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Iodine Enhances the Nutritional Value but Not the Tolerance of Lettuce to NaCl

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Abstract: Positive stress or essential and nonessential elements can improve nutritive values (biofortification) of edible plants. In the present study, we evaluate (i) the effect of moderate salinity on lettuce biofortification, evaluated as nutritional bioactive compound accumulation, and (ii) the role of iodine in enhancing salt tolerance by increasing photorespiration and the content of antioxidants in lettuce. Physiological (gas exchange and chlorophyll fluorescence emission) and biochemical (photosynthetic pigment and bioactive compound) analyses were performed on lettuce plants grown under moderate salinity (50 mM NaCl alone or 50 mM NaCl in combination with iodine, KIO_3). Our results show that NaCl + iodine treatment improves the nutritional value of lettuce in terms of bioactive compounds acting as antioxidants. More specifically, iodine enhances the accumulation of photosynthetic pigments and polyphenols, such as anthocyanins, under salt but does not improve the salt tolerance. Our findings indicate that iodine application under moderate salinity could be a valid strategy in plant biofortification by improving nutritional bioactive compound accumulation, thus exercising functional effects on human health.

Keywords: nonessential elements; moderate salinity; biofortification; gas exchange; *Lactuca sativa* L.

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1. Introduction

Biofortification consists in developing crops with bioavailable micronutrients in edible parts [1,2], and generally it can be achieved by breeding, agronomic, and transgenic approaches [3]. The application of positive stress (eustress) also can trigger an accumulation of bioactive compounds in edible plants [4].

Salinity, in particular an elevated salt concentration, may affect plant growth in several ways [5–8], negatively affecting the crop yield. However, at low or moderate salt concentration, yields are mildly affected or not affected at all [9], and nutritive and/or bioactive compounds can be accumulated [10,11]. Santander et al. [12] found a greater phenolic concentration and antioxidant activity in lettuce plants grown under moderate salinity (50 mM NaCl) with no effect on photosynthetic activity and plant biomass; however, severe salinity (150–200 mM NaCl) reduced the antioxidant capacity and plant biomass. An improvement of functional compounds was also found in *Perilla frutescens*, a novel food, exposed from mild to moderate salinity conditions [13]. Thus, appropriate irrigation management of horticultural crops through a mild–moderate salinity could be a means to enhance the nutraceutical value of crops because a salt-induced reshuffling of plant metabolism would increase the accumulation of nutritional bioactive compounds, thus enriching the functional quality of fresh vegetables with positive outcomes on human wellbeing.

Biofortification of vegetables to increase the mineral concentration in edible organs can also be achieved by adding essential and nonessential beneficial micronutrients [3], generally via mineral fertilizers (agronomic fortification). Several studies on mineral biofortification have been carried out in the past [14], and recently the attention has been focused on the use of iodine (I). For a long time, this element has been considered a nonessential nutrient for plants; however, now it has gained a rising interest because recent research has shown evidence for a nutritional role of iodine in vegetables [15]. Plants can utilize two iodine forms: iodide (I^-) and iodate (IO_3). Owing to their ability to absorb and accumulate exogenous iodine into the edible organs, horticultural crops are the best candidates to test the outcomes of I biofortification [16]. To date, it is not well understood which form of iodine is more adequate in inducing valuable results on plant biomass or inducing plant biofortification; however, it is ascertained that iodine concentrations ranging from 10^{-6} to 10^{-4} M exert positive outcomes [17].

It has been demonstrated that iodine can improve salt tolerance in plants [18], depending on variables such as the sources of iodine, their concentration, and type of application. Salt tolerance is obtained by an increase in antioxidant content, and strictly depends on the species and the form of the ion that is applied. The last aspect is very interesting because negative effects have been reported in plants fertilized with I^-, whereas positive results were derived from the application of $IO_3{}^-$ [19]. In particular, the growth with $IO_3{}^-$ improves nitrogen metabolism and photorespiration in lettuce plants [20].

In the present study, we evaluate, through combined physiological and biochemical analyses: (i) the effect of the moderate salinity on the biofortification of lettuce, a species largely cultivated and consumed for human diet worldwide, assessing the nutritional bioactive compounds accumulation; (ii) the role of $IO_3{}^-$ in improving the salt tolerance and promoting a higher antioxidant–bioactive compound accumulation. Based on the findings of Blasco et al. [20], we hypothesize that the addition of iodine to soil leads to an increase in photorespiration and antioxidant content in lettuce plants, thereby enhancing their tolerance to moderate salinity.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seedlings of lettuce cv Bionda liscia were transplanted 20 days after sowing (DAS) in 0.5 L pots filled with peat soil (90% peat and 10% sand, organic carbon content 31% *w*/*w*, C/N 25) and grown during autumn–winter 2021–2022. After one week from transplanting, plants were subjected to three treatments: (i) fertilization with nutrient solution (Control, EC: 1.8 dS m^{-1}, pH: 7.5), (ii) fertilization with nutrient solution added with NaCl (3 g L^{-1}, 52 mM, EC: 7.0 dS m^{-1}, pH: 7.5), and (iii) fertilization with nutrient solution added with NaCl (3 g L^{-1}, 52 mM, EC: 7.0 dS m^{-1}, pH: 7.5) plus KIO_3 (0.09 mg L^{-1}, 53 µg I). Plants were grown in a greenhouse equipped with a white LED illumination system (T5-60, 14-Watt LED T5 tube, Driwei) at the following growth conditions: 100 µmol photons m^{-2} s^{-1} at the top of canopy and 16 h/8 h light/dark photoperiod. Plants were fertilized weekly—for a total of 6 weeks—until the harvest by supplying 50 mL nutrient solution in which NaCl or NaCl + KIO_3 were dissolved. The control received only the nutrient solution (2 g L^{-1}) with the following mineral composition: total N 20%, N-NO_3 3.6%, N-NH_4 3.6%, N-CH_4N_2O 12.1%, P_2O_5 40%, K_2O 20%, B 0.05%, Cu 0.02%, Fe 0.4%, Mn 0.2%, Mo 0.02%, and Zn 0.02%. Each treatment consisted of 5 plants in three replicates. The plants were harvested 60 DAS, when leaves were moderately expanded. The harvest time was chosen based on a previous study [21], which demonstrated that a high post harvest quality is expected when fresh-cut lettuce presents moderately expanded leaves. At the harvesting, biometrical, ecophysiological, and biochemical determinations were carried out on leaves of different plants. The fresh vegetable quality was evaluated in terms of nutritional bioactive compounds such as: chlorophylls, carotenoids, polyphenols (flavonoids and anthocyanins), and antioxidant capacity. Soluble proteins content was also determined.

