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Biostimulatory Action of a Plant-Derived Protein Hydrolysate on Morphological Traits, Photosynthetic Parameters, and Mineral Composition of Two Basil Cultivars Grown Hydroponically under Variable Electrical Conductivity

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Abstract: Hydroponics is a viable alternative to open field cultivation for year-round vegetable production in urban areas. However, the total dependence on external chemical inputs (fertilizers) makes these systems often less environmentally sustainable. In this perspective, the use of biostimulants could represent a valuable and eco-friendly tool to limit the excessive use of fertilizers without a negative impact on the yield. To this end, our work aimed to evaluate the productive and physiological response of two cultivars of 'Genovese' basil (Eleonora and Italiano Classico) for the industrial production of "pesto" grown for 22 days in two nutrient solutions with different electrical conductivity (1 and 2 dS m^{-1}) and the application of two doses of protein hydrolysates (0.15- and 0.30-mL L^{-1} of Trainer® in the nutrient solution). The mineral profile was evaluated by ion chromatography coupled with a conductivity detector, while pigments were evaluated by UV-Vis spectrophotometry. Generally, the nutrient solution concentration did not significantly affect the fresh yield of the two cultivars tested. On the contrary, the use of the maximum dose of biostimulant (BT_2 = 0.30 mL L^{-1} of nutrient solution) increased fresh yield, leaf area, and ACO_2 by 20.7, 27.5, and 17.6%, respectively, compared with the control. Using the lowest dose of biostimulant (BT_1 = 0.15 mL L^{-1} of the nutrient solution) reduced nitrate by 6.6% compared with the control. The results obtained showed that basil cultivation in a floating raft system combined with biostimulant in the nutrient solution could be an excellent solution to improve productivity, reduce nitrate, and cut fertilizer costs.

Keywords: *Ocimum basilicum* L.; biostimulants; floating raft system; nutrient solution concentration; ion chromatography; nitrate

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1. Introduction

Climate change and rapid unplanned urbanization aggravate the erosion of agricultural land, a valuable nonrenewable resource. A situation imperiled by the steady growth of the world's population (which will reach 10 billion by 2050) challenges the agricultural sector to adopt extensive cropping systems and techniques to ensure food security [1,2]. In this scenario, hydroponics is an effective and practical solution to meet the rapidly changing needs of agriculture. Soilless crops provide better space optimization, including abandoned urban areas that are not suitable for traditional agriculture, where environmental conditions do not interfere, leading to higher yields because of a higher-density setup [3]. Not least, the sudden change in the lifestyle of consumers in the most industrialized countries, who are increasingly conscious of their "waistlines" and are pressured to eat fast meals to keep up with their hectic lives, has increased the consumption of fresh-cut herbs (such as *Lactuca sativa* L., *Spinacia Oleracea* L., *Beta vulgaris* L., *Eruca sativa* Mill.),

which are increasingly grown in soilless systems [4]. However, leaning completely on nutrient solutions that exceed plants' nutrient exigencies undermines the sustainability of hydroponic systems [5,6].

As widely observed in the field, even in superintensive agricultural sectors such as soilless systems, biostimulants increase nutrient use efficiency, partially reducing the use of traditional chemical input while improving crop yield and quality [7–11]. However, an in vivo understanding of the physiological and molecular influence of biostimulants is still under investigation to clarify and improve their efficiency [12]. Therefore, the beneficial effects depend on the mode and timing of application, dose, and composition [7]. Among the different categories of nonmicrobial biostimulants are plant-derived protein hydrolysates (PH) that differ from the rest in the distinctive functions they perform. PHs are produced from organic waste biomass, recycling by-products deriving from anthropogenic activities with positive repercussions from both economic and ecological points of view [13–15]. These hydrolysates are a heterogeneous mixture of oligopeptides, polypeptides, and amino acids (e.g., aspartic acid, glutamic acid, and essential amino acids), produced primarily by enzymatic processes [16]. The latter, as observed by Noroozlo et al. [17] and Souri and Hatamian [18], play different crucial roles in plant metabolism.

As reported in the literature, PHs represent a successful ecological strategy to reduce chemical input by promoting the availability, uptake, and metabolic use of macro and micronutrients and improving crop production and quality performance, especially under suboptimal growth conditions [16,19]. The abovementioned beneficial PHs effects are attributable to signal peptides with a hormone-like activity that can stimulate shoot growth, modulate root architecture, and improve nutrient uptake [20,21]. The biostimulation activity of PHs triggers molecular and physiological processes involving increased hormonal activities, enzymatic antioxidants (catalase, ascorbate peroxidase, superoxide dismutase, peroxidase, and glutathione reductase), nonenzymatic secondary metabolites and pigments, and the activation of processes and key enzymes involved in C metabolism and the nitrogen cycle (GOCAT, GS, NiR, and NR) [2,22]. As pointed out by several authors [23–25], the use of PHs is a potential eco-friendly and effective solution to overcome the environmental problems resulting from excessive use of fertilizers, often produced from nonrenewable resources [26].

However, an investigation of PHs effects should be carried out on a wider range of staple foods and medicinal plants. Among the latter, basil (*Ocimum basilicum* L.) is undoubtedly one of the most cultivated in Italy, with a total annual production of approximately 8000 tons [27] for the gastronomic sector, as young leaves are the main ingredient in typical regional dishes (pesto sauce and pizza Margherita) [28]. In addition, the great morphological and phytochemical variability of the *Ocimum* genus [29] has also allowed this medicinal plant to find wide use in the pharma-cosmetic sector [30]. The necessity to meet the growing demands of the food processing industry, which requires a deseasonalized and well-standardized production, has pushed the whole production sector towards hydroponic cultivation of Genovese basil [30]. As well as guaranteeing higher yields, these systems can improve functional and organoleptic quality while reducing the incidence of pests and pathogens.

