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Boron Biofortification of *Portulaca Oleracea* L. Through Soilless Cultivation for a New Tailored Crop

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Abstract: Purslane (*Portulaca oleracea* L.) is a wild edible plant, traditionally consumed in the Mediterranean area and recently proposed as a new ready-to-eat vegetable; it is also called the "vegetable for long life" because of the high contents of several healthy compounds. Although boron (B) is not considered to be essential for humans, a daily intake of about 2 mg to obtain positive effects on aging in adult men and women has been suggested. In this study, two genotypes of purslane (wild collected and commercial variety) are grown by using a hydroponic system with three boron (B) levels in the nutrient solution (NS) (0.3 mg/L—control, 3 mg/L—low level of biofortification, and 6 mg/L—high level of biofortification) in order to increase the B content in the edible parts of the plant. The crop yield, color traits, and content of glucose, fructose, total phenols, chlorophylls, carotenoids, mineral elements (Al, B, Ca, Cr, Fe, K, Mg, Mn, Na, and Zn), nitrate, and oxalate are analyzed. Independent of the genotype, the B content in edible purslane was successfully increased in comparison with the control, obtaining 1.8- to 10.7-fold higher values of B tissue concentrations by using, respectively, 3 and 6 mg/L of B in the NS without affecting crop performances. From a nutritional point of view, the average daily intake of B could be satisfied by consuming about 75 or 48 g of purslane, grown by using 3 and 6 mg/L B level in the NS, respectively. Apart from B and Fe, the content of mineral elements in edible parts of purslane was not strongly influenced by different B levels in the NS but it was affected by genotypes. A lower sugar content was found in wild purslane grown with the highest B level. A higher content of both chlorophylls and carotenoids was found in the control but only for the commercial genotype. No differences in oxalate content were observed among B levels in the NS, while only in the case of wild genotype, we found a lower nitrate content when a B concentration of 3 mg/L was used in the NS. In conclusion, we demonstrated the possibility of using the floating hydroponic system, combined with specific B concentrations in the NS composition, as a method to calibrate the B uptake in edible parts of purslane.

Keywords: color traits; crop performance; floating system; mineral elements; nitrate; oxalate; *Portulaca oleracea* L.; wild vegetables

1. Introduction

Increasing evidences suggest that healthy eating, based on the adequate consumption of plant-based foods, plays an important role in the prevention of many chronic diseases, such as heart disease, cancer, stroke, diabetes, Alzheimer's disease, and age-related functional decline [1–4]. Vegetables represent one of the most important nutrient sources in the human diet, although their

nutritional value is related to several aspects such as species, cultivar, growing conditions, and production methods.

Purslane (*Portulaca oleracea* L.), a wild edible plant endemic to the Mediterranean area [5,6], has been proposed as a new ready-to-eat vegetable product [7]. This species shows interesting nutritional traits, with particular reference to its high content of several healthy compounds such as mineral elements and ω-3 fatty acids, especially α-linoleic acid [6,8]. Furthermore, it is a vegetable with a very interesting ω-6:ω-3 ratio (less than 2), which has been confirmed to be of major importance for ensuring the proper equilibrium between these two types of essential fatty acids in human body, and consequently for contributing to a good health of the cardiovascular system.[8]. Furthermore, it should be considered that the uptake of ω-3 fatty acids is known for its positive effect on bone health [9]. It is interesting to note that, in certain regions, purslane is also traditionally called the "vegetable for long life", due to its high amounts of dopamine and catecholamine, which has proven useful to prevent cancer and heart related diseases [5].

For the above-mentioned nutritional characteristics, purslane is gaining interest as a healthy vegetable, and it has already been proposed as a target species to obtain vegetable products with improved nutritional value through plant biofortification techniques aimed at enriching plant tissue with specific elements [10].

Boron (B), an element contained in several foods such as fruits, leafy vegetables, legumes, nuts, wine, and beer is not considered to be an essential human nutrient [11]. Nevertheless, there are some evidences regarding the beneficial effects of B intake for humans on Ca metabolism and bone health, probably due to the effects of B on the increased efficiency in the utilization of vitamin D [12,13]. It is interesting to note that B can prevent vitamin D deficiency [12] and increase the levels of steroid hormones in serum by influencing their metabolism [14]. Although boron-recommended intakes for humans are not officially provided, [15] reported that an intake between 1.0 and 3.0 mg B per day could be considered adequate to obtain positive effects on aging prevention and longevity.

Although a specific biochemical function of B has not been identified in plants [16], this element is considered to be essential for vegetable growth [17]. Actually, B is frequently supplied as a foliar fertilizer to improve the nutritional quality of rice [18] and as an element of nutrient solution (NS) for growing soilless vegetables [19]. At the same time, it should be considered that a high B intake in plants, in relation to high concentrations in soil, growing substrate, and/or fertilization practice, could induce toxicity symptoms such as chlorosis and necrosis of leaves as well as the inhibition of plant growth [20]. It is interesting to highlight that the use of soilless systems allows to accurately dose the amount of each element in the NS, potentially avoiding any excess or deficiency of nutrients in plants. Furthermore, by acting on the mineral composition of the NS, it is possible to increase or reduce the concentration of target ions in plant tissue to obtain tailored vegetables for specific nutritional requirements [21–25]. For example, some authors [26,27] reported the use of NS with different potassium (K) levels to obtain low-potassium vegetables suitable for people affected by chronic kidney disease. On the other hand, Gonnella et al.,) [22] proposed the use of iodine-biofortified *Brassica* vegetables for preventing iodine (I) deficiency and related human disorders, while biofortification for silicon and calcium has been proposed to obtain vegetables suitable for women in premenopausal conditions [21–23].

