it was 17.0% and 89.7% higher, respectively, in
235 aeroponics and C2 leaves with respect to the floating system and C1 leaves (Table S3). The I
236 concentration in the control leaves was below the limit of detection against 9.76 to 23.58 mg kg^{-1}
237 FW in KI treated plants.
238 The ITF was higher in KI-treated plants grown in aeroponics, especially in C2 leaves (18.57 against
239 13.89 in C1). On average, the ITF was 89.9 % higher in C2 leaves as compared to C1 leaves (Table
240 S5).
241 A higher leaf nitrate content was found in aeroponics than the floating system, and also in C2 leaves
242 as compared to C1 leaves (Table 2). On average, the nitrate content was 33.5% and 19.3% higher,
243 respectively, in aeroponics and C2 leaves (Table S3).
244 Leaf phenol content was markedly increased (44%) by KI supplementation in C2 leaves of
245 aeroponically-grown plants (Table 3). On average, adding KI to the nutrient solution increased leaf
246 phenol content by 11% (Table S3).
247 Leaf contents of chlorophylls and carotenoids were scarcely influenced by the factors under
248 investigation (Table 2), apart from a significant increase of chlorophyll content observed in C2
249 leaves of plants grown in aeroponics (+20%, on average; Table S3). On average, the chlorophyll
250 content was slightly lower in aeroponics (-8.5%) and higher in plants treated with KI (+5.2%) and
251 in C2 leaves (+9.0%). Only the time of cut affected the carotenoids content, which was higher
252 (+19.5% on average) in C2 leaves (Table S3).
253 In plants grown in aeroponics, the total antioxidant capacity of C1 leaves, as measured by both
254 FRAP (-36.6%) and DPPH (-45.7%) assays was lower than in floating system (Figure 2); no

255 differences between cultivation systems were detected in C2 leaves (Table S4). On average, the
256 total antioxidant capacity of plants was higher in KI-treated plants (+26.3% with DPPH assay and
257 +20.5% with FRAP assay) (Table S4).
258 A significant positive correlation among the total phenols content and the antioxidant capacity of
259 leaves was found. The determination coefficient (r^2) was 0.87 and 0.88, respectively, with DPPH
260 and FRAP assay (Figure 4).
261

262 ## 3.2. Lettuce

263 A significant reduction of leaf FW, DW and area due to KI supplementation was determined in C2-
264 plants, in particular in floating culture (Table 3). For instance, leaf DW was reduced by 69.8% in
265 the floating system and by 30.4% in aeroponics (Table 3). On average, KI supplementation reduced
266 the biomass production (-23.9% DW and -19.9% FW) and leaf area (-20.3%) (Table S6). The
267 biomass production was higher in C2 plants in aeroponics (+83.1% DW, +93.7% FW and +70.6%
268 leaf area) and, on average (+44.1% DW, +38.8% FW and +31.4% leaf area) (Table S6). Shoot FW,
269 DW and leaf area were significantly higher (+186%, +213% and +116% respectively) in aeroponics
270 than the floating culture (Table S6).
271 Leaf DW/FW ratio, Pn and E were not significantly influenced by any factors under investigation
272 (Table 3).
273 The interaction among the time of cutting, hydroponic system and KI concentration significantly
274 affected only the F_v/F_m (Table S7). The supplementation of KI and the cut time slightly reduced
275 F_v/F_m only in C2 leaves and in floating system (Table S7).
276 The supplementation of KI resulted in leaf I accumulation, which occurred to a larger extent in
277 aeroponics and C2 leaves (Table 3). On average, leaf I content in aeroponics was 53.9% higher than
278 in floating system, and 124.8% higher in C2 leaves (Table S8). The I concentration in the control
279 samples was below the limit of detection against 1.55 to 6.55 mg kg^{-1} FW in KI treated plants.

The ITF was higher in KI-treated plants grown in aeroponics, especially in C2 leaves (5.15 against 3.98 in C1). On average, the ITF was higher in C2 leaves (+ 125.7%) and aeroponics (+ 53.8%) as compared to C1 (Table S10).

Leaf nitrate content was not influenced by KI and was higher in the floating system (+33.7%, on average) than aeroponics and in C2 leaves (+25.0%, on average) (Table 3, S8).

No differences were observed among the treatments regarding leaf phenol content (Table 3), which was slightly lower (-26.2%) in C2 leaves of the plants grown in the floating system (Table S8)

ower chlorophyll content was determined in C2 leaves of KI-treated plants grown in the floating system (772 mg kg^{-1} FW) and C1 leaves of aeroponically-grown plants treated (719 mg kg^{-1} FW) or not (739 mg kg^{-1} FW) with KI (Table 3).

On average, the carotenoid content in leaves was 176.8 and 156.3 mg kg^{-1} FW in plants grown, respectively, in the floating system and aeroponics. Treatment with KI moderately reduced (-20.8%, on average) the carotenoid content in leaves (Table S8). In addition, very low content of carotenoids was detected in C2 leaves in KI treated plants grown in the floating system (82.7 mg kg^{-1} FW) (Table 3).

In general, the total antioxidant capacity was higher (+11.9% DPPH; +27.2% FRAP) in the floating system than aeroponics, and in KI-treated plants than the control plants (+8.44% DPPH; +13.46% FRAP) (Figure 3; Table S9).

There was a strong positive correlation among the leaf total phenols content and the antioxidant capacity; the determination coefficient (r^2) was 0.88 and 0.83, respectively, for DPPH and FRAP assay (Figure 4).

4. Discussion

4.1. Iodine biofortification

Growing plants hydroponically with an I concentration of 10 µM in the nutrient solution resulted in effective I biofortification of both basil and lettuce (Fig. 1). In KI treated plants, leaf, the I content

306 ranged between 9.76 and 23.58 mg kg^{-1} FW in basil, and between 1.55 and 6.55 mg kg^{-1} FW in
307 lettuce, depending on the hydroponic system and the harvest time.
308 We calculated that 6 g of fresh basil leaves are contained in approximately 12 g of the Italian
309 'pesto' sauce (an amount used for a single portion of pasta)and can satisfy 40% to 94% of the RDI
310 (EFSA, 2014) of I established for a healthy adult (150 µg day^{-1}; EFSA, 2014). For lettuce, we
311 considered the I content of C1 leaves of KI treated plants as longer exposure to 10 µM KI slightly
312 but significantly reduced the plant growth (Table 3). For lettuce, a serving size of 26 g (Ashfield-
313 Watt et al., 2003) containing 1.55 (floating system) to 3.60 (aeroponics) mg I kg^{-1} FW, satisfies 27
314 % to 62% of the RDI.
315 Using the above-mentioned data for both biofortified basil and lettuce, we calculated the
316 recommended dietary tolerable upper intake of I to be 1,100 µg day^{-1} for an adult of 70 kg body
317 weight (Smoleń et al., 2019). The hazardous amount of fresh leaves consumed per day was 113 to
318 47 g for basil and 710 to 305 g for lettuce.
319 Many experiments have been conducted in hydroponics using different I salts and doses with
320 positive results in terms of biofortification in many species (e.g. tomato, Caffagni et al., 2012;
321 Landini et al., 2011; Li et al., 2017; rice, Mackowiak and Grossl, 1999; spinach, Weng et al., 2008;
322 Zhu et al., 2003), including basil (Incrocci et al., 2019; Kiferle et al., 2019) and lettuce (Blasco et
323 al., 2008, 2011a, 2011b; Smoleń et al., 2014, 2017, 2016; Voogt et al., 2010). Generally, leaf I
324 content increases with the I concentration (Blasco et al., 2011a, 2011b; Incrocci et al., 2019; Kiferle
325 et al., 2019; Voogt et al., 2010; Zhu et al., 2003) and the period of I supplementation (Mackowiak
326 and Grossl, 1999; Voogt et al., 2010). Our results are in agreement with these findings. In fact, I
327 content was significantly higher in C2 leaves which were exposed to a longer period of I
328 supplementation and had a higher leaf area with respect to C1 leaves (Table 2 and 3). After root
329 uptake, iodine is transported to the shoot in the xylem while its redistribution through the phloem is
330 low (Mackowiak and Grossl, 1999; Smoleń et al., 2014). Therefore, I accumulation in leaf tissues
331 depends on plant transpiration (Smoleń et al. (2014) and the differences observed between C1 and

C2 leaves in both basil and lettuce (Figure 1 and 2) could be explained by the difference in the amount of water transpired.

