

# Soilless cultivation systems to produce tailored microgreens for specific nutritional needs

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## Abstract

**BACKGROUND:** The awareness of the importance of following dietary recommendations that meet specific biological requirements related to an individual's health status has significantly increased interest in personalized nutrition. The aim of this research was to test agronomic protocols based on soilless cultivation for providing consumers with new dietary sources of iodine (I), as well as alternative vegetable products to limit dietary potassium (K) intake; proposed cultivation techniques were evaluated according to their suitability to obtain such products without compromising agronomic performance.

**RESULTS:** Two independent experiments, focused on I and K respectively, were conducted in a commercial greenhouse specializing in soilless production. Four different species were cultivated using three distinct concentrations of I (0, 1.5 and 3 mg L<sup>-1</sup>) and K (0, 60 and 120 mg L<sup>-1</sup>). Microgreens grown in I-rich nutrient solution accumulate more I, and the increase is dose-dependent. Compared to unbiofortified microgreens, the treatments with 1.5 and 3 mg L<sup>-1</sup> of I resulted in 4.5 and 14 times higher I levels, respectively. Swiss chard has the highest levels of K (14 096 mg kg<sup>-1</sup> of FW), followed by rocket, pea and radish. In radish, rocket and Swiss chard, a total reduction of K content in the nutrient solution (0 mg L<sup>-1</sup>) resulted in an average reduction of 45% in K content.

**CONCLUSION:** It is possible to produce I-biofortified microgreens to address I deficiency, and K-reduced microgreens for chronic kidney disease-affected people. Species selection is crucial to customize nutritional profiles according to specific dietary requirements due to substantial mineral content variations across different species.

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**Keywords:** personalized nutrition; biofortification; iodine deficiency; chronic kidney disease

## INTRODUCTION

The recognition of adhering to personalized dietary recommendations based on individual health requirements has raised significant interest in personalized nutrition.<sup>1</sup> Biofortification, a scientific approach aimed at improving the nutritional quality of plant-based foods, has become a key focus.<sup>2</sup> Biofortification involves developing staple crops with higher levels of essential micronutrients to address nutritional deficiencies.<sup>3</sup> While the primary goal is to increase specific nutrients such as calcium, silicon, selenium, iodine, iron and vitamins in edible plant parts, it may also involve reducing certain nutrients like potassium (K) and anti-nutrients such as phytate and oxalate, which may be undesirable for individuals with specific physiological conditions.<sup>1,3-6</sup>

In response to these advancements, the production of customized foods tailored to specific consumer groups with distinct nutritional needs has emerged as a significant challenge in food

agriculture.<sup>1</sup> This novel approach aims to optimize the nutritional composition of food to meet the requirements of target

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populations.<sup>1,7,8</sup> By combining personalized nutrition strategies with biofortification techniques, it is possible to address nutritional deficiencies and provide targeted foods that align with individuals' unique dietary needs.

Iodine (I) plays a crucial role in nutrition, particularly in supporting proper thyroid function and overall metabolic health. Adequate I intake is vital for maintaining a healthy thyroid and preventing I deficiency disorders.<sup>9</sup> However, both inadequate and excessive I intake can have adverse effects on thyroid health.<sup>10</sup> In general, the consumption of seafood is not high in geographic areas distant from the sea; furthermore, for agricultural products, the I content depends on the concentrations found in the soil and/or irrigation water.<sup>11</sup>

Potassium plays a significant role in maintaining overall health, but it requires careful attention in the context of chronic kidney disease (CKD). In individuals with impaired kidney function, the body's ability to regulate K levels becomes compromised, leading to potential complications.<sup>12</sup> Managing K intake is crucial for individuals with CKD, and healthcare professionals often recommend dietary restrictions on high-K foods such as bananas, oranges, tomatoes, potatoes and leafy greens.<sup>13</sup>

Various strategies, including genetic engineering, conventional plant breeding methods and agronomic techniques, are employed to produce biofortified and tailored plant materials.<sup>14</sup> Agronomic techniques, in particular, offer a less expensive and accessible approach to modify the nutrient and bioactive compound content in edible plant parts at different growth stages. Soilless systems are optimal techniques for vegetables biofortification, since they enable precise control of plant growth conditions, allowing for the modulation of target nutrient accumulation or reduction, beneficial for human health.<sup>1</sup>

Microgreens, considered as emerging functional foods of the 21st century, are edible plants consumed at the seedling stage. Microgreens can play a role in preserving and enhancing numerous endangered or at-risk local plant varieties. They present an opportunity to safeguard and utilize such genetic resources to cultivate this innovative category of vegetables.<sup>15</sup> They have a short growing cycle of 6–20 days and require minimal space. Industrial production of microgreens takes place under controlled environments such as tunnels, greenhouses and indoor farming facilities, utilizing varying levels of technologies.<sup>15</sup> The nutritional quality of microgreens can be shaped and tailored using agronomic approaches.<sup>16</sup> Starting with these considerations, two independent experiments were conducted using radish, pea, rocket and Swiss chard microgreens. The aim was to test agronomic protocols, suitable to be applied in commercial greenhouse–farm context, for offering consumers new dietary sources of I and alternative vegetable products to limit dietary K intake, in both cases without compromising agronomic crop performance.