In a previous study, we focused on increasing the silicon content in tissue (leaves and petioles) of purslane by using soilless cultivation [10]. However, to the best of our knowledge, there is a lack of information in the literature with regard to the B biofortification of purslane. Furthermore, it is important to highlight that purslane shows a high genetic variability which translates into a potential variability of different agronomic traits [28,29]. In this context, it should be considered that for a successful application of the biofortification strategy, the evaluation of several aspects of the process in relation to different genotype-related traits may be opportune.

Starting from these remarks, the aims of the present study are: (i) to increase the B content in edible parts of purslane without negatively affect crop performance, (ii) to evaluate the effects of different B levels on the content of healthy compounds and anti-nutritional factors in two different genotypes (wild and commercial) of purslane. The general goal is to verify the possibility of using

the floating hydroponic system as a method to calibrate the B uptake in purslane plants to obtain a tailored boron content in edible parts.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

Two experiments were conducted in a plastic greenhouse located at the experimental farm "La Noria" of the Institute of Sciences of Food Production (ISPA-CNR) in Mola di Bari (BA), Southern Italy (41°03′ N, 17°04′ E; 24 m a.s.l.). The first experiment was carried out from 29 June to 29 July, 2016, while the second experiment was carried out from 13 June to 7 July, 2017. Mean air temperature, relative humidity, and photosynthetically active radiation (PAR) inside the greenhouse during the experiments were: 29 °C, 53%, and 221.6 μmol/m²/sec (first experiment) and 30 °C, 47%, and 210.1 μmol/m²/sec (second experiment), respectively. Two different populations of purslane were used: the seeds of wild plants harvested in Policoro (Matera) for the first experiment and commercial seed purchased from the Riccardo Larosa Company (Andria, Italy) for the second experiment.

The seeds were sown in a cell pot containing peat. When seedlings reached the two-true leaves stage (after seven and six days from sowing, respectively, for the first and second experiments) the cell pots were moved on a floating hydroponic system, where plants were grown up to the end of the experiments. An NS composed of rain water, N (140 mg/L—NO_3-N:NH_4-N at a percentage ratio of 80:20), P (50 mg/L), K (200 mg/L), Mg (40 mg/L), S (102 mg/L), and Ca (100 mg/L) was used. Micronutrients were added according to [19], with slight modifications based on the common practice. Boron was added as boric acid to the NS at different concentrations according to the treatments—0.3, 3, and 6 mg/L—corresponding to a control (0.3 mg/L) and two levels of B biofortification (3 and 6 mg/L). The NS pH was measured every three days and it was adjusted to 5.5–6.0 using 1 M H_2SO_4. Moreover, an air pump was used in order to promote oxygenation of the NS and avoid possible root anoxia issues. A randomized complete block design with three replications was used. Each replication constituted of 576 plants.

2.2. Yield, Dry Weight, and Color Analysis

At the harvest (phenological stage of fourth to fifth true leaf, defined as "baby leaf", as reported by Di Gioia et al., [30]), 30 days for the 1st experiment and 24 days after sowing for the 2nd experiment. The yield (expressed as g of fresh weight (FW)/plant) was obtained by collecting 24 plants for each replicate (Figure 1).

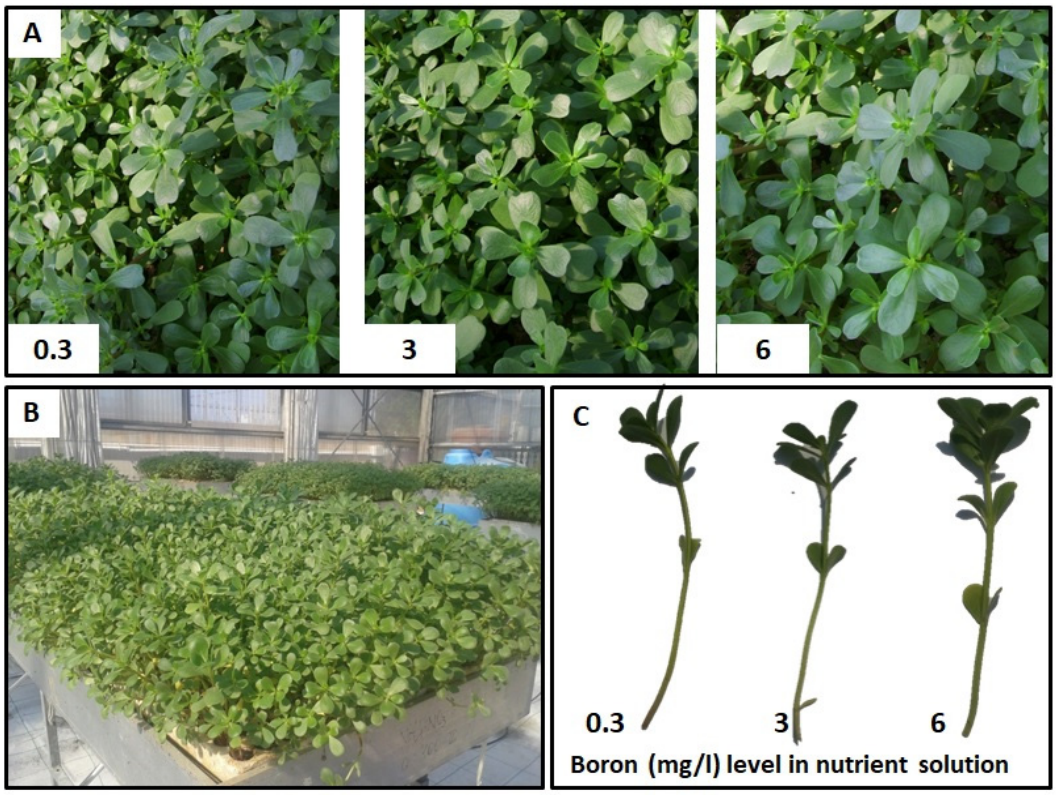

Figure 1. Wild (**A**) and commercial (**B** and **C**) purslane (*Portulaca oleracea* L.) cultivated with three different levels of Boron in nutrient solution.