Aeroponics enhanced leaf I accumulation in both species, although in basil this effect was observed only in the C2-leaves. A more efficient nutrient supply to the roots provided by aeroponics (Blok et al., 2017) may explain a higher I uptake in these plants than those grown in the floating system.

In both the cultivation systems, the ITF of lettuce was lower than the values calculated by Blasco et al. (2008) for lettuce plants fed with a nutrient solution containing 10 µM KI. These differences could be due to the different cultivation system and climatic conditions. Blasco et al. (2008) cultivated the plants in pots in a growth chamber maintained at 25°C/15°C (day/ night). In basil, we found ITF values close to those reported by Incrocci et al. (2019).

4.2. Effects of KI on plant growth and leaf quality

As expected (see Introduction), in both species, I concentration of 10 µM in the nutrient solution have significant effects neither on plant growth (Tables 2 and 3) or on leaf gas exchange (Table S1 and S6) and chlorophyll content (Table 2 and 3). The absence of damages to the photosynthetic apparatus in I-treated plants was coherent with the result of chlorophyll fluorescence measurements. In fact, in all the treatments, the F_v/F_m ratio ranged from 0.78 to 0.83, which are the typical values of healthy plants (Schreiber et al., 1995). The slight reduction of biomass production determined at the second cut in lettuce was likely caused by higher I accumulation in leaf tissues that led to sub-toxic I concentration in the tissues.

Iodine supplementation barely affected leaf quality attributes such as the greenness (data not shown) and the content of nitrates, phenols and pigments (Table 2 and 3).

The European regulation 1258/2011 has set maximum limits for nitrates in some vegetable species such as spinach, rocket salad and lettuce. These limits range between 2000 and 7000 mg kg^{-1} FW, depending on plant species, growing season and environment. They are higher for vegetables grown in fall-winter season and under greenhouse than in spring-summer and in the open field. In our

work, leaf nitrate contents of both species were below the maximum value allowed for summer-grown lettuce and was not affected by the I level in the nutrient solution, which is in agreement with previous findings in radish (Sady et al., 2010; Strzetelski et al., 2010) and sea fennel (Sarrou et al., 2019). In contrast, leaf nitrate content increased in spinach (Smoleń and Sady, 2012) and tomato (Smoleń et al., 2015) treated with non-toxic I concentrations, whereas it decreased in lettuce exposed to toxic KI concentrations (Blasco et al., 2010).

Phenols in plant cells take part in the antioxidant system, which improves the ability of plants to alleviate oxidative stress (Sakihama et al., 2002). An increase of leaf phenolic content following the exposure to toxic I concentrations in the nutrient solution was observed in lettuce ($\geq$10 µM, Blasco et al., 2008; $\geq$40 µM, Smoleń et al., 2017) and sweet basil ($\geq$100 µM, Kiferle et al., 2019; Incrocci et al., 2019) grown in soilless culture. In our work, I concentration of 10 µM exerted no or slight (in C2 leaves of basil grown in aeroponics) effect on the content of total phenols (Table 2 and 3) and the antioxidant capacity (Figure 2 and 3), which were closely correlated (Fig. 4). Total phenols content did not change in I-biofortified carrot (Smoleń et al., 2019) and tomato (Smoleń et al., 2015), with respect to the control.

4.3. Effects of growing system on plant growth and leaf quality

A significant effect of hydroponic system on plant growth was observed only in lettuce, which grew much less in the floating system than aeroponics (Table 3). The lower dissolved oxygen level in the nutrient solution in the floating system (approximately 5 mg L^{-1} against more than 7.0 mg L^{-1} in aeroponics) is the most likely cause of the growth inhibition in lettuce, although a level of 5 mg L^{-1} is considered adequate for many hydroponic crops (Godfrey, 2018). Better aeration of the nutrient solution led to larger leaf area, fresh and dry biomass of hydroponically-grown purslane (Lara et al., 2011), chrysanthemum (Blok et al., 2017; Eveleens and Blok, 2014; Soffer et al., 1991) and ficus (Soffer et al., 1991).

383 A positive relationship between the oxygen concentration in the root environment ranging from 3 to
384 7 mg L^{-1}, and shoot production was found in hydroponically grown lettuce (Ercan and Bayyurt,
385 2014; Tesi et al., 2003). Leaf dry biomass was reduced by 18% in lettuce plants in floating systems
386 with non-aerated nutrient solution (mean oxygen concentration was 4.0 mg L^{-1} with a minimum
387 value of 1.1 mg L^{-1}) with respect to plants grown in aerated solution (oxygen concentration was 6.2
388 mg L^{-1}) (Alvarado-Camarillo et al. 2020).
389 These results suggest that basil is less sensitive than lettuce to the oxygen level in the root zone
390 which is in agreement with previous findings (Kiferle et al., 2012). These authors did not find any
391 significant differences in shoot growth and leaf content of rosmarinic acid between sweet basil
392 plants grown in floating system with aerated or non-areated nutrient solution, having a mean
393 dissolved oxygen concentration of 7.8 and 3.8 mg L^{-1}, respectively. In a study conducted with 35
394 cultivars, when basil plants were grown in NFT (nutrient film technique) system and floating
395 system with dissolved oxygen concentration in the feeding solution above 8.0 mg L^{-1}, the shoot
396 growth was barely affected by the hydroponic system (Walters et al. 2015).
397 We also found that in both species, biomass production was greater in C2 than C1 plants. As plant
398 growth is positively correlated with the cumulative radiation (Horak and Loughin, 2000), a greater
399 leaf biomass and area determined on occasion of the second cut could be ascribed to the longer
400 period of treatment and higher cumulative radiation (Table 1), eventhough the radiation use
401 efficiency (RUE) was, on average, lower in the second growth phase (Table S5 and S10)
402 The higher leaf nitrate content found in C2 plants of both basil and lettuce could be explained by the
403 presence of an extensive root system (data not shown) (Sullivan et al., 2000) and leaf area in these
404 plants with respect to C1 plants, consequently leading to higher nitrate uptake and transportation to
405 the shoot driven by transpiration. The greater root biomass (data not shown) and the better nutrient
406 supply to the roots in aeroponics compared to the floating system (Blok et al., 2017) could also
407 account for the higher leaf nitrate content of basil plants. On the contrary, the higher leaf nitrate
408 content detected in lettuce plants grown in the floating system as compared to aeroponics may

likely be related to a lower growth rate due to reduced level of dissolved oxygen in the nutrient solution. Reduced growth may result in nitrate accumulation when plants are grown with large nitrate availability in the root zone (Devienne-Barret et al., 2000) as generally occurs in hydroponic culture (Yosoff et al., 2015).

