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Nutritional stress suppresses nitrate content and positively impacts ascorbic acid concentration and phenolic acids profile of lettuce microgreens

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Abstract: In the last twenty years, horticulture has focused on producing high-quality food while improving resource use efficiency. The application of nutritional stress is an effective tool to increase the phytochemical content of vegetables while reducing environmental footprint. Microgreens represent an emerging functional food characterized overall by higher levels of phytonutrients than their mature counterparts. The current study examined the effect of using nutrient solution or only distilled water on the nutraceutical performance of lettuce (*Lactuca sativa* L.) microgreens grown in a peat-based substrate. Our results showed that lettuce microgreens can be effectively grown using only substrate nutrients, incurring a 27% decrease in fresh yield, but a significant increase in total ascorbic acid (+187%), anthocyanins (+35%), and total phenolic acids content (+26%). Of utmost interest, is the near absence of nitrates in water-treated microgreens, which renders this category of fresh vegetables ideal for the production of food dedicated to consumers highly sensitive to nitrates, such as infants. Growing microgreens without providing additional nutrients to those found in a typical peat-based substrate is an important potentiality that adds value to this new class of vegetables and also renders them particularly suitable for home cultivation.

Keywords: nutrient deprivation; eustress; mineral profile; anthocyanins; carotenoids; phenolics compounds

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1. Introduction

Modern horticulture has to cope with sub-optimal growth conditions imposed by substantial climate change (Koevoets et al., 2016) and the need to limit resource use to reduce production costs and environmental footprint. The use of intensive and efficient food production systems maximizes crop yield and environmental sustainability without compromising product quality. In recent years, demand for high-quality vegetables has increased rapidly, driven by the growing awareness of society for the health benefits associated with the consumption of fresh products high in nutritional and functional value, and rich in bioactive compounds such as carotenoids, phenolic acids and vitamins (Kyriacou et al., 2016; Bisbis et al., 2018). The biosynthesis and accumulation of phytochemicals depend on several factors including mainly genetic material, followed by environmental conditions, crop management practices and harvest maturity (Weston and Barth, 1997; Rouphael et al., 2012a; El-Nakhel et al., 2020).

Microgreens are immature greens harvested upon the appearance of the second true leaf and they represent a new category of fresh food gaining popularity as a culinary trend due to their organoleptic characteristics and dense bioactive content (Kyriacou et al., 2019a). Microgreens are significantly richer than their mature counterparts in phytonutrients and secondary metabolites (Pinto et al., 2015; Yadav et al., 2019; El-Nakhel et al., 2020), such as ascorbic acid, phenolic acids, anthocyanins and carotenoids, which represent categories of antioxidant properties having compounds that can beneficially modulate human metabolism and reduce the risk of heart disease and certain types of cancer (Della Penna, 1999; Khanam et al., 2012). The genotype is certainly the main factor driving the quantitative and qualitative variation in microgreen's health-beneficial metabolites for humans, often more determinant than cultivation practices and environmental factors (Rouphael et al., 2012a, 2016, 2017c; Kyriacou and Rouphael, 2018).

Lettuce is one of the most cultivated and consumed leafy vegetables in the world, often underestimated in terms of nutritional value. However, lettuce can contribute significantly to the nutritional profile of healthy diets, as it is a noteworthy source of health-promoting phytonutrients such as minerals, carotenoids, folates, flavonoids, phenolic acids, vitamin B9, C, and E (Romani et al., 2002; Kim et al., 2016). Concerning the harvest stage, previous work has demonstrated that lettuce microgreens were more nutrient-packed than mature lettuce in terms of Ca, Mg, and phenolic acids, with ten-fold lower nitrate concentration (El-Nakhel et al., 2020). Concerning cultivation management practices, several studies have shown that the application of positive stress (eustress), such as moderate nutrient deprivation through precise plant nutrition management, can improve the nutritional value of vegetables and reduce the accumulation of anti-nutrients, including nitrate (Tomasi et al., 2015; Colla et al., 2018; Rouphael and Kyriacou, 2018; Rouphael et al., 2018). In response to these practices, vegetable crops activate a series of physiological mechanisms to overcome sub-optimal conditions due to the applied eustress. These mechanisms trigger the biosynthesis and accumulation of secondary metabolites capable of increasing vegetable tolerance to abiotic stress (Orsini et al., 2016). A previous study on lettuce grown in a closed soilless system demonstrated that macronutrient deprivation eustress elicited differential secondary metabolites, increasing the final content of ascorbic acid, anthocyanins and phenolic acids (El-Nakhel et al., 2019). In this perspective, the cultivation of microgreens with minimal nutrient input would be an added value to this emerging class of vegetables.