For the measurement of dry weight (DW) in edible parts, fresh samples were maintained in a forced draft oven at 65 °C until a constant weight was reached. At harvest, the roots of purslane were harvested and abundantly washed with ultrapure H_2O (Milli-Q Millipore 18 M Ω/cm) in order to remove the residue of NS and successively maintained in a forced draft oven at 65 °C until a constant weight was reached.

Color parameters (*L*, *a** and *b**) were measured on the peel surface of ten leaves per replicate with a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in reflectance mode using the CIE*Lab* color scale. Before the measurements, the colorimeter was calibrated with a standard reference with *L*, *a**, and *b** values of 97.55, 1.32, and 1.41, respectively. Hue angle [$h° = \tan^{-1}(b^*/a^*)$] and saturation or chroma [$C = (a^{*2} + b^{*2})^{1/2}$] were then calculated from the primary readings.

2.3. Samples Preparation for Chemical Analysis

For each replication, purslane samples were freeze-dried by a LABCONCO FreeZone® Freeze Dry System, model 7754030, (Kansas City, MI, USA) equipped with a LABCONCO FreeZone® Stoppering Tray Dryer, model 7,948,030 (Kansas City, MI, USA). The freeze-dried samples were ground at 500 µm by using a Retsch laboratory mill (Torre Boldone, BG, Italy) to obtain a homogeneous powder

2.4. Glucose and Fructose Contents

Glucose and fructose contents were determined by ion chromatography (Dionex DX500, Dionex Corporation, Sunnyvale, CA, USA) using a pulsed amperometric detector (PAD) according to the protocol used by Renna et al., [31]. Peak separation was performed using a Dionex CarboPac PA1 separation column (Dionex Corporation) and isocratic elution with 50 mmol/L NaOH.

2.5. Extraction and Analysis of Total Phenols, Chlorophylls, and Carotenoids

The total phenol (TP) content was determined according to the Folin–Ciocalteu method by using the extraction methods reported by D'Imperio et al., (2019) [27]. Briefly, 200 mg of lyophilized sample were mixed with 10 mL of solvent mixture (MeOH:H_2O:CH_3COOH, 79:20:1% *v*/*v*/*v*). The vials were then placed in a sonicator bath at ambient temperature for 30 min, followed by 1 h in a magnetic stirrer. The mixture was centrifuged at 10,000 ×*g* at 4 °C for 10 min and the supernatant was transferred into a volumetric tube. The residue was resuspended in 10 mL of MeOH:H_2O:CH_3COOH (79:20:1% *v*/*v*/*v*), gently mixed manually, and sonicated for an additional 30 min, followed by stirring (1 h) and centrifugation (10,000 ×*g* at 4 °C 10 min). The TP content was determined using gallic acid (R^2 = 0.9991) as a calibration standard by using a Perkin–Elmer Lambda 25 spectrophotometer (Boston, MA, USA).

Chlorophylls and total carotenoid content were determined spectrophotometrically, using the extraction procedure reported by Montesano et al., (2018) [32]. Briefly, lyophilized samples were homogenized in a fresh solution of 80% acetone (C_3H_6O:H_2O, v/v) and stirred for 24 h at room temperature. After extraction, the samples were diluted and filtered by using 0.45 μm (regenerated cellulose, RC) and the absorbance of the extracts were measured at 662, 645 and 470 nm, using a UV-1800 spectrophotometer (Perkin–Elmer Lambda 25 spectrophotometer, Boston, MA, USA).

2.6. Minerals and Anions Analysis Purslane Samples

For Al, B, Ca, Cr, Fe, K, Mg, Mn, Na, and Zn determination, 0.3 g of dried sample were accurately weighted in microwave digestion vessels followed by the addition of 10 mL of 65% HNO_3 (Pure grade, Carlo Erba) and digested in a closed-vessel microwave assisted digestion system (MARS 6, CEM Corporation, Matthews, NC, USA). The digestion procedure was performed in two steps: 15 min to reach 200 °C and 10 min maintained at 200 °C (power set at 900–1050 W; 800 psi). The digested solutions were cooled and quantitatively transferred to a 50 mL volumetric flask. Each solution was diluted to volume with ultrapure H_2O (Milli-Q Millipore 18 M Ω/cm) and filtered using a 0.45 μm filter.

Samples were analyzed with a Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 VDV, Agilent Technologies, Santa Clara, CA, USA) to measure Ca, K, Mg, and Na in radial mode and Al, B, Fe, Mn, Zn, Al, and Cr in axial mode [33].

For nitrate and oxalate determinations, ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was used as reported by D'Imperio et al., (2018) [33]. Briefly, the nitrate and oxalate were extracted from DW samples with 3.5 mM (Na_2CO_3) and 1 mM ($NaHCO_3$) for 30 min. After extraction, the samples were diluted and filtered by using 0.45 μm (RC) followed by Dionex OnGuard IIP (ThermoScientific). The resulting solutions were analyzed by ion chromatography (IC-Dionex DX120, Dionex Corporation) with a conductivity detector by using an IonPac AG14 precolumn and an IonPac AS14 separation column (Dionex Corporation).