5. Conclusions

In our work, the application of a nutrient solution containing 10 µM KI for one or two weeks emerged as an effective strategy for iodine biofortification of basil and baby-leaf lettuce, grown in closed-loop hydroponic systems. Plant growth and leaf quality attributes were not or barely affected by iodine supplementation and the iodine content in the leaves could satisfy 27% to 94% of recommended daily intakes established for human beings, depending on the species and the hydroponic system, and considering a reasonable daily intake of basil or baby-leaf lettuce.

Leaf I accumulation occurred to a greater extent in aeroponics than the floating system in both species.

Lettuce grew much less in the floating system than aeroponics owing to lower dissolved oxygen levels in the nutrient solution as compared to aeroponics. Basil was less sensitive to the oxygen availability in the root zone, since no differences were detected between the two hydroponic systems in terms of plant growth.

Acknowledgements This work was supported by the project "Aeroponica 2.0, Technological development and testing of an aeroponic system for soilless greenhouse cultivation" POR FSE 2014 – 2020, co-funded by EDO Radici Felici Srl.

The authors wish to thank Dr. Chingoileima Maibam, for the language editing of this paper.

6. References

Aburto, N.J., Abudou, M., Candeias, V., Wu, T., 2014. Effect of salt iodization to prevent iodine deficiency disorders: a systematic review with meta-analyses. WHO eLibrary of Evidence for Nutrition Actions (eLENA) 142.

Alshrouf, A., 2017. Hydroponics, Aeroponic and Aquaponic as Compared with Conventional Farming. Am. Sci. Res. J. Eng. Technol. Sci. ISSN 27, 247–255.

Alvarado-Camarillo, D., Valdez-Aguilar, L.A., González-Fuentes, J.A., Rascón-Alvarado, E., Peña-Ramos, F.M., 2020. Response of hydroponic lettuce to aeration, nitrate and potassium in the nutrient solution. Acta Agric. Scand. Sect. B Soil Plant Sci. 70, 341–348. https://doi.org/10.1080/09064710.2020.1730430

Ashfield-Watt, P., Welch, A., Day, N., Bingham, S., 2003. Is 'five-a-day' an effective way of increasing fruit and vegetable intakes? Public Health Nutr. 7, 257–261. https://doi.org/10.1079/phn2003524

Barber, M.J., Notton, B.A., 1990. Spinach Nitrate Reductase : Effects of Ionic Strength and pH on the Full and Partial Enzyme Activities. Plant Physiol. 93, 537–40. https://doi.org/10.1104/pp.93.2.537

Benzie, I.F.F., Strain, J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239, 70–6. https://doi.org/10.1006/abio.1996.0292

Blasco, B., Rios, J.J., Cervilla, L.M., Sánchez-Rodrigez, E., Ruiz, J.M., Romero, L., 2008. Iodine biofortification and antioxidant capacity of lettuce: Potential benefits for cultivation and human health. Ann. Appl. Biol. 152, 289–299. https://doi.org/10.1111/j.1744-7348.2008.00217.x

Blasco, B., Rios, J.J., Cervilla, L.M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Rosales, M.A., Romero, L., Ruiz, J.M., 2011a. Iodine application affects nitrogen-use efficiency of lettuce plants (Lactuca sativa L.). Acta Agric. Scand. Sect. B Soil Plant Sci. 61, 378–383. https://doi.org/10.1080/09064710.2010.492782

Blasco, B., Rios, J.J., Cervilla, L.M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Rosales, M.A., Ruiz, J.M., Romero, L., 2010. Photorespiration Process and Nitrogen Metabolism in Lettuce Plants (*Lactuca sativa* L.): Induced Changes in Response to Iodine Biofortification. J. Plant Growth Regul. 29, 477–486. https://doi.org/10.1007/s00344-010-9159-7

Blasco, B., Ríos, J.J., Leyva, R., Cervilla, L.M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Rosales, M.A., Ruiz, J.M., Romero, L., 2011b. Does iodine biofortification affect oxidative metabolism in lettuce plants. Biol. Trace Elem. Res. 142, 831–842. https://doi.org/10.1007/s12011-010-8816-9

Blasco, B., Rios, J.J., Leyva, R., Melgarejo, R., Constán-Aguilar, C., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.M., Romero, L., Ruiz, J.M., 2011c. Photosynthesis and metabolism of sugars from lettuce plants (Lactuca sativa L. var. longifolia) subjected to biofortification with iodine. Plant Growth Regul. 65, 137–143. https://doi.org/10.1007/s10725-011-9583-0

Blok, C., Jackson, B.E., Guo, X., De Visser, P.H.B., Marcelis, L.F.M., 2017. Maximum plant uptakes for water, nutrients, and oxygen are not always met by irrigation rate and distribution in water-based cultivation systems. Front. Plant Sci. 8, 562. https://doi.org/10.3389/fpls.2017.00562

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Caffagni, A., Pecchioni, N., Meriggi, P., Bucci, V., Sabatini, E., Acciarri, N., Ciriaci, T., Pulcini, L., Felicioni, N., Beretta, M., Milc, J., 2012. Iodine uptake and distribution in horticultural and fruit tree species. Ital. J. Agron. 7, 32. https://doi.org/10.4081/ija.2012.e32

Cataldo, D.A., Maroon, M., Schrader, L.E., Youngs, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6, 71–80. https://doi.org/10.1080/00103627509366547

484 Dai, J.L., Zhu, Y.G., Zhang, M., Huang, Y.Z., 2004. Selecting iodine-enriched vegetables and the
485 residual effect of iodate application to soil. Biol. Trace Elem. Res. 101, 265–276.
486 https://doi.org/10.1385/BTER:101:3:265
487 Dannehl, D., Suhl, J., Miranda, L., Ulrichs, C., 2017. Sustainable Cities: Viability of a Hybrid
488 Aeroponic/Nutrient Film Technique System for Cultivation of Tomatoes Phytomonitoring and
489 Phytocontrol View project Parasitoids View project, waset.org.
490 Devienne-Barret, F., Justes, E., Machet, J.M., Mary, B., 2000. Integrated control of nitrate uptake
491 by crop growth rate and soil nitrate availability under field conditions. Ann. Bot. 86, 995–
492 1005. https://doi.org/10.1006/anbo.2000.1264
493 Díaz-Gómez, J., Twyman, R.M., Zhu, C., Farré, G., Serrano, J.C.E., Portero-Otin, M., Muñoz, P.,
494 Sandmann, G., Capell, T., Christou, P., 2016. Biofortification of crops with nutrients: factors
495 affecting utilization and storage. Curr. Opin. Biotechnol. 44, 115–123.
496 https://doi.org/10.1016/j.copbio.2016.12.002
497 EFSA, 2014. Scientific Opinion on Dietary Reference Values for iodine, EFSA Journal.
498 https://doi.org/10.2903/j.efsa.2014.3660
499 Ercan, N., Bayyurt, R., 2014. The effects of applications which increase the O_2 of the water on yield
500 and quality of lettuce grown in a floating system. Acta Hortic. 1034, 77–84.
501 https://doi.org/10.17660/ActaHortic.2014.1034.8
502 Eveleens, B., Blok, C., 2014. Cultivation of chrysanthemum without substrate. Acta Hortic. 1034,
503 185–192. https://doi.org/10.17660/actahortic.2014.1034.22
504 Godfrey, M., 2018. The Quick Reference Guide for Hydroponic Farmers - Upstart University.
505 https://university.upstartfarmers.com/blog/the-quick-reference-guide-for-hydroponic-farmers
506 Gonnella, M., Renna, M., D'Imperio, M., Santamaria, P., Serio, F., Gonnella, M., Renna, M.,
507 D'Imperio, M., Santamaria, P., Serio, F., 2019. Iodine biofortification of four brassica
508 genotypes is effective already at low rates of potassium iodate. Nutrients 11, 451.
509 https://doi.org/10.3390/nu11020451