## MATERIALS AND METHODS

### Plant materials and experimental conditions

The trials were carried out from December 2020 to January 2021, in a plastic greenhouse at the commercial farm Ortogourmet in Mola di Bari (BA), southern Italy (41°02'16.3" N 17°06'20.3" E m. a.s.l.). Two different and independent experiments were carried out with the aim of producing (i) biofortified I microgreens (Experiment 1) and (ii) tailored microgreens with low K content (Experiment 2). Plants of radish (*Raphanus sativus*), pea (*Pisum*

*sativum*), rocket (*Diplotaxis tenuifolia*) and Swiss chard (*Beta vulgaris*) were grown in plastic trays (130 cm<sup>2</sup>) filled with Sure To Grow substrate. The chemically untreated seeds of radish, pea, rocket and Swiss chard were evenly distributed across on the substrate surface at respective densities of 1.9, 0.64, 3.2 and 1.17 seeds/cm<sup>2</sup>. During the first 4 days, the trays were covered with plastic film for seed germination. On day five, the seedlings were exposed to light inside the greenhouse. The trays were irrigated every day using rainwater until the germination was complete. In both experiments, after germination, trays were irrigated with a half-strength Hoagland nutrient solution (½NS). In both experiments the ½NS was prepared by mixing macro0 and micronutrients with rainwater, resulting in a final concentration (mg L<sup>-1</sup>) of 112 N, 120 K, 80 Ca, 31 P, 16 S, 12 Mg, 0.135 B, 0.56 Fe, 0.055 Mn, 0.0655 Zn, 0.016 Cu and 0.025 Mo. A N<sub>2</sub>NO<sub>3</sub><sup>-</sup>:N<sub>2</sub>NH<sub>4</sub><sup>+</sup> ratio of 84:16 was applied. In Experiment 1, KIO<sub>3</sub> was added to the ½NS at different concentrations – 0, 1.5 and 3 mg L<sup>-1</sup> of I – in order to set up two levels of I dosing, and a control without additional I. Potassium added by KIO<sub>3</sub> was considered in the formulation of the NS and the additional K introduced as KIO<sub>3</sub> was balanced using K<sub>2</sub>SO<sub>4</sub> in the control treatment with no additional I. In Experiment 2, three levels of K were compared: 120, 60 and 0 mg L<sup>-1</sup>. The NS pH was adjusted in both cases to 5.5–6.0 using 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. In both trials the treatments were arranged according to a split-plot experimental design with three replications, where the three NSs were set in the main plots and genotypes in the subplots.

### Yield, dry weight and chemical analysis

At harvest, conducted upon the appearance of the first true leaf, the yield (expressed in kg of fresh weight (FW) m<sup>-2</sup>) was evaluated. After weighing, harvested microgreens were maintained in a forced draft oven at 65 °C until constant weight for the measurement of dry weight (DW).

For Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P and Zn determinations, dry samples (0.3 g) of microgreens were digested in a closed-vessel microwave digestion system (MARS 6, CEM Corporation, Matthews, NC, USA) with 10 mL of HNO<sub>3</sub> (pure grade, Carlo Erba). The digestion procedure was carried out in two steps: 15 min to reach 200 °C and 10 min maintained at 200 °C (power set at 900–1050 W; 800 psi). Each solution was diluted to volume with ultrapure Milli-Q water (18 MΩ cm<sup>-1</sup>) and filtered using a 0.45 µm filter (regenerated cellulose). Samples were analyzed with inductively coupled plasma optical emission spectrometry (5100 VDV, Agilent Technologies, Santa Clara, CA, USA) to measure Ca, K, Mg, P and Na in radial mode and Al, B, Cu, Mn, Mo, Zn and Fe in axial mode.<sup>17</sup>

The quantification of inorganic I in different microgreens species was performed by using the protocol described by Gonnella et al.<sup>18</sup> Briefly, 0.2 g of dry sample was taken and the I content was extracted with ultrapure Milli-Q water (18 MΩ cm<sup>-1</sup>) at 60 °C and stirred for 30 min. After extraction, the samples were allowed to cool to room temperature. The product extract was well mixed and centrifuged at 10 000 × g at room temperature and successively filtered by using 0.45 µm regenerated cellulose filters. The absorbance of samples was determined at 454 nm, using a UV-1800 spectrophotometer (PerkinElmer Lambda 25, Boston, MA, USA). The quantification of I in samples was determined by interpolation with a calibration standard curve (0–10 µg L<sup>-1</sup>; R<sup>2</sup> = 0.9999).

### Percentage of recommended daily allowance and hazard quotient for iodine intake

The daily intake of I and the percentage of coverage of RDA for I (RDA-I) were calculated in relation to the quantity of I in serving size of microgreens,<sup>19</sup> which is generally 20 g. Risk assessment was also performed by using hazard quotient (HQ), considered as the risk to consumer health resulting from the intake of I through the consumption of I-biofortified fresh microgreens, based on a 70 kg adult. The HQ is the ratio of the potential exposure to an organic and/or inorganic substance and it represents the level at which no negative effects are expected. HQ allows estimation of the potential negative effects on health, related to chronic consumption of food (in our case biofortified microgreens). HQ lower than or equal to 1 indicates that adverse effects are unlikely to occur, and thus the food product can be considered to have negligible hazard. For HQ greater than 1, the potential for adverse effects increases.<sup>20</sup> The contribution of I from other nutritional sources was not examined. The HQ was calculated according to the protocol described by the Environmental Protection Agency,<sup>20</sup> using the following equation:  $HQ = ADD/RFD$ , where ADD is the average daily dose of I (mg of I per kg body weight per day) and RFD is the recommended dietary tolerable upper intake level of I (mg of I per kg body weight per day). The I RFD value for a 70 kg adult is  $15.72 \mu\text{g I kg}^{-1} \text{day}^{-1}$  ( $1100 \mu\text{g I day}^{-1}$ ) as suggested by Kessler.<sup>21</sup> The ADD for 20 g portions of microgreens was computed as follows:  $ADD = (MI \times CF \times DI)/BW$ . MI is the I concentration of the microgreens ( $\text{mg kg}^{-1} \text{DW}$ ), CF is the FW to DW conversion factor for plant samples (calculated as the ratio of DW to FW; radish, 0.056 on average; pea, 0.073 on average; rocket, 0.067 on average; Swiss chard, 1.162 on average), DI is the daily intake of microgreens (kg, taken as 20 g) and BW is the body weight (kg) of humans, assumed as 70 kg.