2.7. Statistical Analysis

The effects of different treatments were tested using a one-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.

3. Results

No differences were found in the first experiment as regards yield, dry weight (DW), and color parameters at increasing B level in the NS. The average values of yield and DW were, respectively, 16.22 g/plant and 6.69 g/100 FW, while *L*, *a**, *b**, h°, and C average values were, respectively, 42.76, −13.01, 18.76, 124.75, and 22.83 (Table 1). In the second experiment, the average values of yield and dry weight were 13.2 g/plant and 4.9 g/100 g (FW), respectively, with no differences among treatments (Table 1). By using both high and low boron level in the NS, the *L* value was 3% higher

than the control, while the average values of *a**, *b** h°, and C were, respectively, −10.9, 15.06, 125.89, and 18.59, with no differences among treatments (Table 1).

Table 1. Effects of different boron level in the nutrient solution on yield, dry weight, and color traits in edible parts of wild (first experiments) and commercial (second experiment) purslane.

Boron Level	Yield	Dry Weight	*L*	*a**	*b**	h°	C
mg/L	g/plant	g/100 of FW					
First experiment							
0.3	15.33 ± 0.32	7.12 ± 0.52	42.67 ± 0.89	−13.13 ± 0.52	18.90 ± 1.57	124.81 ± 1.42	23.02 ± 1.55
3	17.71 ± 1.76	7.14 ± 0.21	42.75 ± 0.78	−12.92 ± 0.46	18.25 ± 0.58	125.32 ± 0.73	22.36 ± 0.68
6	15.62 ± 0.32	6.63 ± 0.23	42.86 ± 0.20	−12.97 ± 0.29	19.13 ± 0.56	124.14 ± 0.51	23.11 ± 0.60
Significance	ns	ns	ns	ns	ns	Ns	ns
Second experiment							
0.3	13.40 ± 1.6	4.66 ± 0.33	40.71 ± 0.28 b	−10.67 ± 0.17	14.94 ± 0.46	125.55 ± 0.96	18.36 ± 0.39
3	14.73 ± 2.1	5.16 ± 0.83	41.99 ± 0.66 a	−11.25 ± 0.59	15.44 ± 0.65	126.08 ± 1.0	19.11 ± 0.82
6	13.22 ± 0.6	5.01 ± 014	42.11 ± 0.41 a	−10.77 ± 0.1	14.80 ± 0.28	126.03 ± 0.44	18.31 ± 0.25
Significance	ns	ns	**	ns	ns	Ns	ns

Different letters within each column and experiment indicate that mean values are significantly different, according to the Least Significant Difference ($\alpha = 0.05$). Significance: ns = not significant; ** $p \leq 0.01$ FW: fresh weight.

By using 3 and 6 mg/L of B in the NS, the B tissue content in wild purslane increased, respectively, 6.5- and 10.7-fold with respect to the control (Figure 2A), without causing any symptoms of toxicity in plants. In the second, by using 3 and 6 mg/L of B in the NS, the B content in commercial purslane increased, respectively, 1.85- and 2.74-fold compared to the control (Figure 2B), without causing symptoms of toxicity in plants.

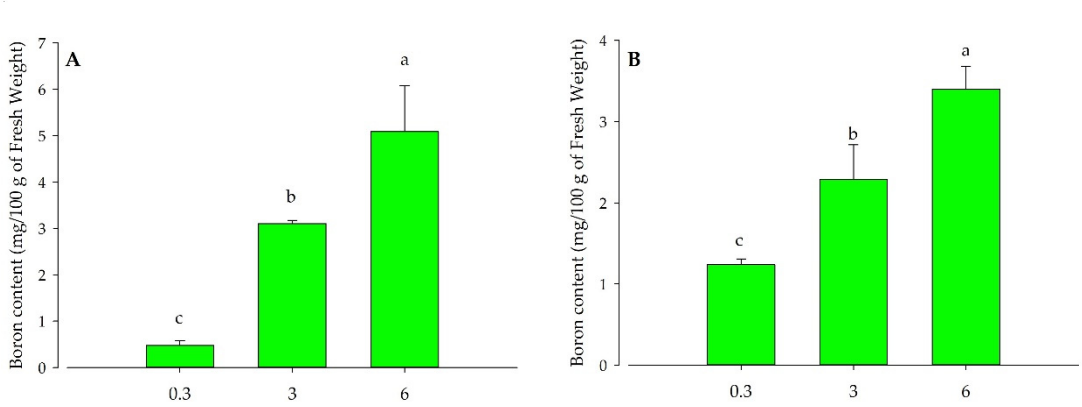

Figure 2. Boron content in edible parts of wild (**A**: firsxperiment) and commercial (**B**: second experiment) purslane, as affected by different boron levels in the nutrient solution, control (0.3 mg/L), and two levels of B biofortification (3 and 6 mg/L). Different letters indicate that mean values are significantly different, according to the Least Significant Difference ($\alpha = 0.05$).

As regards Ca, no differences were observed between the control and treatments. With respect to Fe, the highest content was found in purslane fertigated by using 3 mg/L of B, resulting in being 122.9% higher than the control and 6 mg/L of B. Zinc content was highest in the control, resulting in being 53.2 and 10.3% higher than high and low B levels, respectively (Table 2). The average contents of K, Mg, Na, and Mn were 74.74, 7.78, 0.458, and 0.102 g/kg of DW, respectively, with no differences among treatments. The Al and Cr were low with respect to the limit of quantification (LOQ), as reported in Table 2.