510 Gonzali, S., Kiferle, C., Perata, P., 2017. Iodine biofortification of crops: agronomic biofortifica-
511 tion, metabolic engineering and iodine bioavailability. Curr. Opin. Biotechnol.
512 https://doi.org/10.1016/j.copbio.2016.10.004
513 Gopinath, P., Vethamoni, P.I., Gomathi, M., 2017. Aeroponics Soilless Cultivation System for
514 Vegetable Crops. Chem Sci Rev Lett 6, 838–849.
515 Horak, M.J., Loughin, T.M., 2000. Growth analysis of four Amaranthus species. Weed Sci. 48,
516 347–355. https://doi.org/10.1614/0043-1745(2000)048[0347:GAOFAS]2.0.CO;2
517 Huang, D., Ou, B., Prior, R.L., 2005. The Chemistry behind Antioxidant Capacity Assays. J. Agric.
518 Food Chem. 53, 1841–1856. https://doi.org/10.1021/jf030723c
519 Incrocci, L., Carmassi, G., Maggini, R., Poli, C., Saidov, D., Tamburini, C., Kiferle, C., Perata, P.,
520 Pardossi, A., 2019. Iodine accumulation and tolerance in sweet basil (*Ocimum basilicum* L.)
521 with green or purple leaves grown in floating system technique. Front. Plant Sci. 10, 1494.
522 https://doi.org/10.3389/FPLS.2019.01494
523 Jie, H.E., Kong, L.S., 1998. Growth and photosynthetic responses of three aeroponically grown let-
524 tuce cultivars (*Lactuca sativa* L.) to different rootzone temperatures and growth irradiances
525 under tropical aerial conditions. J. Hortic. Sci. Biotechnol. 73, 173–180.
526 https://doi.org/10.1080/14620316.1998.11510961
527 Jitsuyama, Y., 2013. Responses of Japanese Soybeans to Hypoxic Condition at Rhizosphere Were
528 Different Depending upon Cultivars and Ambient Temperatures. Am. J. Plant Sci. 4, 1297–
529 1308. https://doi.org/10.4236/ajps.2013.46161
530 Kang, H.M., Saltveit, M.E., 2002. Antioxidant capacity of lettuce leaf tissue increases after wound-
531 ing. J. Agric. Food Chem. 50, 7536–7541. https://doi.org/10.1021/jf020721c
532 Katan, M.B., 2009. Nitrate in foods: harmful or healthy? Am. J. Clin. Nutr. 90, 11–12.
533 https://doi.org/10.3945/ajcn.2009.28014
534 Kiferle, C., Ascrizzi, R., Martinelli, M., Gonzali, S., Mariotti, L., Pistelli, L., Flamini, G., Perata, P.,
535 2019. Effect of Iodine treatments on Ocimum basilicum L.: Biofortification, phenolics produc-

tion and essential oil composition. PLoS One 14, 1–23. https://doi.org/10.1371/journal.pone.0226559

Kiferle, C., Maggini, R., Pardossi, A., 2012. Influence of root hypoxia and NaCl salinity on sweet basil (*Ocimum basilicum* L.) grown hydroponically for the production of rosmarinic acid. Agrochimica 56, 257–267.Landi, M., Pardossi, A., Remorini, D., Guidi, L., 2013. Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. Environ. Exp. Bot. 85, 64–75. https://doi.org/10.1016/j.envexpbot.2012.08.008

Landini, M., Gonzali, S., Perata, P., 2011. Iodine biofortification in tomato. J. Plant Nutr. Soil Sci. 174, 480–486. https://doi.org/10.1002/jpln.201000395

Lara, L.J., Egea-Gilabert, C., Niñirola, D., Conesa, E., Fernández, J.A., 2011. Effect of aeration of the nutrient solution on the growth and quality of purslane (*Portulaca oleracea*). J. Hortic. Sci. Biotechnol. 86, 603–610. https://doi.org/10.1080/14620316.2011.11512810

Li, R., Li, D.W., Liu, H.P., Hong, C.L., Song, M.Y., Dai, Z.X., Liu, J.W., Zhou, J., Weng, H.X., 2017. Enhancing iodine content and fruit quality of pepper (*Capsicum annuum* L.) through biofortification. Sci. Hort. 214, 165–173. https://doi.org/10.1016/j.scienta.2016.11.030

Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Mackowiak, C.L., Grossl, R.R., 1999. Iodate and iodide effects on iodine uptake and partitioning in rice (*Oryza sativa* L.) grown in solution culture. Plant Soil 212, 135–143. https://doi.org/10.1023/A:1004666607330

Makri, O., Kintzios, S., 2008. *Ocimum* sp . (Basil): Botany , Cultivation , Pharmaceutical Properties , and Biotechnology. J. Herbs. Spices Med. Plants 13, 123–150. https://doi.org/10.1300/J044v13n03

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

562 Mynett, A., Wain, R.L., 1973. Herbicidal action of iodide: effect on chlorophyll content and photo-
563 synthesis in dwarf bean phaseolus vulgaris. Weed Res. 13, 101–109.
564 https://doi.org/10.1111/j.1365-3180.1973.tb01250.x
565 Pardossi, A., Malorgio, F., Incrocci, L., Tognoni, F., 2006. Hydroponic technologies for greenhouse
566 crops, in: Crops: Quality, Growth and Biotechnology. WFL Publisher, Helsinki, pp. 360–378.
567 Park, H.S., Chiang, M.H., Park, H.S., 1997. Effects of form and concentration of nitrogen in aero-
568 ponic solution on growth, chlorophyll, nitrogen contents and enzyme activities in *Cucumis sa-*
569 *tivum* L. plant. J. Korean Soc. Hortic. Sci. 38, 642–646.
570 Perring, L., Basic-Dvorzak, M., Andrey, D., 2001. Colorimetric determination of inorganic iodine
571 in fortified culinary products. Analyst 126, 985–988. https://doi.org/10.1039/b102423j
572 Ritter, E., Angulo, B., Riga, P., Herrán, C., Relloso, J., San Jose, M., 2001. Comparison of hydro-
573 ponic and aeroponic cultivation systems for the production of potato minitubers. Potato Res.
574 44, 127–135. https://doi.org/10.1007/BF02410099
575 Rouphael, Y., Kyriacou, M.C., Petropoulos, S.A., De Pascale, S., Colla, G., 2018. Improving vege-
576 table quality in controlled environments. Sci. Hortic. (Amsterdam).
577 https://doi.org/10.1016/j.scienta.2018.02.033
578 Sady, W., Strzelecki, P., Rożek, S., Smoleń, S., 2010. Effect of Differentiated Fertilization and Fo-
579 liar Application of Iodine on Yielding and Antioxidant Properties in Radish (*Raphanus sativus*
580 L.) Plants. Ecol. Chem. Eng. A 17, 1189–1196.
581 Saini, R.K., Ko, E.Y., Keum, Y.-S., 2017. Minimally processed ready-to-eat baby-leaf vegetables:
582 Production, processing, storage, microbial safety, and nutritional potential. Food Rev. Int. 33,
583 644–663. https://doi.org/10.1080/87559129.2016.1204614
584 Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H., 2002. Plant phenolic antioxidant and
585 prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxi-
586 cology 177, 67–80. https://doi.org/10.1016/S0300-483X(02)00196-8