Electrical conductivity (EC) and pH were measured at harvesting in a 1:5 soil:distilled water suspension by means of a conductivity meter (Portlab 203) and pH meter (XS Instruments).

2.2. Leaf Gas Exchange and Chlorophyll a Fluorescence Measurement

A Li6400 portable photosynthesis system (Licor Inc., Lincoln, NE, USA) was used to perform simultaneous gas exchange and chlorophyll *a* (Chl a) fluorescence measurement on mature leaves. Measurements were carried out at 400 µmol photons m^{-2} s^{-1}, 25 °C ± 2, RH 45% ± 5, and CO_2 of 400 ppm. To evaluate the potential of photosynthesis and the occurrence of processes other than CO_2 fixation and photorespiration, gas exchange and Chl *a* fluorescence measurements were also measured at elevated CO_2 concentration (800 ppm). Gas exchange parameters: net photosynthesis (A_N), stomatal conductance (G_s), intercellular to ambient CO_2 ratio (C_i/C_a), instantaneous water use efficiency (ratio between net photosynthesis and transpiration—A_N/T_r), were calculated by software operating in the instrument following the equation of von Caemmerer and Farquhar [22]. The fluorescence parameters in the light quantum yield of noncyclic electron transport (Φ_{PSII}) were calculated by Genty et al. [23], while the quantum yield of regulated (Φ_{NPQ}) and non-regulated energy dissipation (Φ_{NO}) were calculated according to Kramer et al. [24]. The total electron transport rate (J_f) was determined as reported in Kral and Edwards [25]), and the electron transport rate to CO_2 (J_c) and to O_2 (J_o) was calculated according to Epron et al. [26] as:

$$J_c = 1/3\ [J_f + 8(A_N + R_d)] \quad (1)$$

$$J_o = 2/3\ [J_f - 4(A_N + R_d)] \quad (2)$$

Dark respiration (R_d) and maximum photochemical efficiency (F_v/F_m) were measured on 30 min dark-adapted leaves following the measurements in the light. Leaves were darkened through an aluminum sheet placed around the leaf, allowing air to circulate.

2.3. Photosynthetic Pigments

The content of total chlorophylls and carotenoids was evaluated following the procedure reported by Lichtenthaler [27]. Pigments were extracted in 100% ice-cold acetone and quantified by measuring absorbance at the wavelengths of 470, 645, and 662 nm. The concentration was expressed in µg cm^{-2}.

2.4. Polyphenols and Soluble Proteins

For all the following assays, samples were preventively powdered with liquid nitrogen and stored at −80 °C until further analysis.

To estimate the total polyphenol content, samples (0.02 g) were extracted in 2 mL of 80% aqueous methanol following the procedure reported in Vitale et al. [28]. The extracts were kept for 1 h at 4 °C and then centrifuged at 11,000 rpm for 5 min. An aliquot (274 µL) was mixed with 274 µL of the Folin Ciocolteau reagent and 1.452 mL of 700 mM sodium carbonate (Na_2CO_3). Samples were incubated for 2 h in darkness. The absorbance was read at 765 nm and the concentration was calculated and expressed as gallic acid equivalent in mg GAE g^{-1} fresh weight (FW) using a gallic acid standard curve.

Total flavonoid content was quantified according to Moulehi et al. [29] and Sun et al. [30]; 250 µL of a diluted methanol sample was added to 75 µL of 5% $NaNO_2$ (sodium nitrite, 150 µL of 10% $AlCl_3$ (aluminum chloride), and 500 µL NaOH (1 M). Then, the mixture was adjusted with distilled water to a final volume of 1.525 mL. After absorbance determination at 510 nm, the flavonoid content was determined using a catechin standard curve and expressed as mg catechin equivalent per gram of fresh weight (mg CE g^{-1}FW).

The anthocyanin content was analyzed according to Mancinelli et al. [31] and Chung et al. [32]. Samples (0.05 g) were extracted with acidified methanol (1% HCl) for 24 h at 4 °C in the dark. Samples were centrifuged and the supernatants were measured spectrophotometrically at 530 and 657 nm. The extinction coefficient of 31.6 M^{-1} cm^{-1}

was used to convert absorbance values into concentrations of anthocyanins, using the following equation: anthocyanin content (μmol g^{-1}) = [(A_{530} − 0.33 × A_{657})/31.6] × [volume (mL)/weight (g)].

The antioxidant capacity was evaluated by the ferric-reducing antioxidant power (FRAP) assay according to George et al. [33] and Vitale et al. [34]. Samples (0.250 g) were extracted with 60:40 (*v*/*v*) methanol/water solution. After centrifugation, an aliquot of extract (150 μL) was mixed with the FRAP reagents (2.5 mL of 300 mM acetate buffer pH 3.6, 250 μL of 10 mM tripyridyltriazine (TPTZ) and 250 μL of 12 mM $FeCl_3$). After the sample incubation for 1 h in the darkness, the absorbance was read at 593 nm. The total antioxidant capacity was quantified as μmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per gram of fresh weight (μmol TE g^{-1} FW) using the Trolox a standard curve.