### Statistical analysis

The effects of different treatments were tested using a two-way analysis of variance (ANOVA) followed by means separation using Fisher's protected least-significant difference (LSD) at  $P = 0.05$ . The statistical software Statistica 10.0 (StatSoft, Tulsa, OK, USA) was used for the analysis.

## RESULTS AND DISCUSSION

### Experiment 1

The purpose of this experiment was to offer consumers a new dietary source of I as microgreens of radish, pea, rocket and Swiss chard biofortified with I aimed at addressing the deficiency of this element, which is common in several European countries.<sup>22</sup> Specifically, the agronomic trials were carried out in a commercial greenhouse to verify how experimental protocols, extensively documented by other studies,<sup>23,24</sup> can be readily applied in a commercial greenhouse context. The impact of I levels and the microgreen species used on the productive and nutritional chemical parameters is reported in Table 1. Each species of microgreens exhibits unique growth and development traits, characteristics that directly affect their production and dry matter (DM) parameter. In our study, radish and pea showed a higher yield than rocket and Swiss chard (Table 2). Conversely, Swiss chard exhibited the highest DM content, followed by pea, rocket and radish. Interestingly, the addition of I to the NS did not impact the agronomic performance of the four microgreen species investigated, and the observed differences were solely attributable to genotype as highlighted in Table 2. This outcome underscores

**Table 1.** Summary of results for all analyzed parameters performed in Experiment 1

Determination	Source of variance		
	Species (S)	Iodine treatment (I)	Interaction S × I
	Significance		
Yield	***	ns	ns
Dry matter	***	ns	ns
Iodine content	***	***	***
Daily Intake of I with 20 g of microgreens	***	***	***
% RDA-I	***	***	***
Hazard quotient	***	***	***
Al	***	ns	***
B	***	***	***
Cu	***	***	***
Fe	***	**	ns
Mn	***	***	***
Mo	***	***	***
Zn	**	ns	ns
Ca	***	*	**
Mg	**	ns	ns
Na	***	***	***
K	***	**	**
P	***	ns	ns

Data were subjected to two-way ANOVA, and LSD multiple comparison test was used for evaluating the differences among means. ns, not significant.  
 \* $P < 0.05$ ;  
 \*\* $P < 0.001$ ;  
 \*\*\* $P < 0.0001$ .

the feasibility of achieving I-biofortified microgreens of radish, pea, rocket and Swiss chard without compromising the profitability potential for farmers.

In Table 1 and Fig. 1(A), a significant interaction between I levels and species is reported. In general, microgreen vegetables grown in an I-rich NS accumulate higher levels of I (Fig. 1(A)). Additionally, the increase in I content in all microgreen species was found to be dose-dependent. Compared to unbiofortified microgreens, the treatments with 1.5 and 3  $\text{mg L}^{-1}$  of I resulted in 4.5 and 14 times higher I levels, respectively (Table S2). Furthermore, it was observed that, at the same concentration of I in the NS, Swiss chard microgreens exhibited higher values of this nutrient compared to other species. In fact, Swiss chard biofortified with a concentration of 3  $\text{mg L}^{-1}$  of I accumulated substantial amounts of I, reaching a value of  $865 \mu\text{g}$  ( $100 \text{g FW}^{-1}$ ).

Various studies have indicated that the uptake of I by vegetables is influenced by both its concentration and existing form in the environment.<sup>25</sup> The soilless system has been recognized as an effective approach for producing biofortified vegetables enriched in I, as demonstrated by other researchers in studies involving lettuce,<sup>26,27</sup> different genotypes of *Brassicaceae*,<sup>19</sup> spinach<sup>25</sup> and Swiss basil.<sup>27</sup>

Furthermore, the accumulation of I in the leaves primarily depends on its transport through the xylem.<sup>28</sup> This implies that plant species with edible leaves serve as more effective candidates for I biofortification. The increase in I content in vegetable