Thus far, the scientific community has focused its research mainly on the evaluation of the effects induced by microbial biostimulants on basil, and only recently, Rouphael et al. [21] investigated the effects of biostimulants based on plant and animal origin protein hydrolyses (Trainer® and Siapton®) on sweet basil cultivar Gecom grown in agricultural soil. To the authors' knowledge, this work is the first to test the effect of PHs applied directly and constantly in contact with the root zone of basil grown in a floating system. The integration of biostimulants into traditional cropping systems could be a beneficial resource for reducing chemical inputs. In the light of this, our study analyzes in detail if and how the use of biostimulants of plant origin can reduce the use of chemical fertilizers for hydroponic basil production. The present study constitutes a continuation of our previous work, where the nutritive, aroma profile, and phytochemical aspects of two basil cultivars indicated that, in

certain conditions, the application of PH can improve the functional quality attributes (i.e., total phenolic concentration) of Genovese basil for pesto. Taking into account the importance of soilless basil cultivation, we evaluated the productive, mineral composition, and physiological response to root integration of a PH (Trainer®) at two doses on two cultivars of Genovese basil (Eleonora and Italiano Classico) grown at two levels of nutrient solution concentration.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

A floating raft system (FRS) experiment was carried out at the Department of Agriculture, University of Naples "Federico II", Portici, Italy (40°48′ N, 14°20′ E, 29 m.s.l.) from June 9 to June 30, 2020, in a passively ventilated greenhouse. A trifactorial randomized complete block experimental design was used, in which two different nutrient solution concentrations (NSC) (1 dS m^{-1} and 2 dS m^{-1}, hereafter NSC_1 and NSC_2, respectively), two basil cultivars (*Ocimum basilicum* L.) (Eleonora, Enza Zaden, Enkhuizen, NH, The Netherlands and Italiano Classico, La Semiorto Sementi, Sarno, Italy) and two doses of biostimulants (0.15 and 0.30 mL L^{-1}, hereafter BT_1 and BT_2, respectively) plus an untreated control were considered as factors. Each experimental treatment was replicated three times (n = 3) for a total of 36 experimental units, each consisting of a polystyrene tray containing 54 plants floating in a tank filled with 35 L of nutrient solution. Both nutrient solutions (NSC_1 and NSC_2) were prepared from osmosis water and contained the same concentrations of micronutrients (15 µM iron, 9 µM manganese, 0.3 µM copper, 1.6 µM zinc, 20 µM boron, and 0.3 µM molybdenum). NSC_1 was obtained by halving the macronutrient concentration of NSC_2 characterized by: 14.0 mM nitrate, 4.5 mM calcium, 5.0 mM potassium, 1.75 mM sulfur, 1.5 mM phosphorus, 1.5 mM magnesium, and 1.0 mM ammonium. For each tank, the nutrient solution oxygenation was provided by a submersible pump (Aquaball 60, Eheim, STU, Deizisau, Germany), and pH was continuously monitored and maintained at values of 5.8 ± 0.2. At transplanting (9 June), the commercial biostimulant Trainer® (plant PH obtained by enzymatic hydrolysis of legume biomass; Supplementary Table S1) was applied directly to the nutrient solution at two different doses (0.15 mL L^{-1} and 0.30 mL L^{-1}). To prevent large fluctuations in EC, pH, and ionic concentrations, the nutrient solutions were completely renewed from all tanks weekly.

2.2. Harvest and Soil Plant Analysis Development Index (SPAD), Leaf Gas Exchange, and Chlorophyll Fluorescence Determination

At the end of the experiment (30 June, 22 days after transplanting (DAT)), twenty-five plants from each experimental unit were collected to perform biometric measurements. The selected plants were separated into leaves and stems to determine fresh weights (g $plant^{-1}$), stem diameter (cm), node number, leaf-to-stem ratio, and leaf area (cm^2) using ImageJ software version 1.50 (US National Institutes of Health, Bethesda, MD, USA). The sampled material was placed in a ventilated oven at 65 °C for approximately 72 h and then stored for mineral analysis.

At harvest, measurements of the SPAD index were made on the adaxial side of twenty fully expanded young leaves per experimental unit using a portable SPAD-502 m (Konica Minolta Co., Ltd., Osaka, Japan). At 22 DAT, on the same leaves used for the determination of the SPAD index, between 10:30 and 12:30, via a portable fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA), measurements of the maximum quantum efficiency of PSII (expressed as Fv/Fm) were made. Chlorophyll fluorescence was performed after adaptation of leaves to darkness (for at least 10 min) using specific leaf clips. The maximum quantum efficiency of photosystem II (PSII) Fv/Fm was calculated as (Fm − F0)/Fm, where F0 and Fm were the ground fluorescence signal and maximum fluorescence intensities in the dark-adapted state, respectively. The determination of net CO_2 assimilation rate (ACO_2; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}), and transpiration (E; mmol H_2O m^{-2} s^{-1}) was performed using an LI-6400 (LI-COR Biosciences, Lincoln, NE, USA). The CO_2 of the gas exchange analyzer chamber was set at ambient values

(approximately 400 ppm) and photosynthetically active radiation at 1000 µmol m^{-2} s^{-1}. Instantaneous water use efficiency (WUEi) was calculated as ACO_2/E.

2.3. Determination of Minerals

For the determination of minerals, 0.25 g of finely ground dried sample (MF10.1 cutting head mill, IKA®, Staufen im Breisgau, Germany), sieved (MF0.5, 0.5-mm hole; IKA®, Staufen im Breisgau, Germany), and extracted in ultrapure water (Arium® Advance EDI (Sartorius, Goettingen, Germany) by stirred water bath (80 °C for 10 min; SW22, Julabo, Seelbach, Germany), were analyzed by ion chromatography (ICS 3000, Thermo Fisher Scientific™ Dionex™, Sunnyvale, CA, USA) according to the method described by Formisano, et al. [31]. An analytical column IonPac CS12A, an IonPac CG12A precolumn, and a self-healing electrolyte suppressor CERS5000 (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) while an IONPAC® ATC-HC 9 × 75 mm trap, an IONPAC® AG11-HC 4 × 50 mm guard column and an IONPAC® AG11-HC 4 × 50 mm column were used for anions and cations, respectively. Each treatment was analyzed in triplicates, and the results, except for nitrate (expressed as mg kg^{-1} fresh weight-fw), were expressed as g kg^{-1} dw. All columns were purchased from Thermo Fisher Scientific™ Dionex™ (Sunnyvale, CA, USA).