Total soluble protein content was determined following Bradford [35] and Im et al. [36]. Briefly, samples (0.200 g) were extracted in 0.2 M potassium phosphate buffer (pH 7.8 + 0.1 mM EDTA) and centrifuged. The supernatant was added to the dye reagent, and the absorbance was read at 595 nm. The total soluble proteins were quantified using a bovine serum albumin (BSA) calibration curve and expressed as mg BSA eq g^{-1} FW.

2.5. Statistical Analysis

The statistical software SigmaPlot 12.0 was used to perform graphics and statistical analysis. Data were analyzed by one-way ANOVA followed by the Holm–Sidak post hoc test. Data shown in tables and figures are means ± standard error (SE).

3. Results

3.1. Plant Growth

Mineral fertilization added with salt (NaCl) did not alter soil pH but increased the electrical conductivity, as compared to the control (Table 1).

Table 1. pH and electrical conductivity (EC) in soil at the harvesting of control plants, and plants fertilized with nutrient solution added with salt (NaCl) or with salt plus iodine (NaCl + KIO_3). Data are means ± SE.

Parameters	Control	NaCl	NaCl + KIO_3
pH_{H2O}	7.28 ± 0.03 [a]	7.16 ± 0.03 [a]	7.18 ± 0.01 [a]
EC (dS m^{-1})	1.80 ± 0.00 [a]	6.70 ± 0.05 [b]	6.55 ± 0.13 [b]

Different letters indicate significant differences among treatments. $p < 0.05$.

Salt did not affect the plant growth (Table 2). The shoot biomass and the number of leaves were comparable among the treatments and no visible difference in plant appearance was observed.

Table 2. Main biometrical characteristics at the harvesting of control plants, and plants fertilized with nutrient solution added with salt (NaCl) or with salt plus iodine (NaCl + KIO_3). Data are means ± SE.

Parameters	Control	NaCl	NaCl + KIO_3
Shoot (g FW p^{-1})	36.60 ± 1.40 [a]	36.00 ± 1.05 [a]	34.20 ± 1.39 [a]
N° leaves	35.80 ± 1.11 [a]	34.20 ± 0.58 [a]	34.40 ± 0.51 [a]

Different letters indicate statistically significant differences among treatments. $p < 0.05$.

3.2. Bioactive Compounds and Soluble Proteins

The chlorophyll and carotenoid content was significantly affected by the treatment with salt plus iodine but not by the salt alone (Table 3). The application of only NaCl caused a 74% reduction in total polyphenols, but increased the anthocyanin content and the FRAP antioxidant capacity by 95% and 21%, compared to control, respectively. (Table 3). The NaCl + KIO_3 treatment improved the total polyphenol content in lettuce leaves compared

to NaCl treatment, and produced a higher anthocyanin accumulation in leaves. Conversely, the combination NaCl + KIO_3 induced a reduction in antioxidant capacity by 42% compared to control and by 52% compared to NaCl treatments. No effect on soluble proteins and flavonoids was observed among the treatments.

Table 3. Chemical composition of control plants and plants fertilized with nutrient solution with salt (NaCl) added or with salt plus iodine (NaCl + KIO_3). Data are means ± SE.

Parameters	Control	NaCl	NaCl + KIO_3
Chlorophylls (a + b) (μg cm^{-2})	49.78 ± 0.92 [a]	53.02 ± 2.52 [a]	64.68 ± 2.76 [b]
Carotenoids (x + c) (μg cm^{-2})	10.03 ± 0.13 [a]	10.97 ± 0.46 [a]	12.99 ± 0.40 [b]
Total polyphenols (mg GAE g^{-1} FW)	0.42 ± 0.08 [c]	0.11 ± 0.02 [a]	0.18 ± 0.02 [b]
Anthocyanins (μmol g^{-1} FW)	0.021 ± 0.006 [a]	0.041 ± 0.003 [b]	0.119 ± 0.007 [c]
Flavonoids (mg CE g^{-1} FW)	6.78± 0.42 [a]	6.31± 0.34 [a]	6.16± 0.20 [a]
Antioxidant capacity (μmol TE g^{-1} FW)	0.43 ± 0.01 [a]	0.52 ± 0.02 [b]	0.25 ± 0.01 [c]
Soluble proteins (mg BSA eq g^{-1} FW)	1.61± 0.07 [a]	1.47± 0.05 [a]	1.56± 0.03 [a]

Different letters indicate significant difference among treatments. $p < 0.05$.

3.3. Leaf Gas Exchange and Chl a Fluorescence Measurement

Salt treatments did not affect leaf gas exchange both at ambient and at elevated CO_2 concentration (Figure 1). According to the limitation imposed on photosynthesis by the current CO_2 concentration in atmosphere, the net photosynthesis (A_N) measured under elevated CO_2 was significantly greater than under ambient CO_2 (Figure 1a), promoting a greater instantaneous water use efficiency (Figure 1d). Under ambient CO_2 concentration, salt treatments determined a significant increase in Φ_{PSII} as compared to control (Figure 2a). The stimulation of Φ_{PSII} by salt treatments induced a lower thermal dissipation (Φ_{NPQ}) of absorbed light (Figure 2b) but no change in nonregulated energy dissipation (Φ_{NO}) (Figure 2c). Under elevated CO_2 the photochemical efficiency (Φ_{PSII}) reflected the behavior of photosynthesis, resulting higher under elevated than under ambient CO_2 concentration and was not affected by salt treatments (Figure 2a), as observed for Φ_{NPQ} and Φ_{NO}. Under ambient CO_2, a higher J_f/A_G ratio was measured in response to salt treatments compared to the control (Figure 2d). This result indicates an increase in electron flow toward O_2 reduction (photorespiration), in contrast to CO_2 reduction (photosynthesis) (Figure 2e,f), which significantly declined under both NaCl and NaCl + KIO_3 treatments.