Table 2. Effects of different boron level in the nutrient solution the content of Ca, K, Mg, Na, Fe, Mn, and Zn in edible parts of wild (first experiment) and commercial (second experiment) purslane.

Boron Level	Ca	K	Mg	Na	Fe	Mn	Zn
mg/L	g/kg of DW				mg/kg of DW		
First experiment							
0.3	10.26 ± 0.3 ab	75.77 ± 4.72	7.99 ± 1.28	0.470 ± 0.1	24.54 ± 4.27 b	107.86 ± 25.2	83.33 ± 8.47 a
3	9.21 ± 0.57 b	71.54 ± 1.91	7.52 ± 0.33	0.460 ± 0.1	51.20 ± 5.8 a	100.80 ± 0.92	54.40 ± 7.60 c
6	11.11 ± 0.55 a	76.92 ± 0.40	7.83 ± 1.42	0.445 ± 0.2	21.40 ± 2.6 b	99.53 ± 18.41	75.53 ± 11.7 b
Significance	*	ns	ns	ns	***	Ns	***
Second experiment							
0.3	11.91 ± 1.3	88.50 ± 8.8	7.29 ± 0.78	1.98 ± 0.12	314.94 ± 68 b	217.13 ± 47 a	119.54 ± 5.6
3	10.39 ± 1.8	76.52 ± 10.6	7.37 ± 0.16	2.12 ± 0.23	958.21 ± 215 a	168.73 ± 21b	151.70 ± 23.6
6	13.32 ± 0.36	88.56 ± 4.0	7.95 ± 0.26	2.25 ± 0.26	316.95 ± 47 b	155.37 ± 11 b	138.64 ± 15.6
Significance	ns	ns	ns	ns	*	*	ns

Different letters within each column and experiment indicate that mean values are significantly different, according to the Least Significant Difference ($\alpha = 0.05$). Significance: ns = not significant; *$p \leq 0.05$; *** $p \leq 0.001$. DW: dry weight. Al and Cr < limits of quantification (LOQ), 1.1 and 0.82 mg/kg dry matter respectively. The LOQ of the method was calculated as follows: 10 × mean of standard deviation (n. 15).

In the second experiment, the use of 3 mg/L of B in the NS allowed to obtain an Fe content 2.9-fold higher than other treatments, while the highest Mn content was found in the control, resulting in being 34% higher than other treatments (Table 2). The average contents of Ca, K, Mg, Na, and Zn were, respectively, of 11.86, 84.53, 7.54, 2.12, and 0.14 g/kg DW, without differences among treatments (Table 2). Furthermore, in this case, Al and Cr were low with respect to the limit of quantification (Table 2).

Purslane fertigated by using 3 mg/L of B level showed the lowest content of nitrate, resulting 32% lower than other samples (Table 3). The average oxalate concentration was 4.73 g/kg FW with no differences among treatments, while the content of glucose and fructose decreased, respectively, of 38% and 32% by using high B level (Table 3). In the second experiment, the average values of NO_3, oxalate, glucose and fructose were, respectively, of 0.32, 4.08, 1.63, and 1.53 g/kg FW, without differences among treatments (Table 3).

Table 3. Effects of different boron levels in the nutrient solution on the content of nitrate, oxalate, glucose, and fructose in edible parts of wild (first experiment) and commercial (second experiment) purslane.

Boron Level	NO_3	Oxalate	Glucose	Fructose
mg/L	g/kg FW			
First experiment				
0.3	0.33 ± 0.03 a	4.8 ± 0.47	4.90 ± 1.06 a	3.79 ± 0.35 a
3	0.23 ± 0.02 b	4.55 ± 0.11	4.65 ± 027 a	4.06 ± 0.44 a
6	0.34 ± 0.05 a	4.85 ± 0.42	2.94 ± 0.31 b	2.67 ± 0.22 b
Significance	*	ns	*	**
Second experiment				
0.3	0.37 ± 0.04	3.86 ± 0.39	2.01 ± 0.27	1.68 ± 0.21
3	0.28 ± 0.02	4.41 ± 0.65	1.25 ± 0.98	1.31 ± 0.18
6	0.29 ± 0.01	3.96 ± 0.26	1.64 ± 0.19	1.60 ± 0.03
Significance	ns	ns	ns	ns

Different letters within each column and experiment indicate that mean values are significantly different, according to the Least Significant Difference ($\alpha = 0.05$). Significance: ns = not significant; *$p \leq 0.05$; ** $p \leq 0.01$. FW: fresh weight.

In the first experiment, the highest total phenol content was found in control conditions, resulting in being 34.5% higher than other treatments, while the average values of CHLa, CHLb,

CHLtot, and carotenoids were 7.31, 1.14, 8.45, and 2.59 mg/100 g of FW, respectively, with no differences among treatments (Table 4). In the second experiment, by using both low and high B levels in the NS, the content of CHLa, CHLb, CHLt, and carotenoids decreased, respectively, of 40%, 55%, 45%, and 41% compared to the control, while the average total phenol content was of 106.75 mg/100 g FW without differences among treatments (Table 4).

Table 4. Effects of different boron levels in the nutrient solution on the content of total polyphenols, chlorophyll (A and B), and carotenoids in the edible parts of wild (first experiment) and commercial (second experiment) purslane.