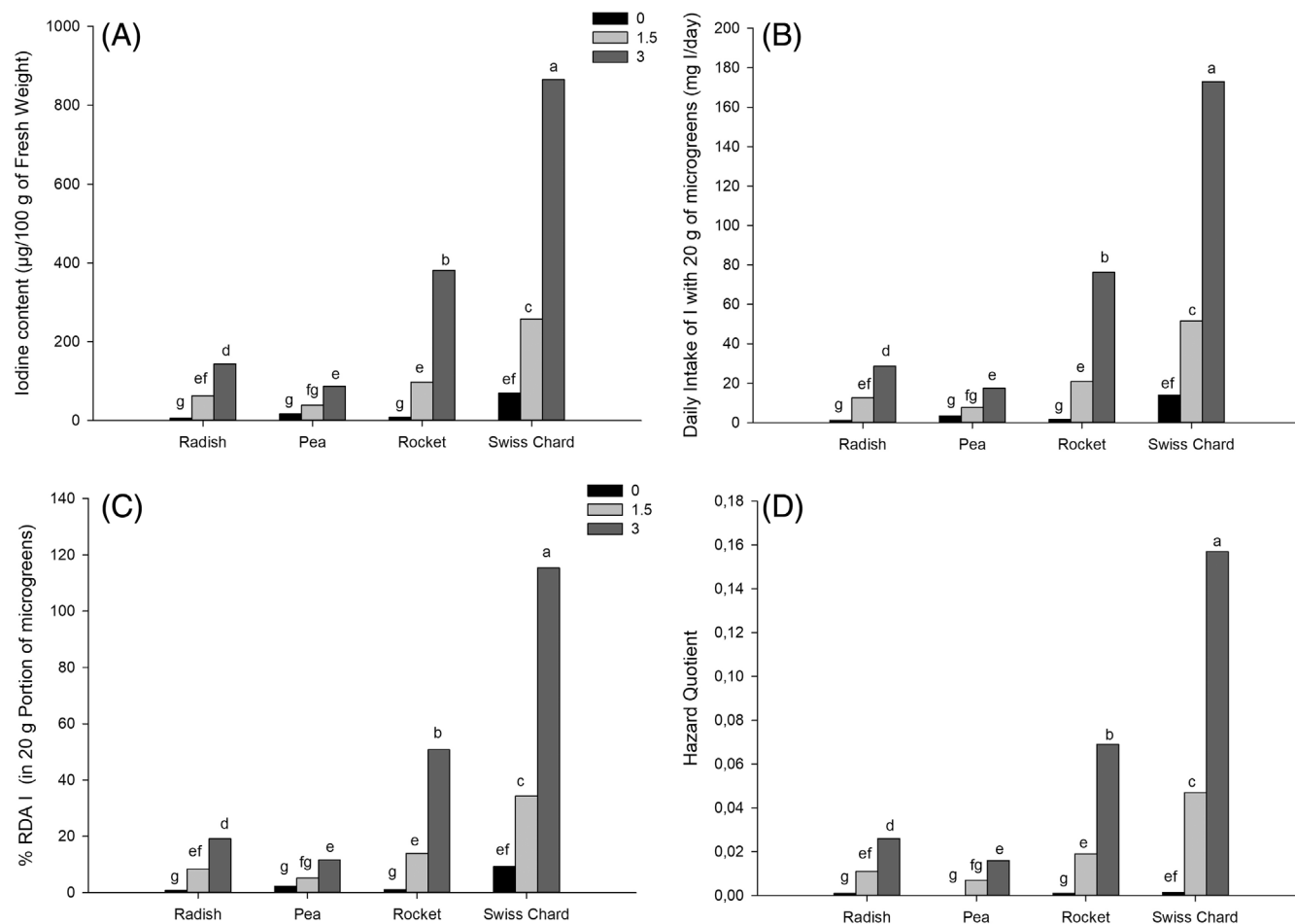
microgreens can have a significant impact on the daily intake of I for individuals consuming these vegetables as reported in Fig. 1(B).

Figure 1(B)–(D) shows, respectively, DI, RDA-I coverage (for men and women) and HQ for I intake through the consumption of 20 g of microgreens, an average portion size for this type of product.<sup>19</sup>

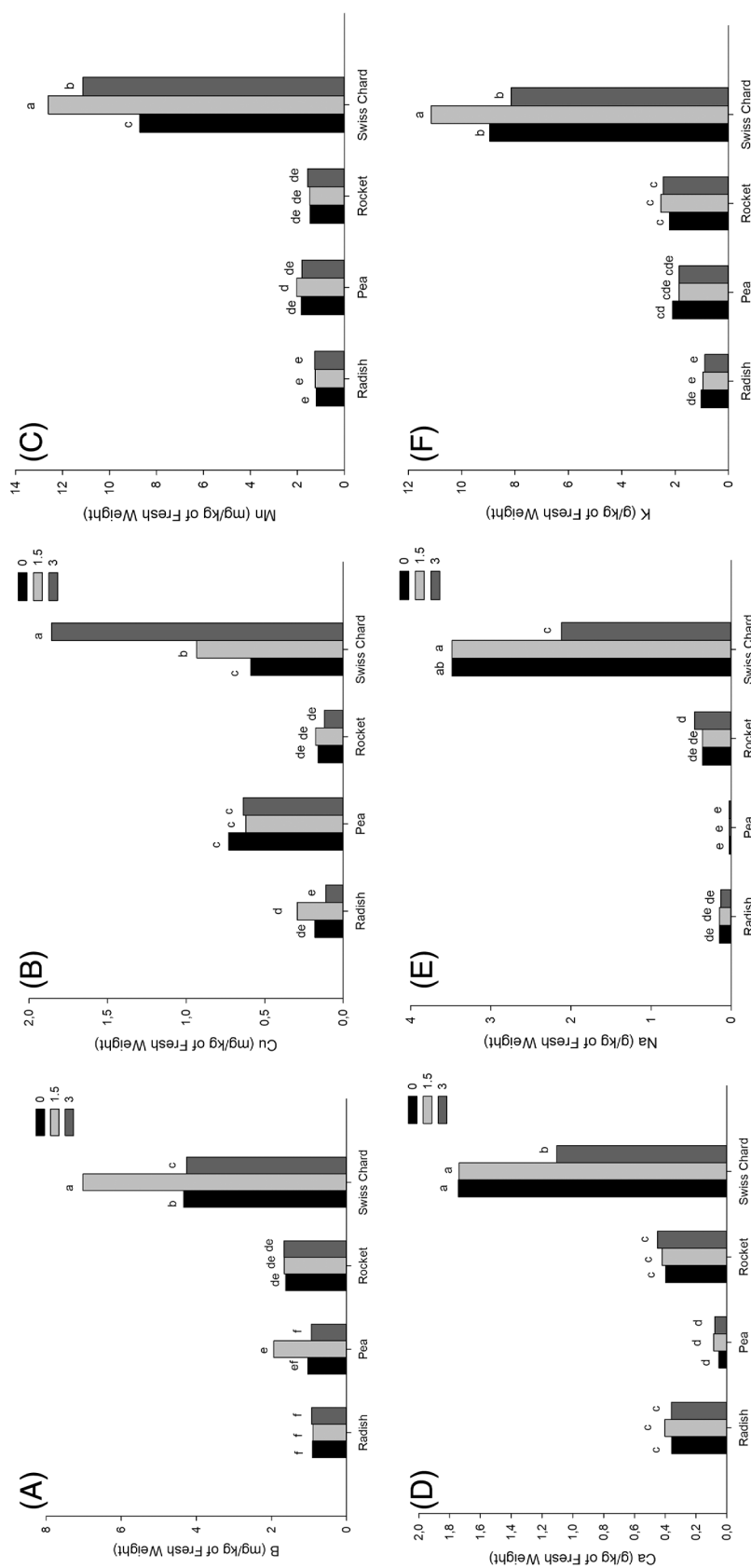
**Table 2.** Dry matter, yield, Fe, Zn, Mg and P content of radish, pea, rocket and Swiss chard microgreens as affected by different levels of I in the NS (Experiment 1)