587 Salachas, G., Savvas, D., Argyropoulou, K., Tarantillis, P.A., Kapotis, G., 2015. Yield and nutri-
588 tional quality of aeroponically cultivated basil as affected by the available root-zone volume.
589 Emirates J. Food Agric. 27, 911–918. https://doi.org/10.9755/ejfa.2015-05-233
590 Sarrou, E., Siomos, A.S., Riccadona, S., Aktsoglou, D.C., Tsouvaltzis, P., Angeli, A., Franceschi,
591 P., Chatzopoulou, P., Vrhovsek, U., Martens, S., 2019. Improvement of sea fennel (*Crithmum*
592 *maritimum* L.) nutritional value through iodine biofortification in a hydroponic floating sys-
593 tem. Food Chem. 296, 150–159. https://doi.org/10.1016/j.foodchem.2019.05.190
594 Schreiber, U., Bilger, W., Neubauer, C., 1995. Chlorophyll Fluorescence as a Nonintrusive Indica-
595 tor for Rapid Assessment of In Vivo Photosynthesis, in: Ecophysiology of Photosynthesis. pp.
596 49–70. https://doi.org/10.1007/978-3-642-79354-7_3
597 Sharma, R., Bhatia, S., Kaur, P., 2018. Influence of packaging and storage conditions on biochemi-
598 cal quality and enzymatic activity in relation to shelf life enhancement of fresh basil leaf. J.
599 Food Sci. Technol. 55, 3199–3211. https://doi.org/10.1007/s13197-018-3250-7
600 Smoleń, S., Baranski, R., Ledwożyw-Smoleń, I., Skoczylas, Ł., Sady, W., 2019. Combined biofor-
601 tification of carrot with iodine and selenium. Food Chem. 300, 125202.
602 https://doi.org/10.1016/j.foodchem.2019.125202
603 Smoleń, S., Kowalska, I., Sady, W., 2014. Assessment of biofortification with iodine and selenium
604 of lettuce cultivated in the NFT hydroponic system. Sci. Hortic. (Amsterdam). 166, 9–16.
605 https://doi.org/10.1016/j.scienta.2013.11.011
606 Smoleń, S., Ledwożyw-Smoleń, I., Halka, M., Sady, W., Kováčik, P., 2017. The absorption of io-
607 dine from 5-iodosalicylic acid by hydroponically grown lettuce. Sci. Hortic. (Amsterdam).
608 225, 716–725. https://doi.org/10.1016/J.SCIENTA.2017.08.009
609 Smoleń, S., Sady, W., 2012. Influence of iodine form and application method on the effectiveness
610 of iodine biofortification, nitrogen metabolism as well as the content of mineral nutrients and
611 heavy metals in spinach plants (*Spinacia oleracea* L.). Sci. Hortic. (Amsterdam). 143, 176–
612 183. https://doi.org/10.1016/J.SCIENTA.2012.06.006

Smoleń, S., Skoczylas, Ł., Ledwożyw-Smoleń, I., Rakoczy, R., Kopeć, A., Piątkowska, E., Bieżanowska-Kopeć, R., Pysz, M., Koronowicz, A., Kapusta-Duch, J., Pawłowski, T., 2016. Iodine and selenium biofortification of lettuce (*Lactuca sativa* L.) by soil fertilization with various compounds of these elements. Acta Sci. Pol. Hortorum Cultus 15, 69–91.

Smoleń, S., Wierzbińska, J., Sady, W., Kołton, A., Wiszniewska, A., Liszka-Skoczylas, M., 2015. Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (*Solanum lycopersicum* L.). Sci. Hortic. (Amsterdam). 188, 89–96. https://doi.org/10.1016/j.scienta.2015.03.023

Soffer, H., Burger, D.W., 1988. Effects of dissolved oxygen concentrations in aero- hydroponics on the formation and growth of adventitious roots. J. Amer. Soc. Hort. Sci. 113, 218–221.

Soffer, H., Burger, D.W., Lieth, J.H., 1991. Plant growth and development of Chrysanthemum and Ficus in aero-hydroponics: response to low dissolved oxygen concentrations. Sci. Hortic. (Amsterdam). 45, 287–294. https://doi.org/10.1016/0304-4238(91)90074-9

Strzetelski, P., Smoleń, S., Rożek, S., Sady, W., 2010. The effect of diverse iodine fertillization on nitrate accumulation and content of selected compounds in radish plants (*Raphanus sativus* L.). Hortorum Cultus 9, 65–73.

Sullivan, W.M., Jiang, Z., Hull, R.J., 2000. Root morphology and its relationship with nitrate uptake in Kentucky bluegrass. Crop Sci. 40, 765–772. https://doi.org/10.2135/cropsci2000.403765x

Tesi, R., Lenzi, A., Lombardi, P., 2003. Effect of salinity and oxygen level on lettuce grown in a floating system, in: Acta Horticulturae. pp. 383–387. https://doi.org/10.17660/ActaHortic.2003.609.58

Voogt, W., Holwerda, H.T., Khodabaks, R., 2010. Biofortification of lettuce (*Lactuca sativa* L.) with iodine: The effect of iodine form and concentration in the nutrient solution on growth, development and iodine uptake of lettuce grown in water culture. J. Sci. Food Agric. 90, n/a-n/a. https://doi.org/10.1002/jsfa.3902

639 Walters, K.J., Currey, C.J., 2015. Hydroponic Greenhouse Basil Production: Comparing Systems
640 and Cultivars. Horttechnology 25, 645–650.
641 Welburn, A.R., Lichtenthaler, H., 1984. Formulae and program to determine carotenoids and chlo-
642 rophyll a and b of leaf extracts in different solvents: Advances in photosyntesis research. Vol.
643 II. Martinus Njhorff/Dr. W.
644 Weng, H.X., Yan, A.L., Hong, C.L., Xie, L.L., Qin, Y.C., Cheng, C.Q., 2008. Uptake of different
645 species of iodine by water spinach and its effect to growth. Biol. Trace Elem. Res. 124, 184–
646 194. https://doi.org/10.1007/s12011-008-8137-4
647 Yosoff, S.F., Mohamed, M.T.M., Parvez, A., Ahmad, S.H., Ghazali, F.M., Hassan, H., 2015. Pro-
648 duction system and harvesting stage influence on nitrate content and quality of butterhead let-
649 tuce. Bragantia 74, 322–330. https://doi.org/10.1590/1678-4499.0453
650 Zhu, Y.G., Huang, Y.Z., Hu, Y., Liu, Y.X., 2003. Iodine uptake by spinach (*Spinacia oleracea* L.)
651 plants grown in solution culture: Effects of iodine species and solution concentrations. Envi-
652 ron. Int. 29, 33–37. https://doi.org/10.1016/S0160-4120(02)00129-0
653 Zimmermann, M.B., Jooste, P.L., Pandav, C.S., 2008. Iodine-deficiency disorders. Lancet 372,
654 1251–1262. https://doi.org/10.1016/S0140-6736(08)61005-3

Table 1. Timetable and environmental conditions related to the experiment.