Boron Level	Total Phenols	CHLa	CHLb	CHLt	Carotenoids
mg/L	mg/100 g FW				
First experiment					
0.3	143.8 ± 17.2 a	7.31 ± 1.0	0.86 ± 0.22	8.17 ± 1.16	2.67 ± 0.18
3	103.6 ± 8.9 b	6.97 ± 0.67	0.78 ± 0.40	7.75 ± 0.92	2.35 ± 0.24
6	110.2 ± 17.7 b	7.67 ± 0.41	1.78 ± 1.02	9.44 ± 1.11	2.75 ± 0.10
Significance	**	ns	ns	ns	ns
Second experiment					
0.3	123.63 ± 13.1	16.1 ± 1.9 a	6.9 ± 1.0 a	23 ± 2.9 a	9.8 ± 0.86 a
3	110.18 ± 35.8	10.7 ± 1.2 b	3.7 ± 0.7 b	14.5 ± 1.9 b	6.5 ± 0.80 b
6	86.44 ± 10.3	8.5 ± 0.7 b	2.4 ± 0.8 b	10.9 ± 1.5 b	4.9 ± 0.57 b
Significance	ns	**	**	**	**

Different letters within each column and experiment indicate that mean values are significantly different, according to the Least Significant Difference ($\alpha = 0.05$). Significance: ns = not significant; ** $p \leq 0.01$. FW: fresh weight.

The boron level in the NS did not affect the dry weight (22.89 g/plant, on average) of wild purslane roots (Table 5). The control showed the lowest K content, resulting in being 43% lower than the other B levels. The average content of Ca, Mg, Na, Fe, Mn, Al, and Zn were, respectively, 13.19, 4.36, 997, 2316, 142, 2405, and 302 mg/kg DW, without differences among the treatments (Table 5). The B and Cr were low with respect to the limit of quantification (Table 5). No differences were found as regards the dry weight (10.66 g/plant, on average) of commercial purslane roots when different boron levels in the NS were used, while the roots of plants fertigated with 6 mg/L of B showed an Al content 63% lower than other treatments. At the same time, the average concentrations of Ca, Mg, Na, Fe, Mn, and Zn were, respectively, of 10.82, 3.09, 733, 922, 103, and 264 mg/kg DW, without differences among treatments (Table 5). The B and Cr were low with respect to the limit of quantification (Table 5).

Table 5. Effects of different boron levels in the nutrient solution on dry weight and content of Ca, K, Mg, Na, Fe, Mn, Al, and Zn in wild (first experiment) and commercial (second experiment) purslane roots.

Boron Level	Dry Weight	Ca	K	Mg	Na	Fe	Mn	Al	Zn
mg/L	g/plant		g/kg of DW		mg/kg of DW				
First Experiment									
0.3	17.00 ± 4.7	14.67 ± 0.8	13.73 ± 1.8 b	3.83 ± 0.46	733 ± 62	2426 ± 273	138.7 ± 19	2409 ± 212	364 ± 11
3	21.96 ± 5.1	11.76 ± 1.7	23.95 ± 0.6 a	4.24 ± 0.53	1034 ± 230	2429 ± 271	155.7 ± 20	2888 ± 590	260 ± 45
6	29.63 ± 8.5	13.13 ± 2.8	24.32 ± 1.5 a	5.00 ± 0.51	1226 ± 150	2094 ± 213	131.7 ± 9.4	1919 ± 557	284 ± 48
Significance	ns	ns	*	ns	ns	ns	ns	ns	ns
Second Experiment									
0.3	11.49 ± 1.9	9.67 ± 1.6	54.1 ± 4.5	2.88 ± 0.1	698 ± 8.4	871 ± 65	92.9 ± 7.8	734 ± 141 a	279 ± 67
3	10.52 ± 2.4	10.45 ± 0.7	51.8 ± 3.4	3.12 ± 0.4	752 ± 43	1046 ± 42	91.4 ±	818 ± 150a	251 ± 26
6	9.96 ± 2	12.33 ± 3.7	58.6 ± 24.3	3.27 ± 0.3	749 ± 87	849 ± 112	126.2 ±	290 ± 22 b	262 ± 45
Significance	ns	ns	ns	ns	ns	ns	ns	**	ns

Different letters within each column and experiment indicate that mean values are significantly different, according to the Least Significant Difference (α = 0.05). Significance: ns = not significant; *$p \leq 0.05$; ** $p \leq 0.01$. DW: dry weight. B and Cr < limits of quantification (LOQ), 1.7 and 0.82 mg/kg DW, respectively. The LOQ of the method was calculated as follows: 10 × mean of standard deviation (n. 15).

4. Discussion

The present study reports scientific evidences on the effectiveness of a cultivation process, based on a soilless system combined with specific B concentrations in the NS, for the B biofortification of purslane. Taking into consideration the high genetic variability intrinsic in this species [28,29], the possibility to obtain a B enrichment in edible parts was tested over two different genotypes (wild and commercial) of *P. oleracea* L.

Increasing B levels in the NS significantly boosted the B content in edible parts of purslane (Figure 2) with no differences in yield and DW (Table 1). Our results are in agreement with several authors [34–39], who reported significant increases of B in different species as a consequence of high concentrations of this element in growing media and/or NS. In fact, B moves from the roots with the transpiration stream and accumulates in growing points of leaves and stems [40]. In addition, the transport of B from roots to shoots increased linearly according to the increase in the concentration of B in the rooting medium [17].

Nevertheless, it should be considered that in response to high B levels [38,39], a yield reduction and symptoms of toxicity in purslane plants was observed. Landi et al [37] observed a strong reduction of yield and DW as well as symptoms of toxicity also in basil plants by using 20 mg/L of B in the NS, although this concentration promoted B uptake in the edible parts of two basil cultivars. In our study, no visible symptoms of toxicity in plants were observed, and no differences of color parameters were detected, with the exception of little *L* value variation in the second experiment (Table 1). This indicates that the color of boron-enriched purslane is almost similar to the control. Therefore, since color, yield, and DW are important traits for assessing crop performance, the results of the present study suggest that the boron biofortification of purslane is effective at a concentration of 3–6 mg/L in the NS without negatively affecting crop performance.