2.4. Determination of Chlorophylls and Carotenoids

The determination of total chlorophylls (chlorophyll a + chlorophyll b) and total carotenoids was performed according to the methods described by El-Nakhel et al. [32] with some modifications. Briefly, 0.50 g of fresh frozen leaves were extracted in the dark (15 min) in ammonia acetone (90% *v/v*; Carlo Erba Reagents Srl, Milan, Italy). Subsequently, the extracts were centrifuged (3000 rpm for 5 min; R-10M (Remi Elektrotechnik Ltd., Mumbai, India), and the pigment concentration was determined by UV-Vis spectrophotometry (DR 4000, Hach Co., Loveland, CO, USA), by reading the absorbances at 647, 664, and 470 nm (for chlorophyll a, b, and carotenoids, respectively). Total chlorophylls were calculated as the sum of chlorophyll a and b. All the pigments were expressed as mg g^{-1} fw.

2.5. Statistics

A two-way analysis of variance (ANOVA) was implemented to assess the significance of the effects and interactions between the factor pairs: Cultivar × Biostimulant Treatment (CV × BT), Biostimulant Treatment × Nutrient Solution Concentration (BT × NSC), and Cultivar × Nutrient Solution Concentration (CV × NSC). One-way ANOVA was used to compare the mean effect of Biostimulant Treatment (BT), whereas Cultivar (CV) and Nutrient Solution Concentration (NSC) were compared according to Student's *t*-test. The statistical significance was determined at the $p < 0.05$ level using the Tukey–Kramer HSD test for CV × BT, BT × NSC, and CV × NSC interactions and for the BT factor. All data were presented as mean ± standard error. All statistical analyses were performed using IBM SPSS 20 (Armonk, NY, USA) package for Microsoft Windows 11.

3. Results

3.1. Yield and Yield Parameters

BT factor had a highly significant main effect ($p \leq 0.001$) on all biometric variables reported in Table 1. Contrary to the effect of CV, the NSC factor did not significantly influence the total fresh weight. Supplementation with biostimulants in the nutrient solution, regardless of doses (BT_1 and BT_2), increased all measured biometric variables compared with the control.

The CV × BT interaction resulted in significant differences for all parameters, except the leaf-to-stem ratio and stem diameter. Particularly, for Eleonora and Italiano Classico, compared with the control, the BT2 dose of biostimulant increased the total fresh weight by 18.99 and 22.63%, respectively. Unlike the other parameters, in Eleonora, the biostimulant did not significantly affect the node number, which increased by 11.78% (on average) in Italiano Classico compared with the control.

Table 1. Analysis of variance and mean comparisons for total fresh weight, leaf-to-stem ratio, node number, and stem diameter of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Total Fresh Weight	Leaf-to-Stem Ratio	Node Number	Stem Diameter
	g Plant^{-1}		n° Plant^{-1}	mm
Cultivar (CV)				
Eleonora	21.02 ± 0.41 a	1.17 ± 0.01 b	4.18 ± 0.05 a	6.03 ± 0.05 a
Italiano Classico	19.91 ± 0.42 b	1.51 ± 0.02 a	3.93 ± 0.06 b	5.52 ± 0.07 b
Biostimulant Treatment (BT)				
Control	18.52 ± 0.24 c	1.31 ± 0.05 c	3.86 ± 0.07 b	5.64 ± 0.11 b
BT_1	20.50 ± 0.25 b	1.37 ± 0.06 a	4.12 ± 0.06 a	5.84 ± 0.12 a
BT_2	22.36 ± 0.28 a	1.35 ± 0.05 b	4.19 ± 0.06 a	5.84 ± 0.08 a
Nutrient Solution Concentration (NSC)				
NSC_1	20.39 ± 0.33	1.32 ± 0.04 b	4.02 ± 0.05 b	5.92 ± 0.06 a
NSC_2	20.53 ± 0.51	1.37 ± 0.05 a	4.09 ± 0.07 a	5.62 ± 0.09 b
CV × BT				
Eleonora × Control	19.06 ± 0.36 c	1.14 ± 0.01	4.08 ± 0.03 ab	5.91 ± 0.12
Eleonora × BT_1	21.31 ± 0.11 b	1.21 ± 0.03	4.26 ± 0.08 a	6.09 ± 0.07
Eleonora × BT_2	22.68 ± 0.45 a	1.18 ± 0.01	4.21 ± 0.12 a	6.08 ± 0.07
Italiano Classico × Control	17.98 ± 0.06 d	1.47 ± 0.02	3.65 ± 0.05 c	5.36 ± 0.07
Italiano Classico × BT_1	19.69 ± 0.06 c	1.54 ± 0.05	3.98 ± 0.05 b	5.58 ± 0.17
Italiano Classico × BT_2	22.05 ± 0.29 ab	1.52 ± 0.03	4.18 ± 0.02 ab	5.61 ± 0.06
BT × NSC				
Control × NSC_1	18.94 ± 0.41 d	1.34 ± 0.08 bc	3.91 ± 0.12 cd	5.84 ± 0.15 b
BT_1 × NSC_1	20.56 ± 0.42 c	1.28 ± 0.06 cd	4.09 ± 0.02 bc	6.10 ± 0.07 a
BT_2 × NSC_1	21.68 ± 0.20 b	1.32 ± 0.06 bcd	4.07 ± 0.06 bc	5.82 ± 0.07 b
Control × NSC_2	18.10 ± 0.11 e	1.27 ± 0.07 d	3.81 ± 0.09 d	5.43 ± 0.10 c
BT_1 × NSC_1	20.44 ± 0.32 c	1.46 ± 0.09 a	4.15 ± 0.12 ab	5.57 ± 0.17 c
BT_2 × NSC_2	23.05 ± 0.32 a	1.37 ± 0.10 b	4.32 ± 0.07 a	5.86 ± 0.16 b
CV × NSC				
Eleonora × NSC_1	21.01 ± 0.31	1.17 ± 0.01 c	4.07 ± 0.03 b	6.12 ± 0.05 a
Eleonora × NSC_2	21.03 ± 0.78	1.18 ± 0.02 c	4.29 ± 0.08 a	5.93 ± 0.08 b
Italiano Classico × NSC_1	19.78 ± 0.53	1.46 ± 0.01 b	3.98 ± 0.08 bc	5.72 ± 0.07 c
Italiano Classico × NSC_2	20.03 ± 0.67	1.56 ± 0.03 a	3.89 ± 0.08 c	5.31 ± 0.06 d
Significance				
CV	***	***	***	***
BT	***	***	***	***
NSC	ns	***	*	***
CV × BT	**	ns	***	ns
BT × NSC	***	***	***	***
CV × NSC	ns	***	***	***

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates (n = 3). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student t-test for cultivar and nutrient solution concentration mean effect and according to Tukey–Kramer HSD test for the rest.