Source of variation	Dry matter	Yield	Fe	Zn	Mg	P
	g (100 g FW) <sup>-1</sup>	kg m <sup>-2</sup>	mg kg <sup>-1</sup> FW		g kg <sup>-1</sup> FW	
Species (S)						
Radish	5.6 d	7.17 a	4.7 c	3.5 c	0.3 c	0.6 c
Pea	7.3 b	5.58 b	10.6 a	7.0 a	0.3 c	0.7 c
Rocket	6.6 c	3.15 c	5.6 b	6.2 b	0.5 b	0.8 b
Swiss chard	16.3 a	1.18 d	10.7 a	6.4 b	1.6 a	1.3 a
Iodine (I)						
0	8.7	4.28	8.3 a	5.9	0.7	0.8
1.5	9.3	4.28	7.9 ab	5.8	0.7	0.8
3	8.8	4.26	7.5 b	5.6	0.6	0.8

Data are expressed as mean of treatment ( $n = 3$ ). Different letters indicate that mean values are significantly different according to the LSD test ( $\alpha = 0.05$ ).



**Figure 1.** (A) Iodine content, (B) DI, (C) RDA-I and (D) HQ for intake of I through consumption of 20 g portions of I-biofortified microgreens, by adult humans (70 kg body weight). Data are expressed as mean of the treatment ( $n = 3$ ). Different letters indicate that mean values are significantly different according to the LSD test ( $\alpha = 0.05$ ).



**Figure 2.** Impact of I content in NS (0, 1.5 and 3 mg L<sup>-1</sup>) on accumulation of inorganic elements (A) B, (B) Cu, (C) Mn, (D) Ca, (E) Na and (F) K in radish, pea, rocket and Swiss chard microgreens. Data are expressed as mean of the treatment ( $n = 3$ ). Significance: \*\*\* $P \leq 0.001$ . Different letters indicate that mean values are significantly different according to the LSD test ( $\alpha = 0.05$ ).

**Table 3.** Summary of results for all analyzed parameters performed in Experiment 2

Determination	Source of variance		
	Species (S)	Potassium treatment (K)	Interaction S × K
		Significance	
Dry matter	***	***	***
Yield	***	***	***
Al	***	*	***
B	***	ns	ns
Ca	***	ns	ns
Cu	***	***	***
Fe	***	**	***
K	***	**	*
Mg	***	ns	ns
Mn	***	**	***
Mo	***	**	ns
Na	***	ns	*
Zn	***	ns	ns
P	***	ns	***

Data were subjected to two-way ANOVA, and LSD multiple comparison test was used for evaluating the differences among means. Means separation within lines by LSD ( $\alpha = 0.05$ ). ns, not significant.  
\* $P \leq 0.05$ ;  
\*\* $P \leq 0.01$ ;  
\*\*\* $P \leq 0.001$ .

The results demonstrate that I biofortification significantly increased these parameters ( $P < 0.001$ ), and variations among microgreens species were observed (Table 1 and Fig. 1(B)–(D)). The biofortified Swiss chard, produced with  $3 \text{ mg L}^{-1}$  of I, exhibited the highest values of DI, RDA-I coverage and HQ. Conversely, the lowest values were observed in unbiofortified radish, pea and rocket (Fig. 1(B)–(D)). Consuming 20 g of biofortified Swiss chard (with  $3 \text{ mg L}^{-1}$  of I) resulted in a 12-fold increase in DI and RDA-I coverage for both males and females, in comparison to unbiofortified vegetables (Fig. 1(B),(C)). All microgreen species tested in this study, after I biofortification, have the potential to contribute toward meeting the recommended daily intake of I. The observed increase in DI and RDA-I coverage underscores the effectiveness of the biofortification protocol employed, indicating its suitability for producing I-biofortified microgreens for various target consumer groups. This approach is particularly beneficial for individuals such as pregnant and breastfeeding women, vegetarians, individuals with specific health conditions and the elderly, who require an elevated DI.<sup>29</sup> Monitoring and regulating the I content in biofortified vegetables is crucial to ensure that individuals can benefit from increased I intake without exceeding safe limits. This involves regular testing and analysis of I levels in vegetables and establishing HQ parameter as reported in other studies.<sup>17,30</sup>