Taking in consideration all these observations, the present study evaluated the effect of applying a nutrient solution or only distilled water onto lettuce microgreens grown in a peat-based substrate. The work aimed to verify the feasibility of cultivating microgreens relying only on substrate nutrients for improving the nutraceutical characteristics of the product, such as the mineral content, ascorbic acid, carotenoids, and phenolic compounds, without excessively compromising fresh yield.

2. Materials and Methods

2.1. Experimental conditions and treatments

The experiment was conducted on lettuce (*Lactuca sativa* L.) microgreens cv. 'Grand Rapids' (Stokes Seeds LTD) in a climate chamber at the Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy. The climate chamber (KBP-6395F, Termaks, Bergen, Norway) was set at 24/18±2 °C day/night temperature with a corresponding relative humidity of 70/80%±5%. The artificial light was delivered by a LED panel (K5 Series XL 750, Kind LED, California, USA) with an emission wavelength range divided into three customizable channels (blue 400-500 nm, green-yellow 500-600 nm, and red 600-700 nm). The radiation setup consisted of an optimal absorption spectrum for photosynthesis with an intensity of 300±15 μmol m^{-2} s^{-1} at canopy level and a 12-h photoperiod.

The adopted sowing density was five seeds cm^{-2}. The germination phase (lasted three days) occurred in darkness at 24 °C and 100% relative humidity in 204 cm^2 plastic trays filled each with 600 mL of peat moss mix (pH 5.48 and EC 282 µS cm^{-1}; Special Mixture, Floragard Vertriebs-GmbH, Oldenburg, Germany). The peat-based substrate had the following composition expressed on a dry matter basis: 11 mg kg^{-1} NO_3, 140 mg kg^{-1} PO_4, 796 mg kg^{-1} K, 2402 mg kg^{-1} Ca, 303 mg kg^{-1} Mg, 235 mg kg^{-1} SO_4, 540 mg kg^{-1} Na.

Microgreens plants were subjected to two different nutrient management, a standard treatment fertigated with a quarter strength Hoagland solution (HS) (pH 6.0±0.2 and EC 400±50 µS cm^{-1}) and one irrigated with only distilled water (DWO) (pH 6.0±0.2 and EC 3 µS cm^{-1}). Depending on the treatment, a total of 33 L of quarter strength Hoagland solution or distilled water, per square meter of planted microgreens, was provided during the entire cycle (16 days, from sowing to harvesting). The composition and concentration of the nutrient solution is described in detail in the work of Kyriacou et al. (2020). Table 1 shows the total amounts of macronutrients supplied to both treatments (by the substrate + nutrient solution and by the substrate only) during the entire cycle, calculated in grams per square meter of planted microgreens. Trays were distributed based on a randomized design of two fertigation treatments with three replicates.

Table 1. Total macronutrient supply derived from the peat-based substrate and each of two fertigation treatments (DWO and HS) calculated in grams per square meter of lettuce microgreens planted area.