From a nutritional point of view, it should be considered that although B is not considered to be essential for humans [11], some authors [13,15,41] suggested an average daily intake of B of about 2 mg in order to improve bone health. In our study, we found an average B content of 2.69 and 4.24 mg 100 g FW, respectively, in purslane grown by using 3 and 6 mg/L of B in the NS (Figures 2). Therefore, the suggested intake of B could be satisfied by consuming about 75 g or 48 g, respectively, of purslane grown by using 3 and 6 mg/L of boron in the NS. Rouphael et al [42] suggested that the management of the NS composition in soilless systems can be considered an important strategy for improving the nutritional quality of vegetable products. By using 3 mg/l of B in the NS, we found the highest Fe content in the edible parts of purslane, resulting in being about 2.5-fold higher than the other treatments (Tables 2). These results are in agreement with Parks et al [43] who found that Fe concentration in tomato leaves increased by increasing the B supply to a certain extent, while a further increase in B caused a decrease in Fe concentration. On the other hand, no differences in Fe concentration were observed in leaves of maize and sunflower by increasing the B supply [44,45]. Therefore, it is likely that the Fe concentration in leaves at different B levels depends on the species. At the same time, in our second experiment (commercial seed), we found an average Fe content about 10-fold higher than the first experiment (seed of wild plants). In this regard, it is interesting to note that also Petropoulos et al [29] found a great variability in Fe content among six genotypes of purslane (from 0.16 to 2.34 mg/100 g FW). Therefore, our results suggest that the Fe content in purslane is strongly affected by both the genotype and level of supplied B. As regards Zn, we observed different contents in the edible parts of purslane by varying the B level in the NS but only in the first experiment (Tables 2). At the same time, only in the second experiment, we observed a higher Mn content in the control than other treatments (Tables 2). For all other mineral elements, no differences were found in the edible parts of purslane (Tables 2). These results suggest that, with the exception of Fe, the content of mineral elements in the edible parts of purslane is not or slightly influenced by different B levels in the NS but can be affected by genotypes. Furthermore, Petropoulos et al [29] showed some differences in mineral composition among six genotypes of purslane, while by increasing B levels during purslane growth only little differences in some mineral elements were found by other authors [38,39]. Likewise, also the content of mineral elements in

purslane roots was unaffected by different B levels in the NS with the exception of K in the first experiment (lower content in control than other treatments) and Al in the second experiment (lower content in 6 mg/l of B level than other treatments—Table 5). The literature lacks information regarding the effects of different B levels on mineral element content in purslane roots. The boron level did not affect the root growth in our study (Table 5), these results confirm the absence of toxicity for purslane by using the B levels proposed in this study. A lower root mass in purslane was observed by some authors [38,39], when excess B levels were applied. In addition, it is interesting to note that the ratio between shoots and roots was very different between the two tested genotypes (0.6 in the wild and 1.4 in the commercial), confirming the fact that a wild genotype has a tendency to a greater root system as an ancestral trait aimed to improve plant tolerance to stress conditions.

In this study, we also evaluated the effect of different levels of B in the NS on the glucose and fructose content in the edible parts of purslane. Only in the first experiment, we found for both sugars a lower content in purslane grown with 6 mg/L of B compared to other B treatments (Table 3). In a study aimed to evaluate the effect of B on nitrogen metabolism and sugar levels of sugar beet, Bonilla et al [46] found a decrease of the sugar content in leaves by increasing B concentration beyond 2.5 ppm. In agreement, our results suggest that also in purslane, B can play a role on sugar metabolism. On the other hand, it is important to note that in both experiments, no increase of sugar concentration in purslane was observed by increasing the B levels in the NS. These results confirm the absence of toxicity symptoms in plants of purslane by using the B levels proposed in this study. Yokota and Konishi [47] suggested that the accumulation of soluble carbohydrates in leaves is necessary to alleviate symptoms of toxicity due to high B concentrations.

Phenols, widely distributed in plants, represent an important group of compounds for humans, due to their antioxidant activities and ability to scavenge free radicals. In our study, only in the first experiment, we found differences among treatments, consisting in a phenol content higher in the control than purslane grown by using 3 and 6 mg/L of B (Table 4), while in the second experiment, we found an average total phenol content of 107 mg/100 g FW (Table 4). There is evidence that the B nutritional status of vascular plants influences the phenol metabolism and vice versa, but the specific nature of these interactions is not established [48]. In the leaves of tobacco plants grown both under B deficiency or toxicity, Ruiz et al [49] and Chamacho-Cristobal et al [50] both reported an increase in phenolic content. Boron forms complexes mainly with pectins and phenols in the cell wall and plasma membrane, respectively, resulting in a higher stability of these structures. When the B supply is adequate, more than 60% of this element is in free form in leaves' tissues [51]. In conditions of B, excess phenols may play a role in B compartmentalization rather than in antioxidant activity [52] and improve the response of plants at B treatments.