The BT × NSC resulted in significant differences for all parameters (Table 1). When the biostimulant dose BT_2 was used, the total fresh weight increased by 14.46% in the NSC_1 and 27.34% in the NSC_2 compared with controls. For leaf stem-to-stem ratio and node number parameters, the use of the biostimulant did not determine significant differences in plants grown in NSC_1. In contrast, in NSC_2, the biostimulant, regardless of the dose, increased (on average) leaf-to-stem ratio and node number by 11.41 and 11.15 compared with the control. The highest stem diameter value (6.10 mm) was obtained from the BT_1 × NSC_1 combination.

Regarding the CV × NSC, in Eleonora, different concentrations of the nutrient solution did not lead to significant differences in leaf-to-stem ratio. On the contrary, increasing the concentration of the nutrient solution increased the number of nodes (+5.4%) but decreased

the diameter of the stem (−3.1%). On the other hand, in Italiano Classico, the increase in the concentration of the nutrient solution did not determine significant differences in node number. In contrast, it increased the leaf-to-stem ratio (+6.8%) and decreased the diameter (−7.2%).

3.2. Physiological Parameters

Except for ACO_2, no significant differences were observed between the two cultivars for the main physiological parameters reported in Table 2. The NSC factor significantly affected the SPAD, ACO_2, gs, and E. The BT factor increased all parameters as a function of the biostimulant dose compared with the control.

Table 2. Analysis of variance and mean comparisons for leaf area, SPAD index, Fv/fm, net CO_2 assimilation rate (ACO_2), stomatal conductance (gs), transpiration (E), and instantaneous water use efficiency (WUE) of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Leaf Area	**SPAD Index**	**Fv/Fm**	**ACO_2**	**gs**	**E**	**WUE_i**
	cm^2 $Plant^{-1}$			**μmol CO_2 m^{-2} s^{-1}**	**mol H_2O m^{-2} s^{-1}**	**mol H_2O m^{-2} s^{-1}**	**mol CO_2 mol H_2O^{-1}**
Cultivar (CV)							
Eleonora	309.09 ± 7.00	35.86 ± 0.39 a	0.80 ± 0.00	28.22 ± 0.54 a	1.27 ± 0.03	6.18 ± 0.10	4.57 ± 0.07
Italiano Classico	304.47 ± 9.27	34.37 ± 0.38 b	0.79 ± 0.00	25.85 ± 0.46 b	1.24 ± 0.03	6.16 ± 0.12	4.21 ± 0.06
Biostimulant Treatment (BT)							
Control	266.96 ± 5.42 c	33.22 ± 0.31 c	0.79 ± 0.00 c	24.69 ± 0.31 c	1.18 ± 0.01 c	5.80 ± 0.12 c	4.27 ± 0.08 b
BT_1	313.08 ± 4.30 b	35.75 ± 0.40 b	0.80 ± 0.00 b	27.37 ± 0.65 b	1.26 ± 0.04 b	6.19 ± 0.08 b	4.42 ± 0.11 ab
BT_2	340.30 ± 4.10 a	36.37 ± 0.27 a	0.81 ± 0.00 a	29.04 ± 0.37 a	1.32 ± 0.02 a	6.52 ± 0.13 a	4.47 ± 0.10 a
Nutrient Solution Concentration (NSC)							
NSC_1	308.34 ± 7.60	34.45 ± 0.40 b	0.79 ± 0.00	26.79 ± 0.59 b	1.28 ± 0.03 a	6.01 ± 0.11 b	4.46 ± 0.07
NSC_2	305.23 ± 8.81	35.77 ± 0.39 a	0.80 ± 0.00	27.28 ± 0.55 a	1.24 ± 0.02 b	6.34 ± 0.10 a	4.31 ± 0.08
CV × BT							
Eleonora × Control	273.01 ± 9.26 d	33.91 ± 0.31	0.79 ± 0.00	25.30 ± 0.49 c	1.19 ± 0.02 b	5.81 ± 0.11	4.36 ± 0.04 bc
Eleonora × BT_1	325.41 ± 3.88 b	36.60 ± 0.46	0.80 ± 0.00	29.44 ± 0.28 ab	1.36 ± 0.06 a	6.24 ± 0.12	4.72 ± 0.11 a
Eleonora × BT_2	328.86 ± 2.70 b	37.06 ± 0.33	0.81 ± 0.00	29.92 ± 0.21 a	1.26 ± 0.01 b	6.50 ± 0.18	4.62 ± 0.15 ab
Italiano Classico × Control	260.92 ± 5.36 d	32.53 ± 0.37	0.78 ± 0.00	24.08 ± 0.19 c	1.17 ± 0.01 b	5.80 ± 0.22	4.18 ± 0.15 c
Italiano Classico × BT_1	300.75 ± 2.32 c	34.90 ± 0.46	0.80 ± 0.00	25.31 ± 0.29 c	1.17 ± 0.01 b	6.14 ± 0.10	4.12 ± 0.05 c
Italiano Classico × BT_2	351.75 ± 3.76 a	35.68 ± 0.15	0.80 ± 0.00	28.16 ± 0.49 b	1.38 ± 0.01 a	6.55 ± 0.20	4.32 ± 0.12 bc
BT × NSC							
Control × NSC_1	272.69 ± 9.78 c	32.53 ± 0.39 d	0.78 ± 0.00	24.08 ± 0.24	1.19 ± 0.01 b	5.46 ± 0.11 d	4.41 ± 0.08 abc
BT_1 × NSC_1	316.52 ± 7.15 b	34.81 ± 0.42 b	0.80 ± 0.00	27.46 ± 0.81	1.31 ± 0.08 a	6.43 ± 0.05 b	4.27 ± 0.10 bc
BT_2 × NSC_1	335.80 ± 4.95 a	36.02 ± 0.26 a	0.80 ± 0.00	28.83 ± 0.67	1.32 ± 0.03 a	6.13 ± 0.09 c	4.71 ± 0.13 a
Control × NSC_2	261.24 ± 4.51 c	33.91 ± 0.30 c	0.79 ± 0.00	25.30 ± 0.47	1.17 ± 0.02 b	6.14 ± 0.06 bc	4.13 ± 0.11 c
BT_1 × NSC_1	309.64 ± 5.03 b	36.69 ± 0.42 a	0.80 ± 0.00	27.28 ± 1.10	1.21 ± 0.01 b	5.95 ± 0.03 c	4.58 ± 0.18 ab
BT_2 × NSC_2	344.81 ± 6.42 a	36.72 ± 0.45 a	0.81 ± 0.00	29.26 ± 0.36	1.32 ± 0.03 a	6.92 ± 0.04 a	4.23 ± 0.07 c
CV × NSC							
Eleonora × NSC_1	316.28 ± 6.33 a	35.12 ± 0.48	0.80 ± 0.00	27.91 ± 0.93	1.32 ± 0.05 a	6.08 ± 0.14 bc	4.59 ± 0.10 a
Eleonora × NSC_2	301.91 ± 12.46 b	36.59 ± 0.54	0.80 ± 0.01	28.53 ± 0.59	1.22 ± 0.02 b	6.28 ± 0.15 ab	4.55 ± 0.11 a
Italiano Classico × NSC_1	300.40 ± 13.78 b	33.78 ± 0.58	0.79 ± 0.01	25.67 ± 0.56	1.23 ± 0.04 b	5.93 ± 0.17 c	4.34 ± 0.10 a
Italiano Classico × NSC_2	308.55 ± 13.10 ab	34.95 ± 0.42	0.80 ± 0.00	26.02 ± 0.75	1.25 ± 0.03 ab	6.39 ± 0.15 a	4.07 ± 0.06 b
Significance							
CV	ns	***	ns	***	ns	ns	***
BT	***	***	***	***	***	***	*
NSC	ns	***	ns	*	**	***	**
CV × BT	***	ns	ns	***	***	ns	**
BT × NSC	**	**	ns	ns	*	***	***
CV × NSC	***	ns	ns	ns	***	*	*