The HQ values obtained for all microgreens, regardless of the biofortification process, were below 1 (Fig. 1(D)), indicating a low risk. However, in the case of Swiss chard biofortified with excessive I, the HQ values increased. When HQ exceeds 1, there is a higher likelihood of adverse health effects. Based on our findings, consuming 20 g of our biofortified microgreen products

does not pose any health risks to consumers. This aspect should be carefully considered during the biofortification process. Excessive I levels in microgreens could pose a risk to consumers since microgreens constitute only a small portion of the overall diet, and other food sources can significantly contribute to daily I intake. Despite HQ highlighting that consuming microgreens biofortified with I does not pose a danger to the consumer, it is important to remember that HQ calculations are based on the potential risk associated solely with I present in microgreens and do not account for other food sources, such as seafood and/or salt fortified in I.<sup>31</sup>

Therefore, it is essential to monitor the intake of iodine-biofortified vegetables in order to avoid exceeding the tolerable upper intake level for this nutrient ( $1100 \mu\text{g day}^{-1}$ ).<sup>31</sup>

As presented in Table 2, the Zn, Mg and P content in the four types of microgreens was found to be influenced by genotype but not by I levels. Specifically, pea microgreens exhibited the highest Zn content, followed by rocket and Swiss chard ( $6.3 \text{ mg kg}^{-1}$  FW, on average) and radish. A similar trend was observed for Mg and P, with higher values observed in Swiss chard and lower values in radish and pea (Table 2). The Fe content was found to be influenced by the specific microgreen species, with higher levels observed in pea and Swiss chard (Table 2). Additionally, the Fe content was also affected by the levels of I in the NS, resulting in a limited reduction of Fe as the I concentration increased. Other authors also observed differences in mineral content among species.<sup>19</sup>

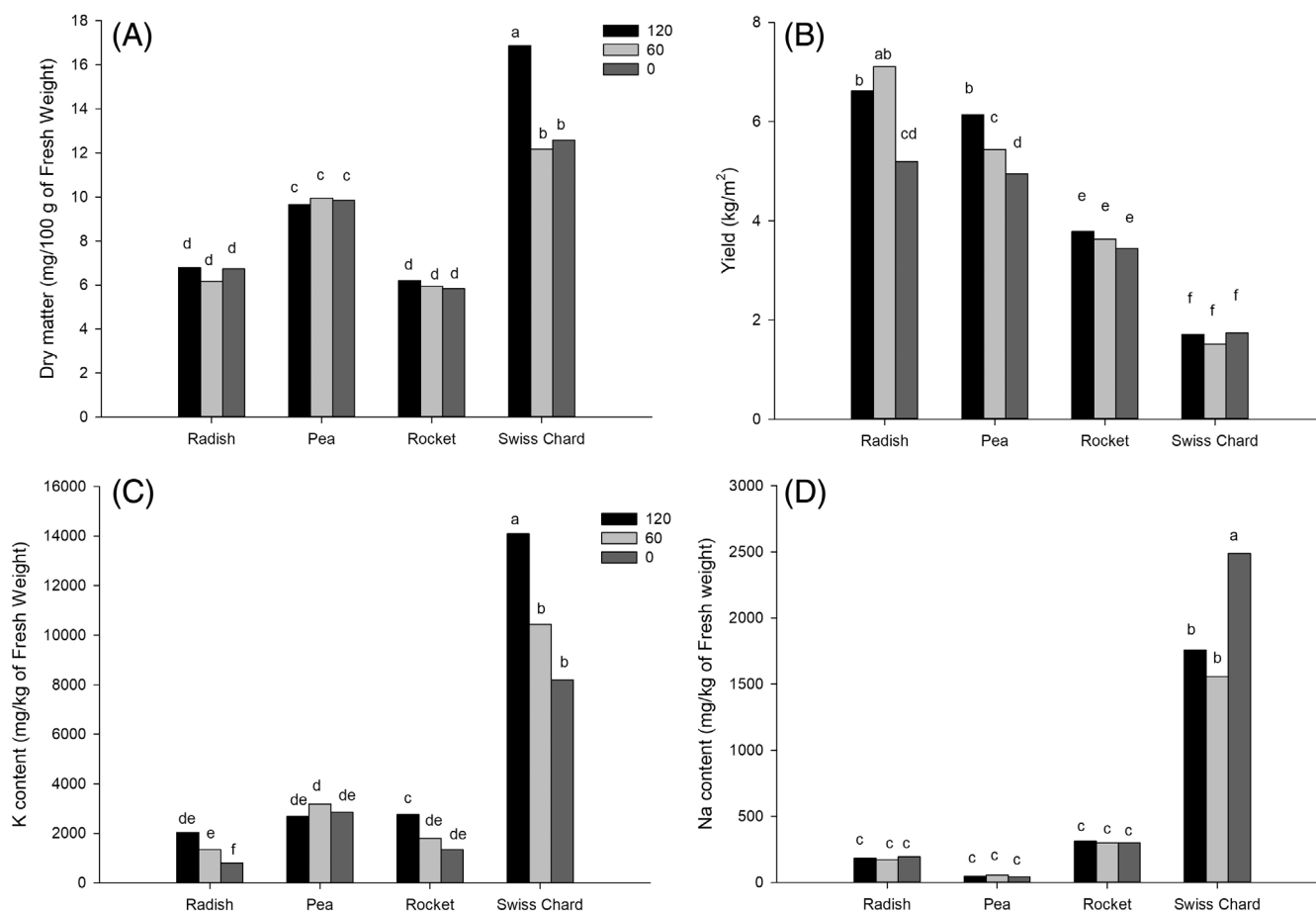
Concerning other mineral elements evaluated (Al, B, Cu, Mn, Mo, Ca, Na and K), an interaction was observed between the species and I treatments in NS (Table 1 and Fig. 2). Among the species included in this study, Swiss chard exhibited a higher sensitivity to I content in the NS, leading to significant variations in the content of B, Cu, Mn, Ca, Na and K. Furthermore, Swiss chard had higher mineral content compared to other microgreen species.