Treatment	NO_3 (g m^{-2})	PO_4 (g m^{-2})	K (g m^{-2})	Ca (g m^{-2})	Mg (g m^{-2})	SO_4 (g m^{-2})	Na (g m^{-2})
DWO (only distilled water)	0.041	0.51	2.94	8.86	1.12	0.87	1.99
HS (Hoagland solution)	4.68	1.07	3.87	10.05	1.31	1.91	2.07

2.2. Colorimetric measurements, harvest and sampling

Before harvest, the CIELAB color space coordinates (L*, a*, and b*) were measured on the surface of the microgreens' canopy using a color meter (CR–400, Minolta Camera Co. Ltd., Osaka, Japan). Chroma and hue angle were calculated according to the following formulas: chroma = $(a^{*2}+b^{*2})^{1/2}$ and hue angle = arctan(b*/a*). The microgreens of both treatments were harvested on the same day (16 days after sowing) when the first two true leaves were fully formed, by cutting just above the substrate. The harvested material was weighed and one part was stored at -80 °C for qualitative analysis, while another part was placed in a forced-air oven to determine the dry weight (dw) and calculate the dry matter percentage. A portion of the material stored at -80 °C was lyophilized, while the remaining frozen fresh material was used for the determination of ascorbic acid and chlorophylls content. Both lyophilized and dried plant material was ground (841–microns screen) for other chemical and qualitative analysis.

2.3. Mineral and nitrate content

Dried microgreens material was used to assess mineral and nitrate content following the method mentioned by Pannico et al. (2019). The analyses were performed through ion chromatography (ICS-3000, Dionex, California, USA) coupled to an electrical conductivity detector. P, K, Ca, Mg, S, and Na were quantified in g kg^{-1} dw, while nitrate was converted to mg kg^{-1} fw based on each sample's original dry matter.

2.4. Total ascorbic acid assessment

For total ascorbic acid (TAA) analysis, 400 mg of frozen fresh material were extracted and assessed at 525 nm through a UV-Vis spectrophotometer (Hach DR 4000; Hach Co, Loveland, CO, USA) following the method of Kampfenkel et al. (1995). The results were expressed as g kg^{-1} dw.

2.5. Extraction and quantification of chlorophylls by spectroscopy

For the extraction of chlorophylls, 500 mg of microgreens frozen fresh material were extracted in ammoniacal acetone using mortar and pestle. The chlorophylls content was determined based on the absorbance at 647 and 664 nm using a spectrophotometer (Hach DR 4000; Hach Co, Loveland, CO, USA). Formulae and extinction coefficients were described in detail by Lichtenhaler and Wellburn (1983). Total chlorophylls content was calculated as the sum of chlorophyll a and b and expressed as mg 100 g^{-1} dw.

2.6. Extraction and quantification of carotenoids by HPLC-DAD

Carotenoids were extracted following the method of Kim et al. (2008) modified by Kyriacou et al. (2019a). Lyophilized microgreens were extracted and quantified in a reverse Phase-HPLC separation through a Shimadzu HPLC LC 10 (Shimadzu, Osaka, Japan) and a 250 × 4.6 mm, 5 µm Gemini C18 column (Phenomenex, Torrance, CA, USA). The samples were eluted with acetonitrile as mobile phase A and ethanol:*n*-hexane:dichloromethane (1:1:1) as phase B. The absorbance of the eluent was measured at 450 nm. Lutein and β-carotene content were expressed as mg kg^{-1} dw.

2.7. Extraction and quantification of anthocyanins and phenolic acids profile by Q Exactive Orbitrap LC-MS/MS

Lyophilized microgreens samples were extracted using the modified procedure reported by Huang et al 2016. 100 mg of sample were extracted with 2.5 mL of methanol/water (70:30, v/v) acidified with formic acid (0.5%). The mixture was sonicated for 30 min at room temperature. The extracts were then centrifuged at 14,000 × g for 10 min at 4 °C and the supernatant was filtered through a 0.2 µm nylon membrane syringe filter (Phenomenex, Italy). Five microliters of the extract were analyzed through a UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Quaternary UHPLC pump and an autosampler device (Dionex Ultimate 3000). The accuracy and calibration of the Q Exactive Orbitrap LC-MS/MS were checked daily using a reference standard. The mass tolerance window was set to 5 ppm in both full scan MS and AIF modes. Data analysis and processing were performed using Xcalibur software v. 3.0.63 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA). All values were expressed in µg g^{-1} dw.