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student *t*-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

In the CV× BT interaction, the biostimulant, regardless of dose, increased leaf area (+19.8%) and ACO_2 (+17.3%) of Eleonora compared with the control. The highest WUEi was obtained from the Eleonora × BT_1 combination. In Italiano Classico, the dose of biostimulant BT_2 determined the highest leaf area (351.75 cm^2 $plant^{-1}$), ACO_2 (28.16 μmol CO_2 m^{-2} s^{-1}), and gs (1.38 mol H_2O m^{-2} s^{-1}).

Relative to the BT × NSC, when plants were grown in NSC_1, the leaf area and SPAD increased as the dose of biostimulant increased. The same trend was also observed with NSC_2 but exclusively for the leaf area, while SPAD increased, on average, by 8.2%, compared with the control. Furthermore, in relation to the more concentrated nutrient solution, the highest gs (1.32 mol H_2O m^{-2} s^{-1}) and E (6.92 mol H_2O m^{-2} s^{-1}) were obtained with the BT_2 dose.

As reported in Table 2, the CV × NSC interaction did not result in significant differences for SPAD, Fv/Fm, and ACO_2. Specifically, in Eleonora, the NSC_2 decreased leaf area and gs by 4.5 and 7.6%, respectively, compared with the NSC_1. In contrast, in Italiano Classico, no significant differences were observed for the above parameters between the different concentrations of nutrient solutions. In Eleonora, E and WUEi did not show significant differences between the different nutrient solutions used. In contrast, in Italiano Classico, the use of the most concentrated solution increased E by 7.7% and decreased WUEi by 6.2%.

3.3. Mineral Profile

As shown in Table 3, the mineral profile was significantly affected by all factors considered in the experiment (CV, BT, and NSC). Except for calcium and magnesium, the CV × BT interaction resulted in significant differences for all the reported parameters (Table 3). The application of biostimulants at both doses did not result in significant differences for nitrate and S compared with the control for both cultivars. However, in Eleonora, the dose of BT_2 increased P (+17.1%) and K (+15%) compared with the control, while in Italiano Classico, it increased only P (+41.6%). Regarding the interaction between BT and NSC, the nitrate of plants grown in NSC_1 showed a significant increase with the dose of BT_2 compared with the corresponding control condition.

The use of the biostimulant in NSC_1 increased K and decreased Ca, compared with the control, while no significant differences were recorded for either macroelement in NSC_2. On the contrary, when plants were grown in NSC_2, a decrease in nitrate concentration (−12.4%) was observed with the BT_1 dose compared with the control. Regardless of the NSC, the highest *p* values were obtained at the dose BT_2. The CV × NSC interaction significantly influenced K and P accumulation only. Specifically, in Eleonora, an increase in K (+13.1%) was observed when NSC_2 was used. On the other hand, the same solution determined the highest *p* values (6.82 g kg^{-1} d.m.) in Italiano Classico.

3.4. Pigments Accumulation

The data presented in Table 4 show that the BT factor significantly affected all parameters. Regarding the CV effect, except for chlorophyll a/b, the highest values for all parameters were obtained in Eleonora. On the other hand, the NSC factor exclusively influenced chlorophyll b, total chlorophylls, and carotenoids. Regarding the CV × BT interaction, in Eleonora, compared with the control, a dose-dependent increase in chlorophyll a and total chlorophylls was observed. In Italiano Classico, the biostimulant, regardless of dose, significantly increased chlorophyll b and total chlorophylls compared with the control. The biostimulant did not significantly change the chlorophyll a/b ratio for both cultivars. The Italian Classico × BT_2 combination recorded the lowest carotenoids value (0.30 mg g^{-1} fw).