As regards chlorophylls and carotenoid content, no differences were observed among treatments during the first experiment, while in the second experiment, we found a higher content of both total chlorophyll (23.0 mg/kg FW) and carotenoids (9.8 mg/100 g FW) in the control than in purslane grown with 3 and 6 mg/L of B (on average 12.7 and 5.7 mg/100 g FW, respectively, for total chlorophylls and carotenoids—Table 4). In a study aimed to evaluate the response of purslane to different levels of B in the growing media, [39] found no differences in chlorophyll content by using 0, 5, and 25 mg B per kg of growing substrate, while a lower content was found by using 10 mg B per kg of growing substrate. Furthermore, for the carotenoids, these authors found a decreased content in purslane grown with 10 mg B per kg of growing. It is important to note that these authors observed a yield reduction only when 25 mg B per kg of growing substrate was used. In another study [38] found an increase of chlorophyll and carotenoid content by using B levels, respectively, ≥16 and ≥8 g per kg of growing substrate, equivalent to B levels toxic for purslane plants. The reduction of chlorophyll content founded in this study could be related with the reduction of photosynthesis as a consequence of structural damage of thylakoids. This, in turn, altered the rate of the electron transport and influenced CO_2 photoassimilation, which can also be limited by stomatal reduction, as suggested by Pereira et al., [53].

Despite some interesting nutritional traits, as already outlined above, it should be considered that purslane is classified as a species rich in oxalate and nitrate, both well-known anti-nutritional

factors. Oxalate intake in humans can reduce the bioavailability of some minerals such as Ca, Mg, K, and Fe, and cause the formation of kidney stones in predisposed subjects [5]. Noonan and Savage [54] reported an oxalate content in purslane ranging from 9.1 to 16.8 g/kg FW, while an amount of 23.4 g/kg FW is indicated by other authors [55]. At the same time, Charfeddine [56] found values not higher than 5.0 g/kg FW in purslane grown by using a floating system. In this context, it has been proposed that the accurate calibration of the NO_3-N:NH_4-N ratio achievable by using hydroponic cultivation (floating system in the case of the present study) allows to reduce the synthesis of organic acids, such as oxalic ones, and/or optimize the activity of the oxalic acid oxidase avoiding a high accumulation of oxalates in leaves and stems [5]. In agreement, we found an oxalate content ranging from about 4.1 to 4.7 g/kg FW (Tables 3), without differences between B treatments, by using a NO_3-N:NH_4-N ratio of 80:20.

Nitrate, per se, is relatively non-toxic, but after ingestion, its reaction products and metabolites, such as nitrite, nitric oxide, and N-nitroso compounds can negatively impact on human health [57]. Purslane is considered as a species rich in NO_3, since its content generally exceed 2500 mg/kg FW, although wild plants of purslane gathered in Southern Italy showed a NO_3 content ranging from 360 to 2100 mg/kg FW [5]. In our study we found a NO_3 content not higher than 370 mg/kg FW. Furthermore, we observed the lowest NO_3 content (226 mg/kg FW) during the first experiment when 3 mg/L of B was used (Table 3). Bonilla et al [46] found a decrease of NO_3 in sugar beet leaves when the B level in the NS was increased from 0.05 to 2.5 mg/L, while by increasing the B concentration beyond 2.5 mg/L, an increase of NO_3 in leaves was observed. At the same time, these authors detected an increase of nitrate-reductase activity when the B level in the NS was increased from 0.05 to 2.5 mg/L, while by increasing the B concentration beyond 2.5 mg/L, a decrease of enzyme activity was observed. Therefore, these authors hypothesized a dose-dependent specific action of B on the nitrate-reductase activity which affects NO_3 accumulation or its assimilation into nitrogen organic forms. At the same time, the absence of significant differences of NO_3 content between treatments during the second experiment highlights that, apart from the B availability for plants, other factors (i.e., light exposure and genotype) can play a substantial role on the nitrate-reductase activity [58,59]. In this context, it is well-known that a lower light exposure can increase the NO_3 content in plants. It is possible that the average slightly higher NO_3 contents in the second experiment could be also due to the lower photosynthetically active radiation measured (210.1 and 221.6 μmol/m^2/sec, respectively for second and first experiment). At any rate, it could be interesting to evaluate the NO_3 content in purslane found in our study in relation to the tolerable levels for NO_3 in foodstuffs. To this aim, it is important to note that regarding vegetables, the European Regulation [60] reports maximum levels of nitrate only for the "rucola" group (*Eruca sativa* L., *Diplotaxis* spp., *Brassica tenuifolia* L. and *Sisymbrium tenuifolium* L.), spinach (*Spinacia oleracea* L.), and lettuce (*Lactuca sativa* L.). The European Regulation fixed the maximum levels of 6000 and 7000 mg NO_3 per kg FW for "rucola" (respectively, for harvesting in the spring-summer and autumn-winter periods), 2000 and 3500 mg NO_3 per kg FW for spinach (respectively, for frozen and fresh products) and from 2000 to 5000 mg NO_3 per kg FW for lettuce, depending on several factors such as cultivar type ("iceberg" type or other ones), growing system (in open air or under cover), and growing period (spring-summer or autumn-winter). Considering these maximum levels, our results show the successful hydroponic production of boron-biofortificated purslane with also a relatively low content of NO_3.

5. Conclusions

In the present study, we demonstrated the possibility of using the floating hydroponic system as a method to calibrate the B uptake in edible parts of purslane by acting on B concentration in the NS. The results suggest that B biofortification of purslane is effective at a concentration of 3–6 mg/L in the NS without negatively affecting crop performance. The robustness of our findings is confirmed by the fact that the proposed biofortification strategy was tested on two different purslane genotypes (wild and commercial) and the B biofortification was successful in both cases. From a nutritional point of view, the average daily intake of B could be satisfied by consuming reasonably sized portions of this vegetable. The boron biofortified purslane obtained in this study can be

proposed as a tailored vegetable for people with specific nutritional requirements for which B assumption is beneficial. In particular, it is important to mention that the achievements of this study on B biofortification add to those already achieved by this research group on the biofortification of vegetables with other elements beneficial for bone health (namely silicon and calcium).

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