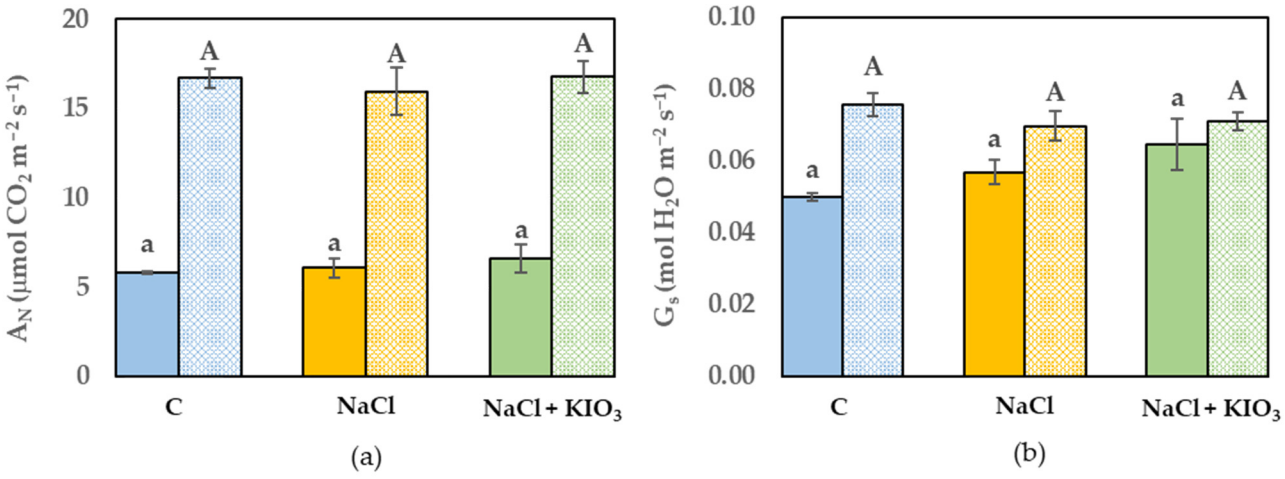

Figure 1. *Cont.*

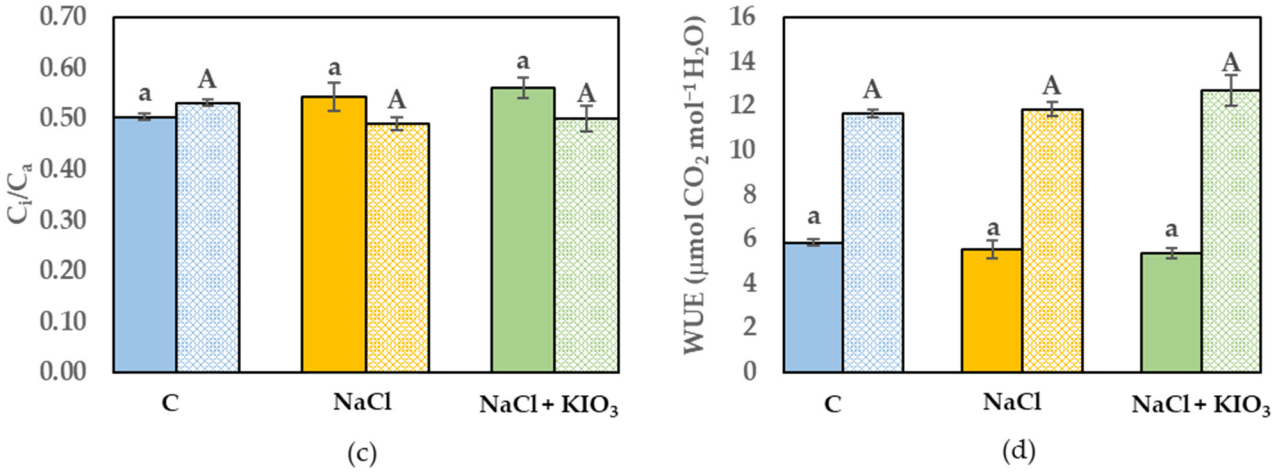

Figure 1. (**a**) Net photosynthesis (A_N); (**b**) stomatal conductance (G_s); (**c**) intercellular to ambient CO_2 concentration ratio (C_i/C_a); (**d**) instantaneous water use efficiency (WUE) measured in plants fertilized with nutrient solution (C), in plants fertilized with nutrient solution *plus* salt (NaCl), and in plants fertilized with nutrient solution *plus* salt and iodine (NaCl + KIO_3). Filled bars: measurements to ambient CO_2; cross-linked bars: measurements to elevated CO_2. Data are means ± SE. Different letters indicate statistical difference among treatments ($p < 0.05$).