Regarding the BT × NSC interaction, regardless of the concentration of the nutrient solution, the use of biostimulant at both doses resulted in higher values of chlorophyll b and total chlorophylls compared with control conditions. In contrast, the use of biostimulants in NSC_1 reduced carotenoids compared with the control. In Eleonora, the more concentrated solution reduced carotenoids (−10.2%) compared with what was obtained from the same cultivar grown in NSC_1.

Table 3. Analysis of variance and mean comparisons for the mineral concentration of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Nitrate	P	K	Ca	Mg	S
	(mg kg^{-1} fw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)	(g kg^{-1} dw)
Cultivar (CV)						
Eleonora	2988.16 ± 122.67 a	6.56 ± 0.15 b	53.17 ± 1.43 a	10.27 ± 0.35 b	3.32 ± 0.06 a	0.95 ± 0.02 b
Italiano Classico	2584.55 ± 92.73 b	7.58 ± 0.37 a	48.51 ± 0.81 b	13.22 ± 0.54 a	3.16 ± 0.05 b	1.46 ± 0.04 a
Biostimulant Treatment (BT)						
Control	2766.18 ± 181.42 b	6.36 ± 0.13 b	47.48 ± 1.88 c	13.13 ± 0.82 a	3.32 ± 0.07 a	1.20 ± 0.08 ab
BT_1	2583.26 ± 123.40 c	6.61 ± 0.27 b	51.13 ± 1.14 b	10.96 ± 0.56 b	3.21 ± 0.06 ab	1.17 ± 0.07 b
BT_2	3009.64 ± 97.30 a	8.24 ± 0.40 a	53.91 ± 1.00 a	11.13 ± 0.56 b	3.18 ± 0.07 b	1.24 ± 0.10 a
Nutrient Solution Concentration (NSC)						
NSC_1	2434.36 ± 90.26 b	6.6 ± 0.18 b	48.34 ± 1.26 b	12.25 ± 0.62 a	3.37 ± 0.03 a	1.26 ± 0.06 a
NSC_2	3138.35 ± 75.61 a	7.55 ± 0.36 a	53.34 ± 1.00 a	11.23 ± 0.51 b	3.11 ± 0.06 b	1.14 ± 0.07 b
CV × BT						
Eleonora × Control	2901.45 ± 336.83 ab	6.24 ± 0.18 c	48.93 ± 3.83 bc	11.92 ± 0.33	3.43 ± 0.05	1.00 ± 0.02 b
Eleonora × BT_1	2837.60 ± 142.44 b	6.14 ± 0.09 c	54.32 ± 0.61 ab	9.44 ± 0.45	3.29 ± 0.11	0.94 ± 0.03 b
Eleonora × BT_2	3225.43 ± 49.60 a	7.31 ± 0.10 b	56.25 ± 0.15 a	9.44 ± 0.36	3.23 ± 0.11	0.91 ± 0.05 b
Italiano Classico × Control	2630.90 ± 155.07 bc	6.48 ± 0.18 bc	46.03 ± 0.16 c	14.35 ± 1.50	3.22 ± 0.13	1.40 ± 0.10 a
Italiano Classico × BT_1	2328.92 ± 144.35 c	7.07 ± 0.48 bc	47.94 ± 1.12 c	12.47 ± 0.51	3.13 ± 0.03	1.40 ± 0.03 a
Italiano Classico × BT_2	2793.84 ± 143.40 b	9.18 ± 0.59 a	51.57 ± 1.49 abc	12.82 ± 0.30	3.14 ± 0.08	1.56 ± 0.04 a
BT × NSC						
Control × NSC_1	2226.11 ± 69.60 d	6.28 ± 0.21 cd	43.34 ± 1.33 c	14.35 ± 1.48 a	3.41 ± 0.05	1.31 ± 0.13 a
BT_1 × NSC_1	2270.56 ± 117.17 d	6.02 ± 0.09 d	49.30 ± 1.66 b	11.16 ± 0.36 b	3.30 ± 0.08	1.22 ± 0.11 ab
BT_2 × NSC_1	2806.42 ± 150.99 c	7.50 ± 0.20 b	52.39 ± 1.81 ab	11.23 ± 0.48 b	3.38 ± 0.04	1.24 ± 0.11 ab
Control × NSC_2	3306.25 ± 152.67 a	6.45 ± 0.16 cd	51.62 ± 2.63 ab	11.92 ± 0.39 ab	3.23 ± 0.14	1.09 ± 0.05 b
BT_1 × NSC_1	2895.95 ± 119.00 bc	7.20 ± 0.42 bc	52.96 ± 1.26 ab	10.75 ± 1.11 b	3.11 ± 0.07	1.12 ± 0.10 ab
BT_2 × NSC_2	3212.85 ± 48.35 ab	8.99 ± 0.67 a	55.43 ± 0.49 a	11.03 ± 1.07 b	2.99 ± 0.04	1.23 ± 0.19 ab
CV × NSC						
Eleonora × NSC_1	2606.97 ± 148.52	6.31 ± 0.21 b	49.91 ± 2.43 b	10.62 ± 0.19	3.41 ± 0.04	1.01 ± 0.01
Eleonora × NSC_2	3369.35 ± 74.66	6.82 ± 0.18 b	56.43 ± 0.29 a	9.91 ± 0.68	3.22 ± 0.09	0.89 ± 0.03
Italiano Classico × NSC_1	2261.76 ± 71.54	6.88 ± 0.28 b	46.78 ± 0.48 b	13.88 ± 0.96	3.32 ± 0.05	1.51 ± 0.03
Italiano Classico × NSC_2	2907.35 ± 73.34	8.27 ± 0.63 a	50.25 ± 1.34 b	12.55 ± 0.46	3.00 ± 0.04	1.40 ± 0.07
Significance						
CV	***	***	***	***	***	***
BT	***	***	***	***	*	**
NSC	***	***	***	***	***	***
CV × BT	*	***	***	ns	ns	***
BT × NSC	***	***	***	**	ns	***
CV × NSC	ns	***	***	ns	ns	ns

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates (n = 3). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student t-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

Table 4. Analysis of variance and mean comparisons for pigments concentration of Eleonora and Italiano Classico basil cultivars grown in floating raft system under two different nutrient solutions treatments and two rates of biostimulant application.