2.8. Statistical analysis

The two treatments effects were analyzed by an unpaired Student's t-test and all data are shown as mean ± standard error (SPSS 20 software package). The experimental design consisted of three randomized units per treatment, and all qualitative analyses were conducted on three different analytical samples per treatment.

3. Results

3.1. Fresh biomass yield, dry matter content and leaf colorimetric parameters

Lettuce microgreens were harvested in both treatments at 16 days after sowing, upon the appearance of the first two true leaves. Fresh biomass was significantly affected by the two treatments, with DWO plants showing a 27% decrease in fresh yield compared to the HS treated plants (Figure 1). In particular, the yield values were 1.56 kg m^{-2} and 2.14 kg m^{-2} in DWO and HS microgreens, respectively. In contrast, DWO plants achieved a significantly higher percentage of dry matter (5.27%) than those fed with the nutrient solution (4.36%) (Figure 1). No significant differences among the treatments were found in the colorimetric parameters of lettuce leaves (Table 2).

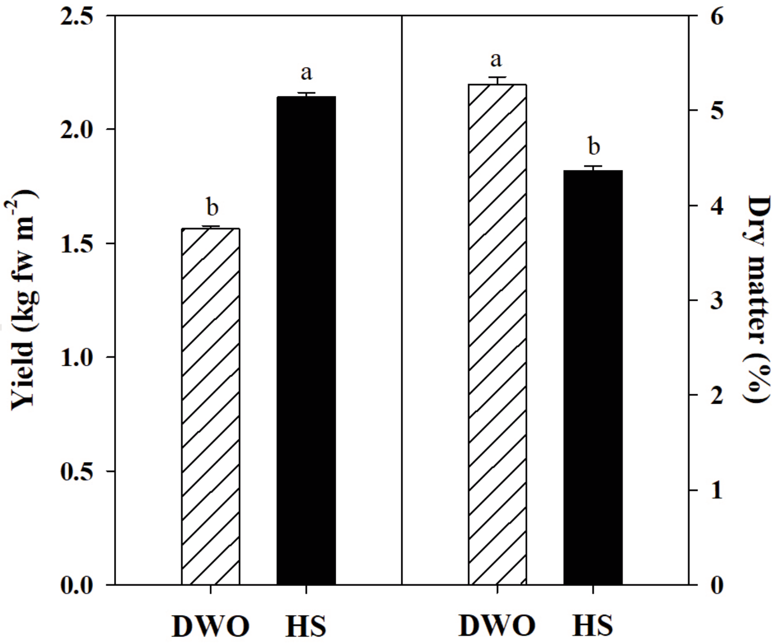

Figure 1. Fresh yield and dry matter percentage of lettuce microgreens as influenced by fertigation treatments (DWO: only distilled water, HS: Hoagland solution). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$). All data are expressed as mean ± standard error, n = 3.

Table 2. Colorimetric components L*, a*, b*, chroma, and hue angle of lettuce microgreens as influenced by fertigation treatment.

Treatment	L*	a*	b*	Chroma	Hue angle (°)
DWO (only distilled water)	49.56 ± 0.78	-9.36 ± 0.14	31.94 ± 0.46	33.29 ± 0.45	106.3 ± 0.26
HS (Hoagland solution)	49.08 ± 1.64	-8.96 ± 0.25	31.39 ± 0.27	32.65 ± 0.30	105.9 ± 0.37
t-test	ns	ns	ns	ns	ns

All data are expressed as mean ± standard error, *n* = 3. ns Non-significant at $p \leq 0.05$. Fertigation treatments are compared according to Student's t-test.