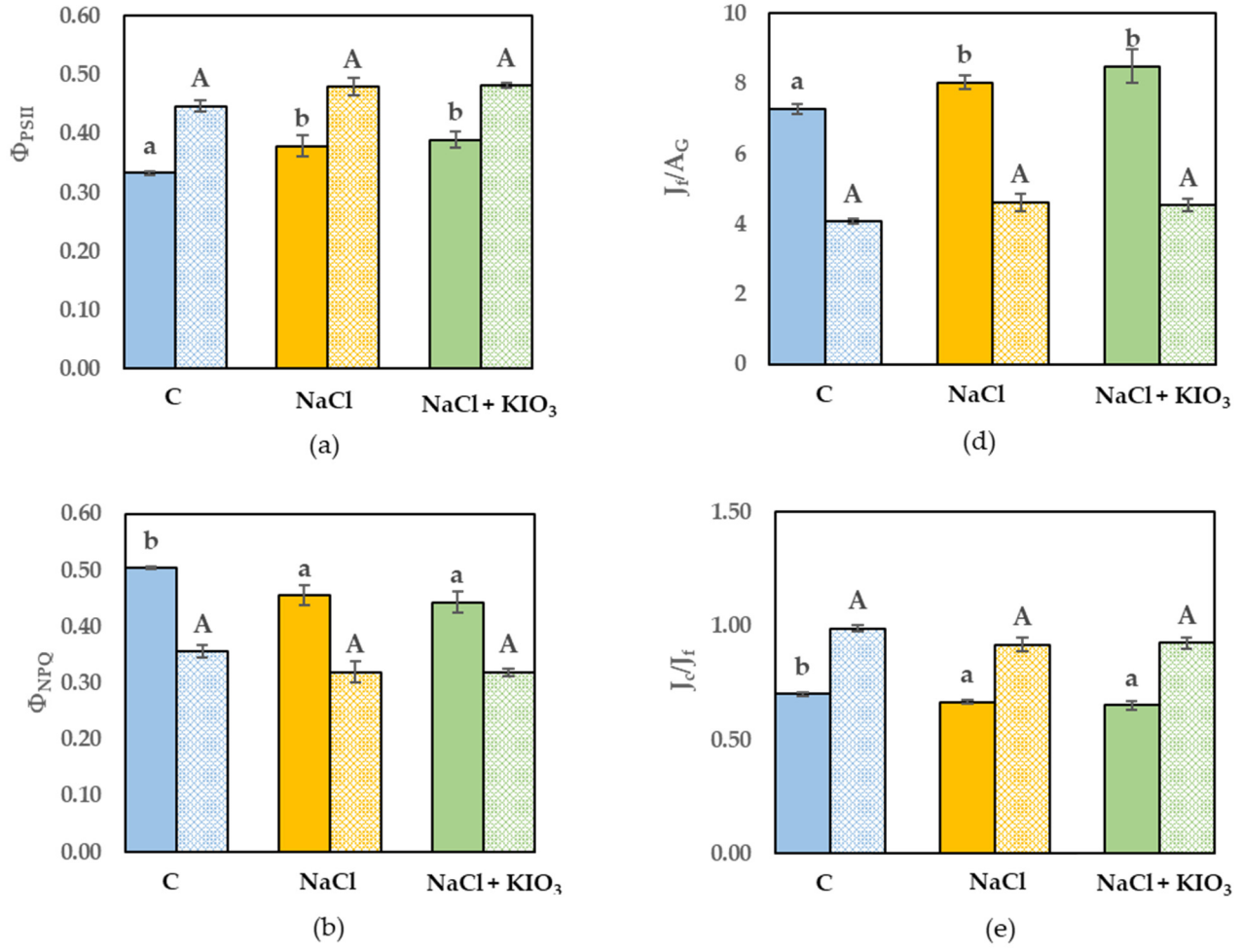

Figure 2. *Cont*.

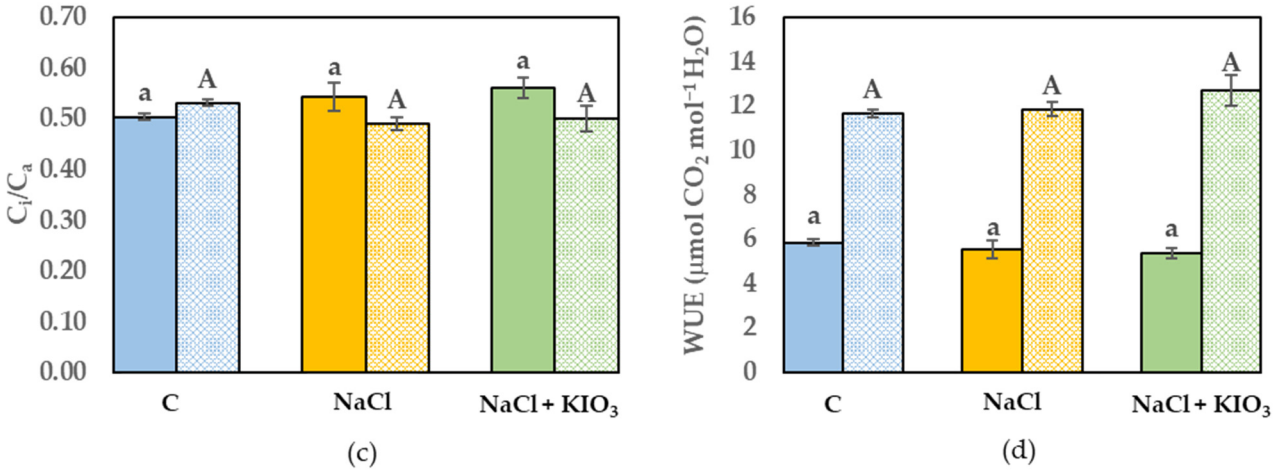

Figure 2. (**a**) Quantum yield of noncyclic electron transport (Φ_{PSII}); (**b**) quantum yield of regulated energy dissipation (Φ_{NPQ}); (**c**) quantum yield of nonregulated energy dissipation ((Φ_{NO}); (**d**) electron transport rate to gross photosynthesis ratio (J_f/A_G); (**e**) electron transport rate to CO_2 and total electron transport rate ratio (J_c/J_f); (**f**) electron transport rate to O_2 and total electron transport rate ratio (J_o/J_f) measured in plants fertilized with nutrient solution (C), in plants fertilized with nutrient solution *plus* salt (NaCl), and in plants fertilized with nutrient solution *plus* salt and iodine (NaCl + KIO_3). Filled bars: measurements to ambient CO_2; cross-linked bars: measurements to elevated CO_2. Data are means ± SE. Different letters indicate statistical difference among treatments ($p < 0.05$).