	Chlorophyll a	Chlorophyll b	Total Chlorophylls	Carotenoids	Chlorophyll a/b
	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	
Cultivar (CV)					
Eleonora	1.13 ± 0.02 a	0.67 ± 0.02 a	1.75 ± 0.03 a	0.37 ± 0.01 a	1.703 ± 0.04
Italiano Classico	1.07 ± 0.02 b	0.63 ± 0.02 b	1.68 ± 0.03 b	0.34 ± 0.01 b	1.742 ± 0.06
Biostimulant Treatment (BT)					
Control	1.02 ± 0.01 b	0.55 ± 0.02 b	1.55 ± 0.02 c	0.36 ± 0.02 a	1.875 ± 0.07 a
BT_1	1.11 ± 0.02 a	0.68 ± 0.01 a	1.74 ± 0.01 b	0.36 ± 0.01 a	1.645 ± 0.03 b
BT_2	1.16 ± 0.03 a	0.71 ± 0.03 a	1.84 ± 0.03 a	0.34 ± 0.01 b	1.647 ± 0.07 b
Nutrient Solution Concentration (NSC)					

Table 4. *Cont.*

	Chlorophyll a	Chlorophyll b	Total Chlorophylls	Carotenoids	Chlorophyll a/b
	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	mg g^{-1} fw	
NSC_1	1.10 ± 0.02	0.62 ± 0.02 b	1.68 ± 0.03 b	0.37 ± 0.01 a	1.806 ± 0.06
NSC_2	1.10 ± 0.02	0.68 ± 0.02 a	1.74 ± 0.04 a	0.34 ± 0.01 b	1.639 ± 0.04
CV × BT					
Eleonora × Control	1.05 ± 0.01 c	0.60 ± 0.01 b	1.61 ± 0.01 c	0.37 ± 0.01 a	1.740 ± 0.03 ab
Eleonora × BT_1	1.11 ± 0.04 b	0.68 ± 0.02 ab	1.73 ± 0.01 b	0.36 ± 0.01 a	1.637 ± 0.04 b
Eleonora × BT_2	1.23 ± 0.02 a	0.73 ± 0.05 a	1.91 ± 0.04 a	0.37 ± 0.01 a	1.732 ± 0.13 b
Italiano Classico × Control	1.00 ± 0.02 c	0.51 ± 0.03 c	1.50 ± 0.03 d	0.36 ± 0.03 a	2.009 ± 0.13 a
Italiano Classico × BT_1	1.11 ± 0.02 b	0.67 ± 0.01 ab	1.76 ± 0.02 b	0.35 ± 0.02 a	1.653 ± 0.04 b
Italiano Classico × BT_2	1.09 ± 0.02 bc	0.70 ± 0.01 a	1.78 ± 0.02 b	0.30 ± 0.01 b	1.562 ± 0.01 ab
BT × NSC					
Control × NSC_1	1.03 ± 0.01	0.53 ± 0.04 d	1.54 ± 0.04 c	0.41 ± 0.01 a	1.976 ± 0.13
BT_1 × NSC_1	1.13 ± 0.03	0.68 ± 0.01 b	1.74 ± 0.01 b	0.34 ± 0.02 bc	1.663 ± 0.04
BT_2 × NSC_1	1.15 ± 0.05	0.65 ± 0.01 bc	1.78 ± 0.02 b	0.35 ± 0.02 bc	1.779 ± 0.10
Control × NSC_2	1.02 ± 0.02	0.58 ± 0.01 cd	1.57 ± 0.03 c	0.32 ± 0.01 c	1.774 ± 0.04
BT_1 × NSC_1	1.10 ± 0.03	0.68 ± 0.02 b	1.75 ± 0.02 b	0.37 ± 0.01 ab	1.627 ± 0.04
BT_2 × NSC_2	1.17 ± 0.03	0.78 ± 0.03 a	1.91 ± 0.04 a	0.32 ± 0.01 c	1.515 ± 0.06
CV × NSC					
Eleonora × NSC_1	1.15 ± 0.03 a	0.64 ± 0.01	1.73 ± 0.03	0.39 ± 0.01 a	1.793 ± 0.06
Eleonora × NSC_2	1.10 ± 0.03 ab	0.69 ± 0.04	1.77 ± 0.06	0.35 ± 0.00 b	1.614 ± 0.06
Italiano Classico × NSC_1	1.05 ± 0.02 b	0.59 ± 0.04	1.64 ± 0.05	0.34 ± 0.02 b	1.819 ± 0.12
Italiano Classico × NSC_2	1.09 ± 0.03 ab	0.66 ± 0.03	1.72 ± 0.05	0.33 ± 0.02 b	1.664 ± 0.05
Significance	***	**	***	***	ns
CV	***	***	***	***	***
BT	ns	***	***	***	***
NSC	**	*	***	***	***
CV × BT	ns	***	*	***	ns
BT × NSC	**	ns	ns	**	ns
CV × NSC					

*, **, and *** significant effect at the $p \leq 0.05$, 0.01, and 0.001 level, respectively. ns—nonsignificant effect. Data represent means ± standard error of 3 replicates ($n = 3$). Treatment means within each column followed by different letters denote significant differences ($p < 0.05$) according to the Student *t*-test for cultivar and nutrient solution concentration mean effect and according to the Tukey–Kramer HSD test for the rest.

4. Discussion

Hydroponic systems are increasingly popular and widely used to improve the yield of leafy vegetables such as basil (*Ocimum basilicum* L.) and, not least, are valuable tools for understanding how the combined action of preharvest factors affects the leaves' characteristics. For this purpose, we evaluated the supplementation of plant-derived protein hydrolysate in two nutrient solutions with different macronutrient concentrations to understand and improve the yield and physiological response of two Genovese basil cultivars grown in a floating raft system.