3.2. Nitrate content and mineral composition

The plant nutrition management significantly affected the nitrate content of lettuce microgreens. Treatment with DWO resulted in a hundred-fold decrease of nitrate content (2.67 mg kg^{-1} fw) than fertilization with nutrient solution (328.9 mg kg^{-1} fw; Table 3). Among the five major analyzed elements, K (19.12-28.14 mg g^{-1} dw) was the main mineral constituent of lettuce microgreens, followed by Ca (7.12-9.07 mg g^{-1} dw), Mg (4.33-5.79 mg g^{-1} dw), P (3.37-4.16 mg g^{-1} dw) and S (0.58-1.03 mg g^{-1} dw). Concerning the influence of treatments, a significant decrease of 32% in K, 21% in Ca, 25% in Mg, 19% in P and 44% in S was observed in DWO plants compared to fertigated ones (Table 3).

Table 3. Nitrate concentration and macronutrient content of lettuce microgreens as influenced by fertigation treatment.

Treatment	NO_3 (mg kg^{-1} fw)	P (mg g^{-1} dw)	K (mg g^{-1} dw)	Ca (mg g^{-1} dw)	Mg (mg g^{-1} dw)	S (mg g^{-1} dw)	Na (mg g^{-1} dw)
DWO (only distilled water)	2.67 ± 0.61	3.37 ± 0.11	19.12 ± 0.45	7.12 ± 0.56	4.33 ± 0.28	0.58 ± 0.16	5.85 ± 0.44
HS (Hoagland solution)	328.9 ± 6.87	4.16 ± 0.08	28.14 ± 0.70	9.07 ± 0.40	5.79 ± 0.20	1.03 ± 0.01	5.25 ± 0.25
t-test	***	**	***	*	*	*	ns

All data are expressed as mean ± standard error, *n* = 3. ns,*,**, *** Non-significant or significant at $p \leq$ 0.05, 0.01, and 0.001, respectively. Fertigation treatments are compared according to Student's t-test.

3.3. Pigments and total ascorbic acid content

Treatment with only distilled water positively affected the content of total ascorbic acid and anthocyanins compared to microgreens treated with nutrient solution (Table 4). In particular, the total content of ascorbic acid and anthocyanins in DWO irrigated plants increased significantly, by 187% and 35% respectively, compared to fertigated ones (Table 4). On the contrary, the carotenoids concentration was negatively impacted by nutrient deprivation (Figure 2). Lutein and β-carotene content of DWO microgreens were significantly lower than that of HS microgreens, reduced by 40% and 19%, respectively (Figure 2). Likewise, chlorophyll a, b and total chlorophylls content in DWO irrigated plants decreased significantly, by 14%, 34% and 19% respectively, compared to fertigated ones (Table 4).

Table 4. Chlorophylls, total ascorbic acid and total anthocyanins of lettuce microgreens as influenced by fertigation treatment.

Treatment	Chlorophyll a (mg 100 g^{-1} dw)	Chlorophyll b (mg 100 g^{-1} dw)	Total chlorophylls (mg 100 g^{-1} dw)	Total ascorbic acid (g kg^{-1} dw)	∑ Anthocyanins (μg g^{-1} dw)
DWO (only distilled water)	6.07 ± 0.17	1.76 ± 0. 15	7.84 ± 0.19	20.81 ± 0.77	31.51 ± 0.96
HS (Hoagland solution)	7.05 ± 0.13	2.67 ± 0.15	9.72 ± 0.47	7.25 ± 1.15	23.37 ± 0.49
t-test	*	*	*	***	**

All data are expressed as mean ± standard error, *n* = 3. *,**, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Fertigation treatments are compared according to Student's t-test.