Basil sowing date	16/05/2019	
Lettuce sowing date	23/05/2019	
Transplant date	05/06/2019	
Start of treatment	11/06/2019	
	Cut 1	Cut 2
Harvest date	19/06/2019	03/07/2019
Days of treatment	8	14
Mean air temperature (°C)	29.5	33.1
Mean air relative humidity (%)	58.5	53.0
Mean daily solar radiation (MJ m^{-2} day^{-1})	17.6	19.5
Cumulative solar radiation (MJ m^{-2})	140.5	273.2

Table 2. Fresh (FW) and dry (DW) shoot biomass, dry matter content (DW/FW), leaf area and the content of nitrates, phenols, chlorophylls and carotenoids in fresh leaves of basil plants grown under greenhouse in different hydroponic system (HS; floating system, FS, and aeroponics, AE) and iodine (I) concentrations in the nutrient solution: 0.07 μM (control) and 10.0 μM. Potassium iodide was used to increase the I concentration of the raw water. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut).

Cultivation system	Iodine concentration (μM)	CUT	FW (kg m^{-2})	DW (kg m^{-2})	DW/FW (%)	Leaf area (cm^2 $plant^{-1}$)	Pn (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Nitrates (mg kg^{-1} FW)	Total phenols (mg GAE kg^{-1} FW)	Chlorophylls (mg kg^{-1} FW)	Carotenoids (mg kg^{-1} FW)
FS	0.07	1st cut	2.31	0.194	8.38	213	13.09	8.77	1161	6447 a	1517 a	215.9
		2nd cut	3.08	0.326	10.55	1050	12.57	8.70	1490	5782 ab	1483 a	249.3
	10	1st cut	2.22	0.217	9.75	202	13.20	8.42	1408	6514 a	1514 a	252.1
		2nd cut	2.96	0.303	10.23	1053	12.67	8.27	1550	6638 a	1532 a	288.7
A	0.07	1st cut	2.30	0.195	8.48	262	13.31	7.58	1674	4501 c	1183 c	209.0
		2nd cut	3.07	0.280	9.12	1012	12.78	7.28	2010	5171 bc	1461 a	254.0
	10	1st cut	2.16	0.198	9.18	249	14.27	8.65	1728	4577 c	1326 b	209.1
		2nd cut	2.95	0.284	9.61	999	13.70	8.55	2074	6603 a	1561 a	266.2
Significance												
	HS		ns	ns	*	ns	ns	ns	***	ns	***	ns
	KI		ns	ns	ns	ns	ns	ns	ns	*	*	ns
	Cut		***	***	*	***	ns	ns	***	ns	***	**
	HS x I		ns	ns	ns	ns	ns	ns	ns	**	ns	ns
	I x Cut		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	HS x Cut		ns	ns	ns	ns	ns	ns	ns	*	***	ns
	HS x I x Cut		ns	ns	ns	ns	ns	ns	ns	*	**	ns

Letters following the means (n=3) denote the significant differences regarding the interaction of all tested factors after Duncan's test for P = 0.05. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Table 3. Fresh (FW) and dry (DW) shoot biomass, dry matter content (DW/FW), leaf area and the content of nitrates, phenols, chlorophylls and carotenoids in fresh leaves of lettuce plants grown under greenhouse in different hydroponic system (HS) (floating system, FS, and aeroponics, AE) and iodine (I) concentrations (control: 0.07 µM) in the nutrient solution: 0.07 µM (control) and 10.0 µM. Potassium iodide was used to increase the I concentration of the raw water. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut).

Cultivation system	Iodine concentration (µM)	CUT	FW (kg m^{-2})	DW (kg m^{-2})	DW/FW (%)	Leaf area (cm^2 $plant^{-1}$)	Pn (µmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Nitrates (mg kg^{-1} FW)	Total phenols (mg GAE kg^{-1} FW)	Chlorophylls (mg kg^{-1} FW)	Carotenoids (mg kg^{-1} FW)
FS	0.07	1st cut	1.62 e	0.085d	5.29	178.7 d	13.64	9.05	2956	3874	1062 a	220 a
		2nd cut	1.33 e	0.096d	7.00	192.8 d	13.09	8.95	2893	2474	847 ab	178 ab
	10	1st cut	1.69de	0.091d	5.39	177.8 d	12.84	8.52	2252	4666	1088 a	227 a
		2nd cut	0.50 f	0.029e	5.56	78.9 e	12.32	8.48	2961	3826	772 b	83 c
A	0.07	1st cut	2.55 c	0.166c	6.50	255.4 c	12.83	7.95	1405	2974	739 b	146 bc
		2nd cut	5.56 a	0.358a	6.39	476.8 a	12.32	7.80	2161	3501	953 ab	201 ab
	10	1st cut	2.48cd	0.166c	6.73	245.2 c	14.57	8.55	1563	2810	719 b	138 bc
		2nd cut	4.14 b	0.249b	5.95	377.3 b	13.99	8.45	2207	3786	971 ab	141 bc
Significance												
HS			***	***	ns	***	ns	ns	***	ns	ns	ns
KI			*	*	ns	***	ns	ns	ns	ns	ns	*
Cut			***	**	ns	***	ns	ns	**	ns	ns	ns
HS x I			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
I x Cut			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
HS x Cut			***	***	ns	***	ns	ns	ns	*	***	**
HS x I x Cut			***	***	ns	***	ns	ns	ns	ns	*	**

Letters following the means (n=3) denote the significant differences regarding the interaction of all tested factors after Duncan's test for P = 0.05. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