4. Discussion

4.1. Nutritional Value of Crops

In agricultural practices, many approaches have been utilized aimed to enhance plant growth and nutritional value of crops, such as mild abiotic stress and the use of essential and nonessential elements [4,37,38].

In the present research, it was found that moderate salt levels do not modify the growth of lettuce plants, but improve their nutraceutical value, depending on the imposed salt treatment. Our findings confirm previous studies which demonstrated that eustress, such as the imposition of a mild to moderate salinity, may enhance the organoleptic components of quality in vegetables [12,39]. In our study, the vegetable quality was evaluated in terms of bioactive substances acting as antioxidants. Iodine increased the nonenzymatic antioxidants under moderate salinity, leading to an accumulation of phenolic compounds and, in particular, anthocyanins, more than under saline treatment alone. It is hypothesized that the combination NaCl + KIO_3 may induce an enhancement of enzymes involved in polyphenol synthesis [14]. Similar results have been previously found in lettuce plants [32] biofortified with KIO_3 at <80 μM, a concentration comparable to that used in our study. These bioactive compounds would act as antioxidants in preventing oxidative damages, and it is likely to suppose that the increase in polyphenols and, in particular, anthocyanins, was associated with plant photoprotection, because a significant increase in chlorophyll and carotenoid content occurred in plants fertilized with iodine under moderate salinity. Our results are consistent with the findings of Medrano Macías et al. [19]. It has been previously demonstrated that anthocyanin synthesis is activated by salt stress [40]; however, our results indicated that it can be potentiated by iodine. Our results also indicated that lettuce plants grown under NaCl treatment supplemented with iodine invested more energy in anthocyanin synthesis compared to the control and NaCl plants. The investment of plants in anthocyanins may have reduced the energy at disposal for the synthesis of other secondary metabolites acting as antioxidants, inducing the significant decline observed for the antioxidant capacity measured in plants fertilized with IO_3^-. On the contrary, the lowest energy engaged in anthocyanin synthesis in plants grown with salt without iodine likely allowed a greater synthesis of antioxidants, enhancing the antioxidant capacity.

Our data also indicated that iodine exerted some effect on lettuce nitrogen metabolism. According to Blasco et al. [41] which reports an enhanced nitrogen assimilation and protein synthesis in plants treated with IO_3^-, the increase in total chlorophyll content in lettuce plants suggests that nitrogen is assimilated in organic compounds that serve as a N donor for their synthesis.

4.2. Stress Tolerance

In our experiment, salt did not influence leaf gas exchange, evidencing that a moderate salt level may trigger plant biofortification without detrimental effects on crop yield and plant shape and architecture. Similarly, no unfavorable effect on net photosynthesis in NaCl + KIO_3 plants was observed, confirming that low iodine amounts (<80 μM) are not toxic for plants [42]. Once absorbed, iodine is translocated to chloroplasts via phloem, where it exerts its effects on photosynthesis [43]. Phenolic compounds can bind iodine through an electrophilic H substitution in the aromatic ring [44], likely protecting the photosynthetic apparatus from the mineral toxicity.

Photosynthesis is compromised by salt, generally provided at high concentration in salt-sensitive plants. On the contrary, salt stress-tolerant plants activated protective responses to overcome this form of abiotic stress [8]. In both salt treatments, salt induces a greater partitioning of electrons of the photosynthetic electron chain toward processes other than CO_2 assimilation, as indicated by the greater J_f/A_G ratio compared to the unstressed treatment. Our data showed that the J_f/A_G ratio in lettuce plants ranges from 4 to 5 under elevated CO_2, a typical value indicating a nonphotorespiratory condition for photosynthetic machinery and, in turn, a negligible electron flow to O_2 as an alternative acceptor to CO_2. Salt, alone or in combination with iodine, induces an increase in the electron transport flow toward photorespiration (J_o/J_f), which becomes the main photochemical process other than CO_2 assimilation in dissipating the absorbed light under stress. The increase in photorespiration determines a reduction in regulated thermal dissipation (Φ_{NPQ}) sustained by the xanthophyll cycle, avoiding the rise of processes linked to photooxidation and photoinhibition of photosystems (which absence is proved by steady values of nonregulated thermal dissipation—Φ_{NO-} among treatments). Blasco et al. [20] reported an increase in photorespiration in lettuce plants treated with IO_3^-. These authors found in plants treated with high IO_3^- concentration, an increase in the hydroxypyruvate reductase activity, a key enzyme of the photorespiration cycle. It is likely to suppose that under treatment with moderate salt levels, as in our experimental conditions, iodine did not enhance salt tolerance through an increase in photorespiration. Nevertheless, in both salt treatments the photorespiration rise could have provided carbonated skeletons for the photosynthetic carbon reduction cycle, thus avoiding the decline of photosynthetic activity.

In conclusion, our data show that moderate salinity improves the nutritional value of lettuce, and that iodine promotes a higher accumulation in bioactive compounds such as chlorophylls and carotenoids, polyphenols, and anthocyanins under conditions of moderate salt levels. It is likely that iodine induces a greater synthesis of anthocyanins at the expense of the synthesis of other secondary metabolites that act as antioxidants. Our data indicate that iodine does not improve salt tolerance through an ameliorating of photorespiration; however, iodine application under moderate salt levels could represent a valid strategy for plant biofortification, as it improves the bioactive compound accumulation with its valuable functional effects on human health.