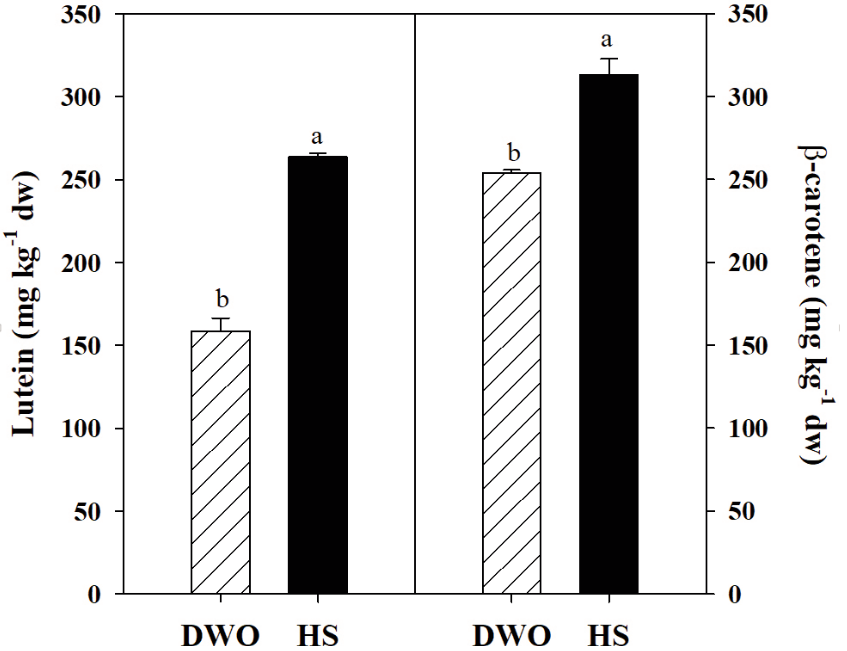

Figure 2. Lutein and β-carotene content of lettuce microgreens as influenced by fertigation treatments (DWO: only distilled water, HS: Hoagland solution). Different letters indicate significant differences according to Student's t-test ($p \leq 0.05$). All data are expressed as mean ± standard error, n = 3.

3.4. Phenolic compounds profiling

Orbitrap LC-MS/MS analysis identified ten different phenolic acids present in lettuce microgreens (Table 5). Irrespective of treatments, the four most abundant phenolic compounds were caffeoyl-quinic acid (893.8 μg g^{-1} dw), quercetin 3-glucoside (130.3 μg g^{-1} dw), rutin (23.61μg g^{-1} dw) and kaempferol 3,7-diglucoside (16.25 μg g^{-1} dw) in decreasing order of magnitude (Table 5). The aforementioned phenolic acids were significantly higher in water-treated plants than those fertilized with nutrient solution. Specifically, in DWO microgreens the concentrations of caffeoyl-quinic acid, quercetin 3-glucoside, rutin and kaempferol 3,7-diglucoside increased by 19%, 95%, 25% and 25%, respectively, compared to fertigated ones. However, no significant differences were found among the treatments on the concentration of synapoyl-hexose, kaempferol 3-glucoside, coumaroyl-diglucoside, ferulic acid, disinapoylgentiobiose and kaempferol 3-hydroxyferuloylsophorotrioside-7- glucoside. Overall, the total quantity of phenolic acids detected in water-treated microgreens was higher by 26% compared to fertigated microgreens (Table 5).

Table 5. Phenolic acids content of lettuce microgreens as influenced by fertigation treatment.