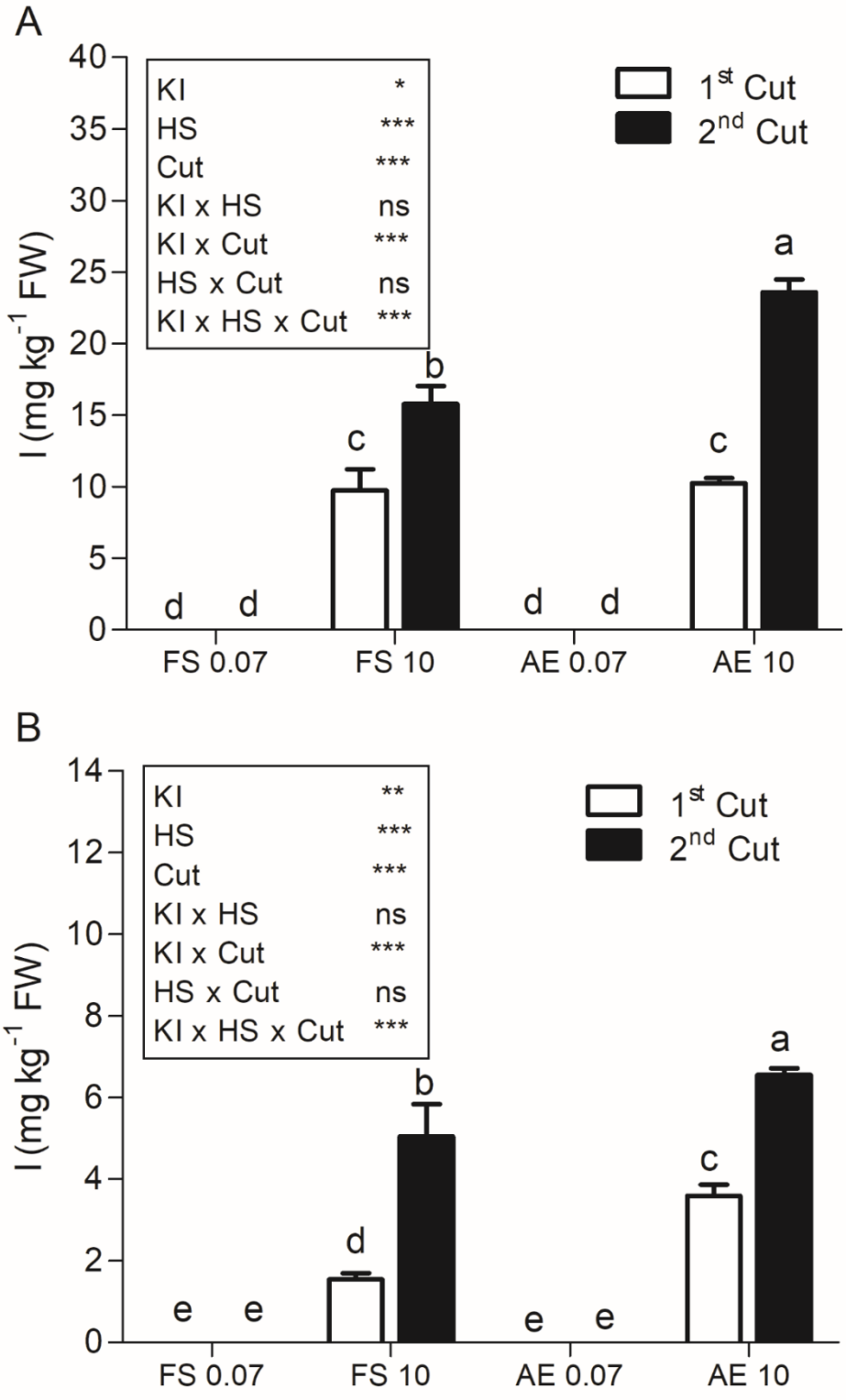

Figure 1. Leaf iodine content in basil (A) and lettuce (B) plants grown under greenhouse conditions with different hydroponic system (HS) (floating system, FS, and aeroponics, AE) and potassium iodide (KI) concentrations (0.07 (control) and 10 μM and) in the nutrient solution. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut). The I concentration in control samples was below the limit of detection.

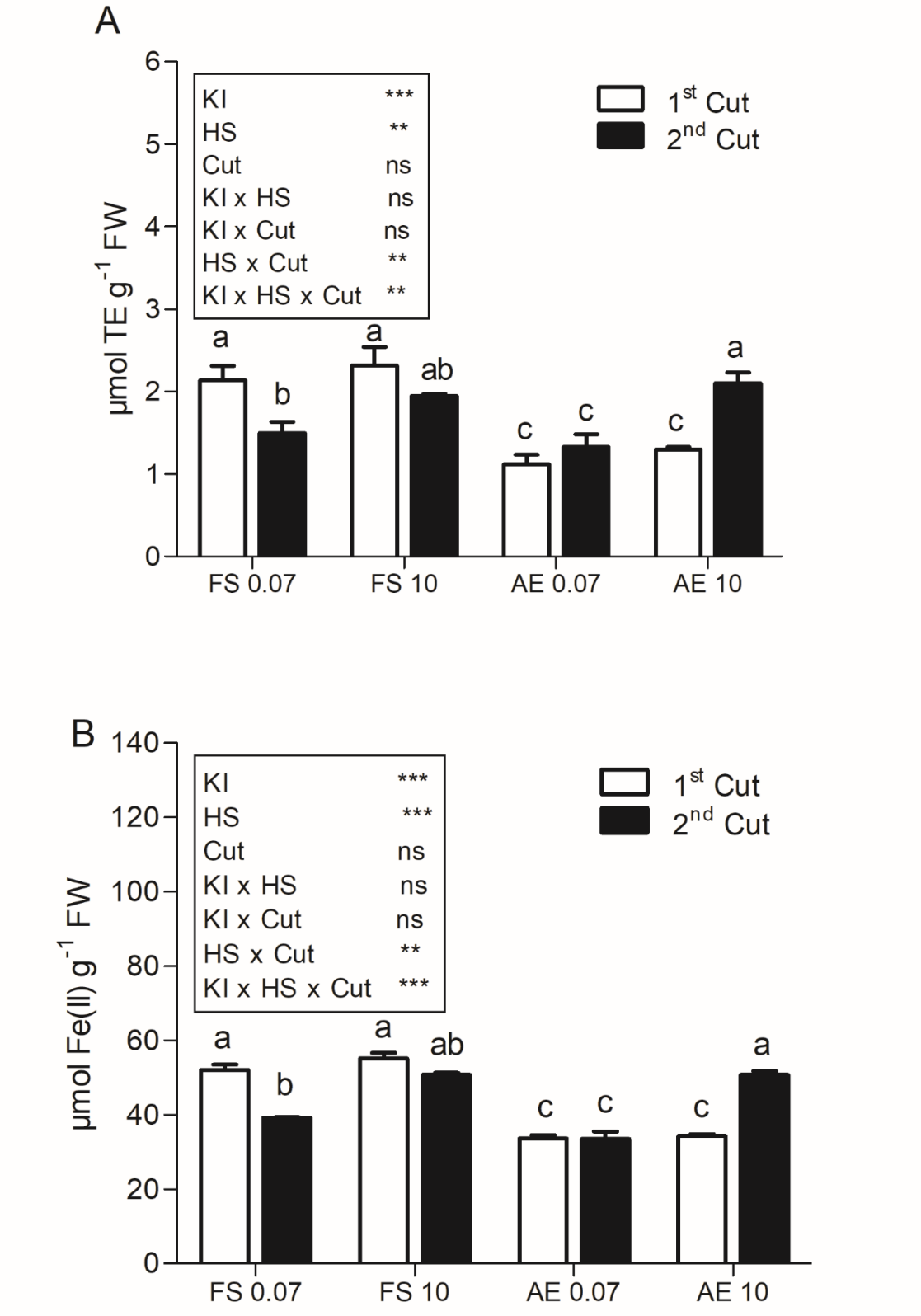

Figure 2. Antioxidant capacity, expressed as µmol Trolox Equivalents g^{-1} FW (DPPH assay) (A) and µmol Fe(II) g^{-1} FW (FRAP assay) (B), of basil plants grown under greenhouse conditions with different hydroponic system (HS) (floating system, FS, and aeroponics, AE) and potassium iodide (KI) concentrations (0.07 (control) and 10 µM) in the nutrient solution. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut).