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References

1. Malorgio, F.; Diaz, K.E.; Ferrante, A.; Mensuali, A.; Pezzarossa, B. Effects of selenium addition on minimally processed leafy vegetables grown in floating system. *J. Sci. Food Agric.* **2009**, *89*, 2243–2251. [CrossRef]
2. Montesano, F.F.; D'Imperio, M.; Parente, A.; Cardinali, A.; Renna, M.; Serio, F. Green bean biofortification for Si through soilless cultivation: Plant response and Si bioaccessibility in pods. *Sci. Rep.* **2016**, *6*, 31662. [CrossRef] [PubMed]
3. Garg, M.; Sharma, N.; Sharma, S.; Kapoor, P.; Kumar, A.; Chunduri, V.; Arora, P. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* **2018**, *5*, 12. [CrossRef] [PubMed]
4. Rouphael, Y.; Kyriacou, M.C. Enhancing quality of fresh vegetables through salinity eustress and biofortification applications facilitated by soilless cultivation. *Front. Plant Sci.* **2018**, *9*, 1254. [CrossRef]
5. Carillo, P.; Annunziata, M.G.; Pontecorvo, G.; Fuggi, A.; Woodrow, P. Salinity Stress and Salt Tolerance. In *Abiotic Stress in Plants-Mechanisms and Adaptations*; Shanker, A., Venkateswarlu, B., Eds.; Inthec Open: London, UK, 2011; Volume 1, pp. 21–38.
6. Cirillo, C.; De Micco, V.; Arena, C.; Pannico, A.; De Pascale, S.; Rouphael, Y. Biochemical, Physiological and Anatomical Mechanisms of Adaptation of *Callistemon citrinus* and *Viburnum lucidum* to NaCl and $CaCl_2$ Salinization. *Front. Plant. Sci.* **2019**, *10*, 742. [CrossRef]
7. Carillo, P.; Cirillo, C.; De Micco, V.; Arena, C.; De Pascale, S.; Rouphael, Y. Morpho-anatomical, physiological and biochemical adaptive responses to saline water of *Bougainvillea spectabilis* Willd. trained to different canopy shapes. *Agric. Water Manag.* **2019**, *212*, 12–22. [CrossRef]
8. De Micco, V.; Arena, C.; Amitrano, C.; Rouphael, Y.; De Pascale, S.; Cirillo, C. Effects of NaCl and $CaCl_2$ Salinization on Morpho-Anatomical and Physiological Traits of Potted *Callistemon citrinus* Plants. *Forests* **2021**, *12*, 1666. [CrossRef]
9. Maggio, A.; Hasegawa, P.M.; Bressan, R.A.; Consiglio, M.F.; Joly, R.J. Review: Unravelling the functional relationship between root anatomy and stress tolerance. *Aust. J. Plant Physiol.* **2001**, *28*, 999–1004. [CrossRef]
10. Rouphael, Y.; Kyriacou, M.C.; Petropoulos, S.A.; De Pascale, S.; Colla, G. Improving vegetable quality in controlled environments. *Sci. Hort.* **2018**, *234*, 275–289. [CrossRef]
11. Rouphael, Y.; Petropoulos, S.A.; Cardarelli, M.; Colla, G. Salinity as eustressor for enhancing quality of vegetables. *Sci. Hort.* **2018**, *234*, 361–369. [CrossRef]
12. Santander, C.; Vidal, G.; Ruiz, A.; Vidal, C.; Cornejo, P. Salinity Eustress Increases the Biosynthesis and Accumulation of Phenolic Compounds That Improve the Functional and Antioxidant Quality of Red Lettuce. *Agronomy* **2022**, *12*, 598. [CrossRef]
13. Rouphael, Y.; Kyriacou, M.C.; Carillo, P.; Pizzolongo, F.; Romano, R.; Sifola, M.I. Chemical Eustress Elicits Tailored Responses and Enhances the Functional Quality of Novel Food Perilla frutescens. *Molecules* **2019**, *24*, 185. [CrossRef]
14. Buturi, C.V.; Mauro, R.P.; Fogliano, V.; Leonardi, C.; Giuffrida, F. Mineral Biofortification of Vegetables as a Tool to Improve Human Diet. *Foods* **2021**, *10*, 223. [CrossRef] [PubMed]
15. Kiferle, C.; Martinelli, M.; Salzano, A.M.; Gonzali, S.; Beltrami, S.; Salvadori, P.A.; Hora, K.; Holwerda, H.T.; Scaloni, A.; Perata, P. Evidences for a nutritional role of iodine in plants. *Front. Plant Sci.* **2021**, *12*, 616868. [CrossRef] [PubMed]
16. Caffagni, A.; Pecchioni, N.; Meriggi, P.; Bucci, V.; Sabatini, E.; Acciarri, N.; Ciriaci, T.; Pulcini, L.; Felicioni, N.; Beretta, M.; et al. Iodine uptake and distribution in horticultural and fruit tree species. *Ital. J. Agron.* **2012**, *7*, 229–236. [CrossRef]
17. Medrano-Macías, J.; Leija-Martínez, P.; González-Morales, S.; Juárez-Maldonado, A.; Benavides-Mendoza, A. Use of iodine to biofortify and promote growth and stress tolerance in crops. *Front. Plant Sci.* **2016**, *7*, 1146. [CrossRef]
18. Leyva, R.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Romero, L.; Ruiz, J.M.; Blasco, B. Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. *Plant Sci.* **2011**, *181*, 195–202. [CrossRef]
19. Medrano Macías, J.; López Caltzontzit, M.G.; Rivas Martínez, E.N.; Narváez Ortiz, W.A.; Benavides Mendoza, A.; Martínez Lagunes, P. Enhancement to salt stress tolerance in strawberry plants by iodine products application. *Agronomy* **2021**, *11*, 602. [CrossRef]
20. Blasco, B.; Rios, J.J.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Rosales, M.A.; Ruiz, J.M.; Romero, L. Photorespiration process and nitrogen metabolism in lettuce plants (*Lactuca sativa* L.): Induced changes in response to iodine biofortification. *J. Plant Growth Regul.* **2010**, *29*, 477–486. [CrossRef]
21. Chiesa, A.; Frezza, D.; Fraschina, A.; Trinchero, G.; Moccia, S.; León, A. Pre-harvest factors and fresh-cut vegetables quality. *Acta Hortic.* **2003**, *604*, 153–159. [CrossRef]
22. von Caemmerer, S.; Farquhar, G.D. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **1981**, *153*, 376–387. [CrossRef] [PubMed]