Phenolic acid	Treatment		t-test
	DWO (only distilled water)	HS (Hoagland solution)	
Caffeoyl-quinic acid (μg g^{-1} dw)	973.2 ± 33.4	814.4 ± 19.6	*
Coumaroyl-diglucoside (μg g^{-1} dw)	2.37 ± 0.11	2.29 ± 0.13	ns
Disinapoylgentionbiose (μg g^{-1} dw)	0.47 ± 0.07	0.44 ± 0.04	ns
Ferulic acid (μg g^{-1} dw)	1.48 ± 0.16	1.67 ± 0.03	ns
Km 3,7-diglucoside (μg g^{-1} dw)	18.03 ± 0.47	14.48 ± 0.85	*
Km 3-glucoside (μg g^{-1} dw)	5.69 ± 0.30	5.33 ± 0.38	ns
Km 3-hydroxyferuloylsophorotrioside-7-glucoside (μg g^{-1} dw)	0.27 ± 0.00	0.28 ± 0.04	ns
Qn 3-glucoside (μg g^{-1} dw)	172.4 ± 10.7	88.26 ± 6.76	**
Rutin (μg g^{-1} dw)	26.25 ± 0.73	20.97 ± 1.26	*
Sinapoyl-hexose (μg g^{-1} dw)	15.91 ± 0.85	14.53 ± 0.89	ns
∑ phenolic acids (μg g^{-1} dw)	1216 ± 41.4	963 ± 25.7	**

All data are expressed as mean ± standard error, *n* = 3. ns,*,** Non-significant or significant at $p \leq 0.05$ and 0.01, respectively. Fertigation treatments are compared according to Student's t-test. Qn = quercetin, Km = Kaempferol

4. Discussion

The genetic material, environmental conditions and cultivation management practices are the main pre-harvest factors that impact the biometric characteristics of microgreens (Kyriacou et al., 2019a, 2019b, 2020). In the present work, plant development of lettuce microgreens does not appear to be affected by nutrient deprivation, as both treatments reached commercial maturity simultaneously (appearance of the first two-true leaves). Sub-optimal nutritional conditions inevitably lead to a more or less severe reduction in yield depending on the vegetable species and the intensity of stress. In previous work on lettuce, grown in a closed soilless system with different concentrations of nutrient solution, a 24% yield decrease was found in plants managed with quarter-strength solution compared to those cultivated with full-strength solution; in contrast, a respective significant increase in dry matter percentage was observed (El-Nakhel et al., 2019). Fallovo et al. (2009) also reported a significant reduction in the marketable yield of lettuce in response to a decrease in nutrient solution concentration. The reduction in fresh biomass due to nutrient deprivation could be associated with both an osmotic effect and nutritional deficiency, especially of N and Mg (Fallovo et al., 2009). N reduction is the most critical nutrient affecting growth compared to other macro-and trace elements (Kopsell et al., 2007). The above findings are fully consistent with our experimental results, in which there was a significant yield reduction in water-treated plants, meanwhile the dry matter content increased conversely. In addition, leaf chlorophyll content has shown to be closely related to nitrogen concentration in the plant (Zebarth et al., 2002). In our work, the reduction in chlorophyll content in WDO plants was in accordance with what was found on two lettuce genotypes subjected to nitrogen deficiency (Becker et al., 2015). The greater sensitivity of chlorophyll b than chlorophyll a to nitrogen reduction agrees with the findings of Berges et al. (1996). Considering that photosystem II contains chlorophyll a and b in equal parts, while photosystem I contains four times more chlorophyll a than b, the authors reported that nitrogen deficiency affected photosystem II more than photosystem I in several microalgae species. On the other hand, the absence of significant differences in colorimetric parameters, denotes that the sub-optimal nutrient conditions did not result in noticeable effects on the leaf appearance of microgreens.

In our work, nutrient deprivation resulted in an analogous reduction in mineral content. Similar results were obtained in previous work on lettuce and other vegetable species grown under suboptimal nutrient conditions (El-Nakhel et al., 2019; Rouphael et al., 2012b; Fallovo et al., 2009; Vernieri et al., 2006). Although the nitrate content of microgreens fertigated with the nutrient solution was far below the limits established by the UE regulation for mature lettuce (2000-4500 mg kg^{-1} fw), the even lower concentration of nitrate recorded in water-treated plants represents a very interesting result. It is well known that high levels of nitrates can be particularly harmful to human health, causing serious physiological disorders, such as methemoglobinemia and blue baby syndrome (Colla et al., 2018). In this regard, infants younger than 12 months are particularly exposed to this risk (Vasco and Alvito, 2011); for this population category, the maximum level of nitrates established in 2002 by the Joint FAO/WHO Expert Committee on Food additives (JEFCA) was reduced to 200 mg kg^{-1} fw with an acceptable daily intake (ADI) of 0-3.7 mg kg^{-1} body weight per day (FAO/WHO, 2003).