In radish, pea and rocket, the I levels did not cause variations in the content of mineral elements; except for slight differences in B content in peas (Fig. 2(A)), as well as Cu content (Fig. 2(B)) in radish. Moreover, only in Swiss chard did the Mn content tend to increase with higher I levels in the NS. However, both pea and Swiss chard exhibited a similar trend, with higher values observed in microgreens grown with  $1.5 \text{ mg L}^{-1}$  of I. Conversely, the Ca and K content tended to decrease in microgreens grown with higher I levels. The mineral composition of microgreens can be influenced by agronomic and genetic factors, as reported by Xiao et al.<sup>32</sup> Different genotypes of microgreens can exhibit different mineral profiles. It is important to note that only the mineral composition of Swiss chard was influenced by the presence of I in the NS as reported in Fig. 2.

## Experiment 2

The main purpose of the second experiment was to provide subjects with impaired kidney function an alternative food product (K-reduced microgreens) to limit K intake. Similarly to the first experiment, the agronomic trial was conducted in a commercial greenhouse to verify how the extensively documented experimental protocols from other studies<sup>7,8,33–35</sup> can be readily applied in a commercial greenhouse context.

The production parameters considered in the present study, DM and yield ( $\text{kg m}^{-2}$ ), were influenced both by the species and by the K levels in the NS (Table 3; Fig. 3(A),(B)). In radish, pea and rocket, the reduction of K in the NS did not modify the DM, while in Swiss chard, K levels of 50 and 0  $\text{mg L}^{-1}$  reduced this parameter



**Figure 3.** Impact of K content in NS (120, 60 and 0 mg L<sup>-1</sup>) on (A) DM dry matter and (B) yield and on the accumulation of inorganic elements (C) Na and (D) K in radish, pea, rocket, and Swiss chard microgreens. Data are expressed as mean of the treatment ( $n = 3$ ). Significance: \*\*\* $P \leq 0.001$ . Different letters indicate that mean values are significantly different according to the LSD test ( $\alpha = 0.05$ ).

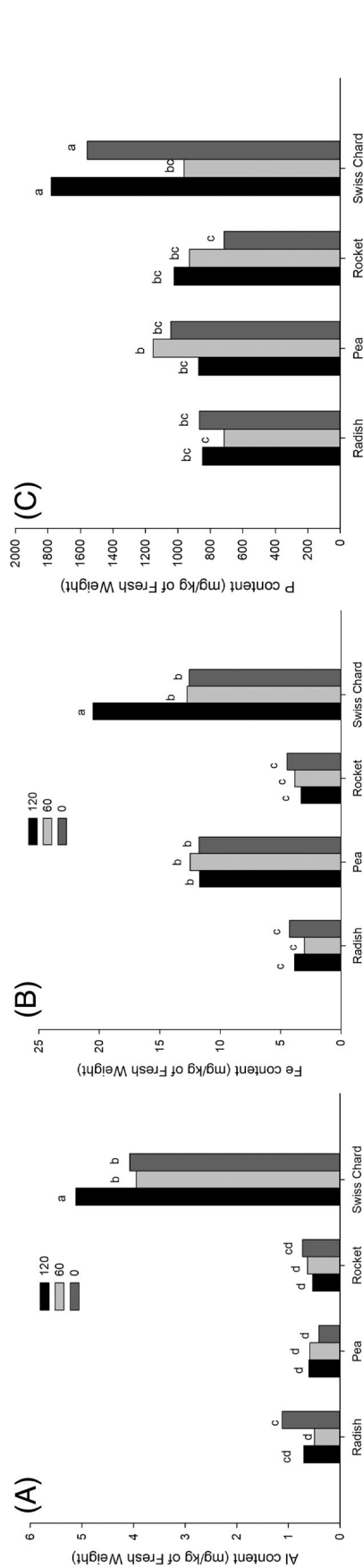
**Table 4.** Effects of different levels of K in NS on content of B, Ca, Mg, Mo and Zn content in radish, pea, rocket and Swiss chard microgreens in Experiment 2

	B	Ca	Mg	Mo	Zn
Species (S)	mg kg <sup>-1</sup> FW				
Radish	1.20 b	1266 b	413 b	0.14 a	3.87 b
Pea	1.29 b	681 c	240 b	0.11 a	8.35 a
Rocket	1.31 b	1641 b	460 b	0.05 b	4.81 b
Swiss chard	3.22 a	3407 a	1895 a	0.05 b	8.08 a
Potassium (K)					
120	1.85	1732	788	0.10 a	6.41
60	1.64	1700	668	0.07 b	5.60
0	1.78	1816	801	0.09 ab	6.82

Data are expressed as mean of treatment ( $n = 3$ ). Different letters indicate that mean values are significantly different according to the LSD test ( $\alpha = 0.05$ ).

by 27% compared to control (Fig. 3(A)). In a previous study, the reduction in K content did not result in any variation in DM in lettuce and chicory microgreens,<sup>7</sup> as well as spinach and Swiss chard at commercial stage of baby leaf.<sup>8</sup> However, other authors have observed a reduction in DM in spinach baby leaves grown with reduced levels of K in the NS.<sup>34</sup>

Regarding production, expressed in kg m<sup>-2</sup>, radish and pea are the highest yielding species, followed by rocket and Swiss chard (Fig. 3(B)). Radish and pea demonstrated abundant production, with yields of 6.19 and 5.51 kg m<sup>-2</sup>, respectively. Conversely, rocket and Swiss chard displayed relatively lower production rates, at 3.62 and 1.65 kg m<sup>-2</sup>, respectively. Furthermore, the



**Figure 4.** Impact of K content in NS (120, 60 and 0 mg L<sup>-1</sup>) on accumulation of inorganic elements (A) Al, (B) Fe and (C) P in radish, pea, rocket and Swiss chard microgreens. Data are expressed as mean of treatment (n = 3). Significance: \*\*\*P ≤ 0.001. Different letters indicate that mean values are significantly different according to the LSD test (α = 0.05).

highest production was observed in radish (7.11 kg m<sup>-2</sup>) cultivated with a low K content in the NS (50 mg L<sup>-1</sup>).