23. Genty, B.; Briantais, J.M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **1989**, *990*, 87–92. [CrossRef]
24. Kramer, D.M.; Johnson, G.; Kiirats, O.; Edwards, G.E. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth Res.* **2004**, *79*, 209–218. [CrossRef]
25. Krall, J.P.; Edwards, G.E. Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiol. Plant.* **1992**, *86*, 180–187. [CrossRef]
26. Epron, D.; Godard, D.; Cornic, G.; Genty, B. Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). *Plant Cell Environ.* **1995**, *18*, 43–51. [CrossRef]
27. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology, PlantCell Membranes*; Academic Press: Cambridge, MA, USA, 1987; pp. 350–382.
28. Vitale, L.; Vitale, E.; Guercia, G.; Turano, M.; Arena, C. Effects of different light quality and biofertilizers on structural and physiological traits of Spinach plants. *Photosynthetica* **2020**, *58*, 932–943. [CrossRef]
29. Moulehi, I.; Bourgou, S.; Ourghemmi, I.; Tounsi, M.S. Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (Citrus reticulate Blanco) and bitter orange (*Citrus aurantium* L.) seeds extracts. *Ind. Crop. Prod.* **2017**, *39*, 74–80. [CrossRef]
30. Sun, B.; da Silva, J.M.R.; Spranger, I. Factors of Vanillin Assay for Catechins and Proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 4267–4274. [CrossRef]
31. Mancinelli, A.L.; Huang Yang, C.P.; Lindquist, P.; Anderson, R.; Rabino, I. Photocontrol of anthocyanin synthesis. *Plant Physiol.* **1975**, *55*, 251–257. [CrossRef]
32. Chung, Y.C.; Chen, S.J.; Hsu, C.K.; Chang, C.T.; Chou, S.T. Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. *Food Chem.* **2005**, *91*, 419–424. [CrossRef]
33. George, B.; Kaur, C.; Khurdiya, D.S.; Kapoor, H.C. Antioxidants in tomato (*Lycopersicum esculentum*) as a function of genotype. *Food Chem.* **2004**, *84*, 45–51. [CrossRef]
34. Vitale, E.; Vitale, L.; Costanzo, G.; Velikova, V.; Tsonev, T.; Simoniello, P.; De Micco, V.; Arena, C. Light spectral composition influences structural and eco-physiological traits of *Solanum lycopersicum* L. cv. 'Microtom' in response to high-LET ionizing radiation. *Plants* **2021**, *10*, 1752. [CrossRef] [PubMed]
35. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
36. Im, J.; Ko, J.; Kim, H.Y.; Ha, B.K. Biochemical responses of Soybean (*Glycine max* L. Merr.) to proton beam irradiation. *Plant Breed. Biotechnol.* **2017**, *5*, 97–105. [CrossRef]
37. Carillo, P.; Soteriou, G.A.; Kyriacou, M.C.; Giordano, M.; Raimondi, G.; Napolitano, F.; Di Stasio, E.; Mola, I.D.; Mori, M.; Rouphael, Y. Regulated Salinity Eustress in a Floating Hydroponic Module of Sequentially Harvested Lettuce Modulates Phytochemical Constitution, Plant Resilience, and Post-Harvest Nutraceutical Quality. *Agronomy* **2021**, *11*, 1040. [CrossRef]
38. Nunes da Silva, M.; Machado, J.; Osorio, J.; Duarte, R.; Santos, C.S. Non-Essential Elements and Their Role in Sustainable Agriculture. *Agronomy* **2022**, *12*, 888. [CrossRef]
39. Kyriacou, M.C.; Rouphael, Y. Towards a new definition of quality for fresh fruits and vegetables. *Sci. Hortic.* **2018**, *234*, 463–469. [CrossRef]
40. Chutipaijit, S.; Cha-um, S.; Sompornpailin, K. High contents of proline and anthocyanin increase protective response to salinity in Oryza sativa L. spp. indica. *Aust. J. Crop Sci.* **2011**, *5*, 1191–1198.
41. Blasco, B.; Rios, J.J.; Leyva, R.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Rosales, M.Á.; Ruiz, J.M.; Romero, L. Does iodine biofortification affect oxidative metabolism in lettuce plants? *Biol. Trace Elem. Res.* **2010**, *142*, 831–842. [CrossRef]
42. Blasco, B.; Rios, J.J.; Leyva, R.; Melgarejo, R.; Constan-Aguilar, C.; Sanchez-Rodrıguez, E.; Rubio-Wilhelmi, M.M.; Romero, L.; Ruiz, J.M. Photosynthesis and metabolism of sugars from lettuce plants (*Lactuca sativa* L. var. longifolia) subjected to biofortification with iodine. *Plant Growth Regul.* **2011**, *65*, 137–143. [CrossRef]
43. Weng, H.X.; Hong, C.L.; Yan, A.L.; Pan, L.H.; Qin, Y.C.; Bao, L.T.; Xie, L.L. Mechanism of iodine uptake by cabbage: Effects of iodine species and where it is stored. *Biol. Trace Elem. Res.* **2008**, *125*, 59–71. [CrossRef] [PubMed]
44. Incrocci, L.; Carmassi, G.; Maggini, R.; Poli, C.; Saidov, D.; Tamburini, C.; Kiferle, C.; Perata, P.; Pardossi, A. Iodine Accumulation and Tolerance in Sweet Basil (*Ocimum basilicum* L.) With Green or Purple Leaves Grown in Floating System Technique. *Front. Plant Sci.* **2019**, *10*, 1494. [CrossRef] [PubMed]