The value of lettuce as a source of beneficial human health compounds such as ascorbic acid and phenolic acids has been demonstrated (Kim et al., 2016, 2018; Kennedy, 2019). In our work, sub-optimal nutrient conditions achieved through deprivation of nutrient supply, triggered the greater accumulation of total ascorbic acid, anthocyanins, and other phenolic acids compared to fertigated lettuce microgreens. These findings are following previous works that reported a greater accumulation of ascorbic acid in lettuce fed with half or quarter-strength solution than a full-strength solution (Shinohara and Suzuki, 1981; El-Nakhel et al., 2019). Similarly, the concentration of phenolic acids has been demonstrated to increase under conditions of nutrient stress (Rouphael et al., 2012b). In particular, other studies found that anthocyanins concentration in lettuce increased under both nitrogen and nutritional deficiencies (Gershenzon, 1984; Akula and Ravishanka, 2011; El-Nakhel et al., 2019). Regarding the phenolic content, our results corroborate those found by other authors who showed a significant increase in phenolic compounds in lettuce exposed to lower nutrient concentrations (Alberici et al., 2007; Becker et al. 2015; Qadir et al., 2017; El-Nakhel et al., 2019). Nitrogen deficiency has proven to be the major mediator of phenolic acid accumulation, followed by phosphorus deficiency and finally potassium deficiency (Chishaki and Horiguchi, 1997). This response can be attributed to the activation of specific genes such as phenylalanine ammonia-lyase (PAL) and L-galactose dehydrogenase, which are involved in the biosynthesis of phenolics and ascorbic acid, respectively. Activation of these genes could also be triggered by stress such as nutrient deprivation or, more specifically, nitrogen deficiency, as confirmed by Becker et al. (2015), who stated that the expression of phenylalanine genes was more pronounced under nitrogen deprivation. Concerning carotenoids content, our findings are in line with those of El-Nakhel et al. (2019) who observed a reduction in lutein and β-carotene in lettuce plants treated with a quarter-strength solution compared to those at full-strength. Lutein and β-carotene are among the major xanthophyll pigments of the lettuce light-harvesting complex of photosystem II and light-harvesting antenna. In addition to being accessory pigments, these compounds probably constitute a pivotal action point for N limitation with adverse effects on the photosynthetic apparatus. Therefore, their concentrations could decrease in response to nutrient reduction because they are part of a photosystem that is being downsized due to nitrogen depletion (Becker et al., 2015).

5. Conclusions

In recent years, the interest of farmers and scientists has focused on producing high-quality food while improving resource use efficiency, such as water and fertilizers, through intensive production systems and environmentally sustainable practices. Our findings demonstrated that lettuce microgreens can be successfully grown using only the available nutrients in the substrate, with a reduction in fresh yield (-27%), but a significant increase in total ascorbic acid (+187%), anthocyanins (+35%), and phenolic acids content (+26%). In this regard, the consistent improvement in phytonutrients leads to an increase in product value by offsetting the loss of yield. The near absence of nitrate observed in water-treated lettuce microgreens is of particular interest for the production of food dedicated to consumers highly sensible to nitrate content, such as infants, or for the preparation of so-called baby-food. Finally, the feasibility of producing microgreens without any additional input of nutrients beyond those found in a typical peat-based substrate is particularly appealing. Thus, microgreens can be easily grown at home using the methods described in this work, becoming a means for consumer access to greater quantities of nutrients per gram plant biomass than store-bought mature lettuce, which has a higher anti-nutrient content, such as nitrate, and a relatively lower nutraceutical value than microgreens.

Conflicts of Interest: The authors declare no conflict of interest.

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