Radish (0 mg L<sup>-1</sup> of K) and pea (60 and 0 mg L<sup>-1</sup> of K) showed a reduction of yield in relation to the decrease in K in the NS (Fig. 3(B)). Similar results have been found by other authors in different microgreens and baby leaf crops<sup>7,34</sup> grown with reduced levels of K in the NS. Potassium is a crucial macronutrient for plants, constituting up to 10% of their DW.<sup>36,37</sup> It plays a fundamental role in plant health, growth and development, with significant effects. Consequently, reducing K concentration in plants without negatively impacting yield and marketable quality is challenging. This difficulty arises from the essential physiological functions of K, such as enzyme activation, osmotic regulation, photosynthesis and translocation of photosynthesis products.<sup>36-38</sup>

The absence of yield differences, as observed in rocket and Swiss chard, could be attributed to a higher K content in the seeds, ensuring a basal K level sufficient for the plant early growth for the main physiological activities in which K is involved.

No differences were found in the content of B, Ca, Mg and Zn in relation to the K content in NS (Tables 3 and 4). Swiss chard was the species with the highest content of B, Ca and Mg, as reported in Table 4. A slight variation in the Mo content was observed in relation to the K content in NS.

Regarding K content, our results, in line with our previous work,<sup>7,8</sup> suggest a species-dependent effect. Independently of the treatment, Swiss chard was the species with highest levels of K (14 096 mg kg<sup>-1</sup> of FW), followed by rocket, pea and radish (average of 2495 mg kg<sup>-1</sup> of FW) (Fig. 3(C)). This highlights the importance of selecting specific species suitable for cultivation processes in order to produce microgreens that are customized to meet specific nutritional requirements, particularly for individuals with CKD. In radish, rocket and Swiss chard, a total deprivation of K in the NS (0 mg L<sup>-1</sup>) resulted in an average reduction of 45% in K tissue content (Table S5). However, there were no significant variations in the K content of peas (average of 2900 mg kg<sup>-1</sup> of FW) as reported in Fig. 3. The findings from this study indicate that the production of microgreens with low K content, associated with species selection, has the potential to decrease the amount of this nutrient consumed per serving.

This result is significant considering that individuals with impaired kidney function typically follow a nutritional approach aimed at limiting K intake by reducing consumption of food sources rich in this nutrient, including vegetables.<sup>39</sup> However, a diet low in fruits and vegetables also results in a reduction of various essential nutrients such as vitamins and bioactive compounds, which generally have health effects, as well as alterations in gut microbiota.<sup>40,41</sup> In this regard, it is important to underline that dietary and nutritional therapy is an integral component of the treatment for patients with CKD and should still be complemented by pharmacological therapy.<sup>39</sup>

As reported in Fig. 3(C), only in Swiss chard, cultivated with the lowest dose of K, was a significant increase (60%) in Na content observed (Fig. 3(D)). This result, in accordance with other studies,<sup>7,8,34</sup> is likely due to a compensatory phenomenon occurring in the plant, where it partially substitutes K with cations, including Na, which have similar roles in physiological processes such as pH control and osmotic regulation.<sup>36</sup>

Compared to the control with 100 mg L<sup>-1</sup> of K, the decrease in K concentration in the NS (50 and 0 mg L<sup>-1</sup>) leads to a significant reduction in Al content only in Swiss chard microgreens,



averaging –22% (Fig. 4(A)). The presence of this mineral element, which is potentially harmful to consumers, is likely due to contamination of the fertilizers used in the preparation of the NS. Similarly to Al, Fe content also exhibits a similar trend. In this case, the reduction of K in the NS results in a significant decrease only in Swiss chard microgreens, averaging –39% (Fig. 4(A),(B)).

As reported by other authors, different species and/or genotypes show varying levels of Al<sup>42</sup> and Fe.<sup>32,43,44</sup> Swiss chard had the highest content of P among the examined species, and furthermore, it was the only species affected by variations in K in the NS in terms of P content (Fig. 4(C)).

## CONCLUSION

We demonstrated the feasibility of producing I-biofortified microgreens as a novel dietary source of I. We also explored the potential of producing K-reduced microgreens to cater to individuals with CKD. In Experiment 1, all species readily accumulated higher levels of I in an I-rich NS, with Swiss chard showing the highest I accumulation. In radish, rocket and Swiss chard, a substantial reduction in K content was achieved when grown with a K-free NS. In addition, our study reveals that K content in microgreens varies significantly depending on the species, with Swiss chard exhibiting the highest K levels, followed by rocket, pea and radish.

The selection of microgreen species is essential to tailor nutritional profiles to individual dietary needs, as the mineral content varies significantly among the different species examined. Future research activities could be aimed at assessing the bioaccessibility of mineral elements in relation to both different microgreen species and the contents of I and K in plant tissue.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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