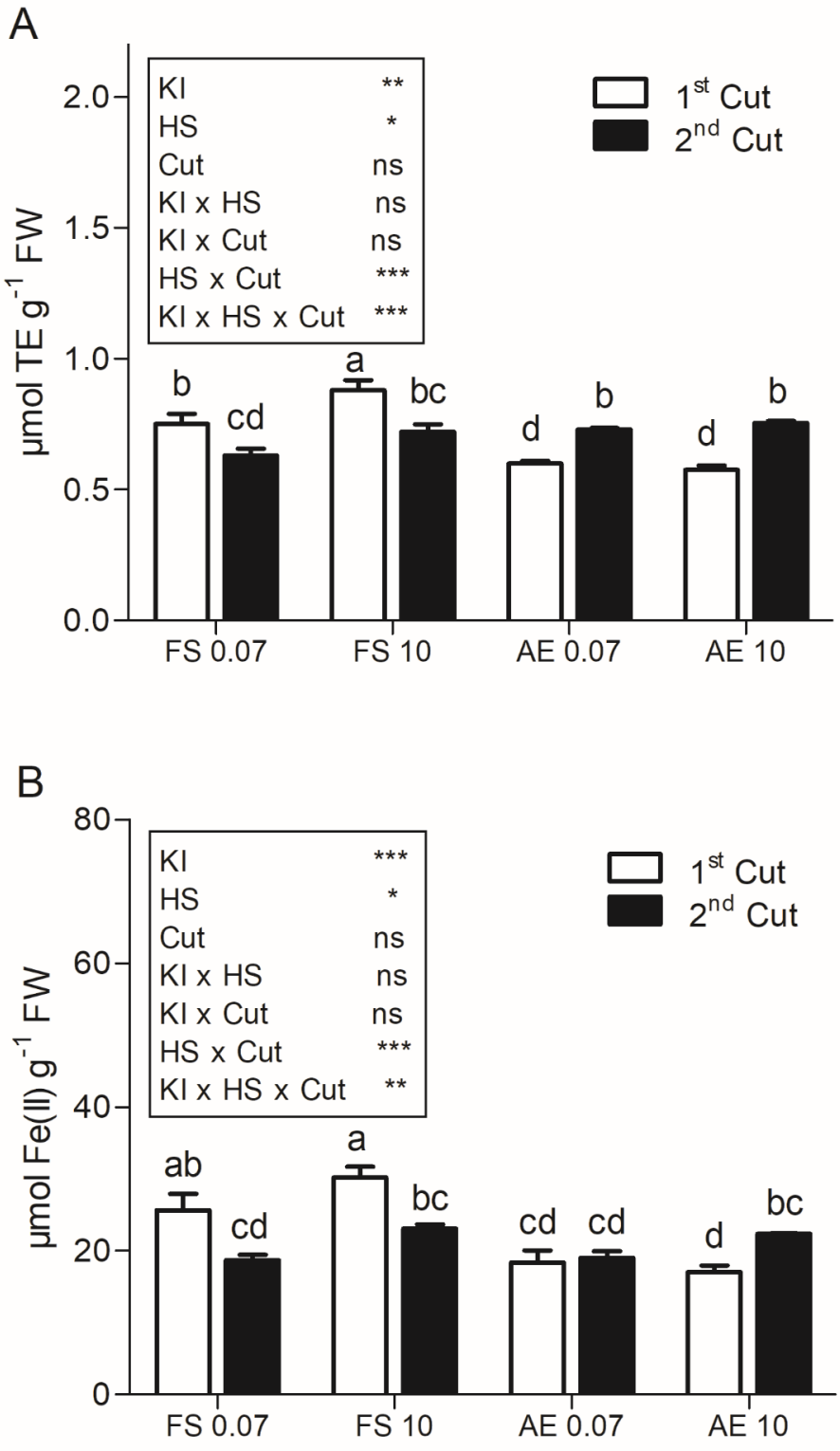

Figure 3. Antioxidant capacity, expressed as µmol Trolox Equivalents g^{-1} FW (DPPH assay) (A) and µmol Fe(II) g^{-1} FW (FRAP assay) (B), of lettuce plants grown under greenhouse conditions with different hydroponic system (HS) (floating system, FS, and aeroponics, AE) and potassium iodide (KI) concentrations (0.07 (control) and 10 µM) in the nutrient solution. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut).

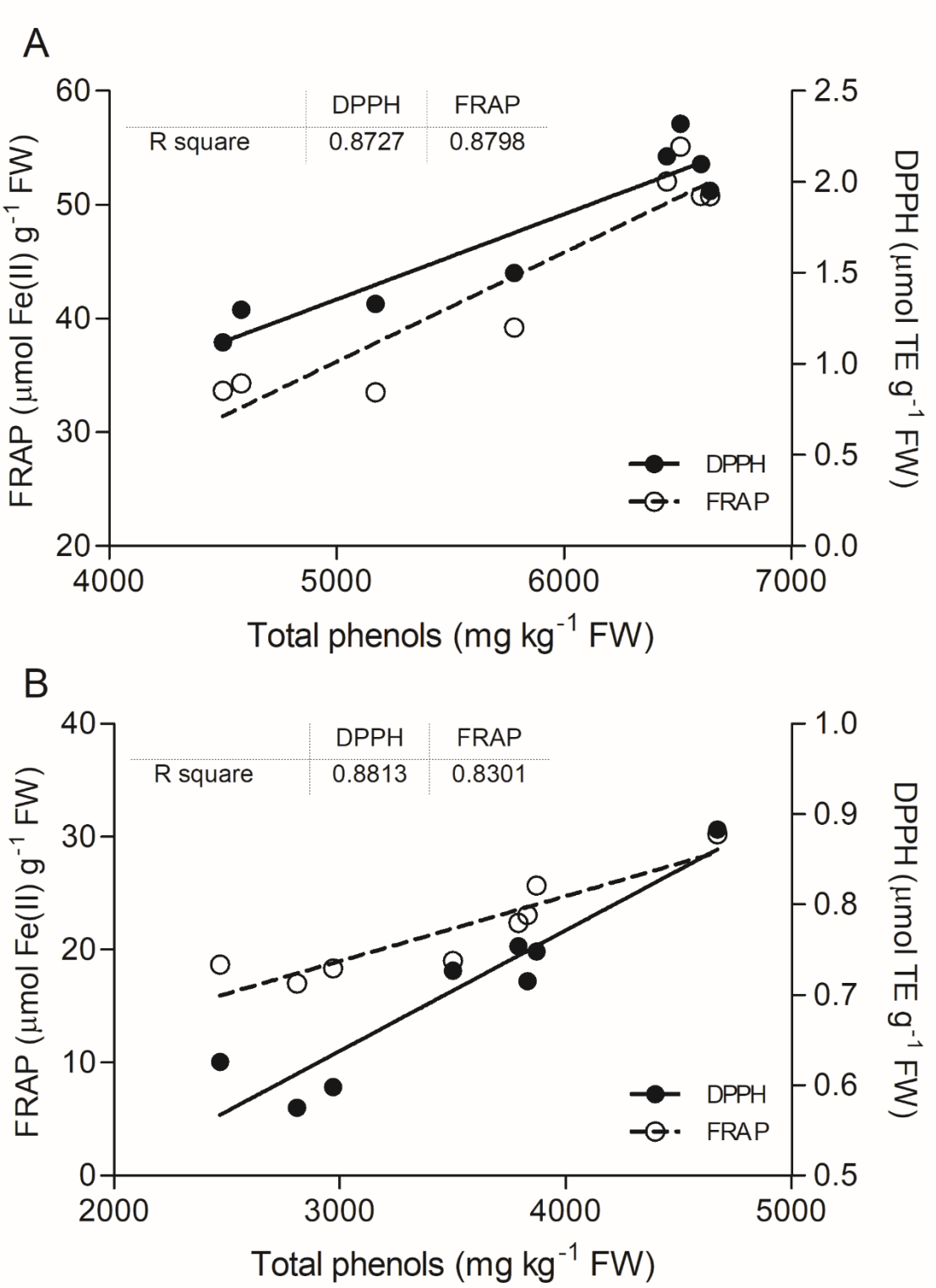

Figure 4. Results of the linear regression between antioxidant capacity (measured by FRAP or DPPH assay) and total phenol content in leaves of basil (A) and lettuce (B) plants grown under greenhouse conditions with different hydroponic system (HS) (floating system, FS, and aeroponics, AE) and potassium iodide (KI) concentrations (control: 0.07 µM) in the nutrient solution. The plants were harvested 8 days after the start of treatment (1st cut) and 14 days later from subsequent regrowth (2nd cut).