The lower unit yields of Genovese basil grown in open fields recorded by Nicoletto et al. [33] and Formisano et al. [34] show that hydroponics, thanks to the higher planting density, the lower abiotic and biotic pressure, and the potentially unlimited availability of nutrients and water, maximizes the production of this leafy vegetable, confirming the results of Ciriello et al. [30].

Furthermore, the better growth conditions of our system also affected leaf dry matter, which was significantly lower compared with the authors' findings mentioned above. Additionally, the leaf-to-stem ratio recorded by Formisano et al. [34] in the open field of the same basil cultivars (Eleonora and Italiano Classico) was lower. The leaf-to-stem ratio is a crucial quality parameter for the industrial production of "pesto sauce", as an excessive fibrousness of the stem extends the processing time needed to process the leaves (increased

temperature), triggering oxidative processes resulting in the blackening of the green sauce with negative impacts on the quality of the final product [28].

Regardless of the NSC and BT factors, the measured parameters significantly depended on the genetic material, although both basil cultivars belonged to the 'Genovese' type. These findings are not surprising since it is well established in the literature that different basil cultivars can have distinct productive and physiological properties [33,35–37]. In particular, Eleonora had a higher fresh yield compared with Italiano Classico. This result is probably attributable to the stem component (>stem diameter and >node number) since the leaf area did not vary significantly among the two cultivars. It should be noted that Eleonora was characterized by higher pigments (chlorophyll a, b, and total and carotenoids), which, in addition to affecting net CO_2 assimilation, resulted in a higher SPAD index. An increase in the SPAD index is often correlated with higher greenness, which is a valuable quality characteristic for leafy vegetables such as basil [38].

In soilless hydroponic systems, in addition to genetic material, the careful management of macronutrients is also crucial to achieving high production and better physiological response. Although no ideal electrical conductivity value is known for different environmental conditions [39], overconcentrated or under concentrated nutrient solutions negatively affect the nutritional status and growth of vegetables [1,39]. However, total fresh weight and ACO_2 were not significantly affected by the NSC at both levels (1 and 2 dS m^{-1}) in either cultivar. Our results probably reflect the fact that the macronutrient concentration in the nutrient solutions was optimal for basil, which was also confirmed by the maximum quantum efficiency of the PSII photochemistry (Fv/Fm) that did not vary significantly in the NSC treatment [40]. Similarly, Hosseini et al. [1] observed a yield reduction at NSC of less than 0.9 ds/m, while Walters and Currey [41] did not show significant differences in Sweet, Holy, and Lemon basil up to 4 ds/m.

Although no production differences were observed for the NSC factor, regardless of CV and BT, the nitrate increased by 28.9% when the nutrient solution concentration was doubled (2 ds/m; Table 3). The higher transpiration (E) of plants grown in 2 ds/m nutrient solutions probably accounts for the observed luxury consumption of nitrate (Tables 2 and 3). Similar results were reported in previous work on basil [42], pakchoi (*Brassica campestris* L. ssp. Chinensis) [39], and lettuce (*Lactuca sativa* L.) [43]. However, as reported by Colla et al. [44], it should be noted that nitrate accumulation in leaves is also strongly dependent on the genetic aspect. Eleonora and Italiano Classico showed different responses to nitrate accumulation in the leaves, with the latter having the lowest value (Table 3). Similar to nitrate, the use of a more concentrated nutrient solution resulted in the luxury consumption of potassium, as evidenced by Walters and Currey [41]. Regardless of the effect of the CV, the increase in potassium in the leaves was coupled with a reduction in magnesium and calcium as the nutrient solution concentration increased, probably because of the well-recognized antagonism between these macroelements [41].

Similar to what was observed by Hosseini et al. [1], our study shows a positive correlation between the concentration of macronutrients in the nutrient solution and the chlorophylls (Table 4). Regardless of CV and BT, the higher chlorophylls a, b, and total chlorophylls obtained in plants grown at EC 2 dS m^{-1} were probably attributable to the higher nitrogen values. Nitrogen is one of the critical constituents of chlorophyll as it is involved in its biosynthesis and the structure of the porphyrin ring [45]. Chenard et al. [46] reported a concomitant increase in carotenoids and chlorophylls in hydroponically grown parsley (*Petroselinum crispum* L. cv. Dark Green Italian), a finding that is not in line with our results. To confirm the unclear dependence of carotenoids on nutrient solution concentration, Fallovo et al. [43] reported that the use of nutrient solutions with different concentrations did not affect the amount of these pigments in lettuce.

Soilless systems, such as the floating graft system, are highly dependent on external chemical input, making them not always environmentally sustainable. For this reason, biostimulant supplementation in the nutrient solution could be a valuable tool to increase the sustainability of hydroponic systems [7,8]. In the present study, regardless of CV

and NSC, total fresh weight, leaf area, leaf-to-stem ratio, node number, and stem diameter increased with protein hydrolysate biostimulant (PHs) in the nutrient solution (Tables 1 and 2). The yield improvements obtained are consistent with what has been reported by several authors on horticultural crops [16,47,48]. The increased percentage of dry matter does not entirely agree with the literature reviewed. Caruso et al. [47], Rouphael et al. [49], and El-Nakhel et al. [32] reported contrasting results after application of PH in arugula (*Diplotaxis tenuifolia* (L.) DC), spinach, and two different species of microgreens (*Daucus carota* L. and *Anethum graveolens* L.), respectively, underlining how interactions between physiologically active compounds of PH are highly dependent on plant species, environmental and growth conditions, dose, and application time [50]. However, interestingly, supplementation of PHs in the less concentrated nutrient solution ensured higher fresh yield than what was obtained from control x NSC_2, confirming in part that the use of amino acids to replace a part of nitrogen fertilization can support the yield and growth of leafy vegetables in hydroponics [51].

Although the physiological and biochemical mechanisms underlying the effects of biostimulants are still unclear, we hypothesize that the improved fresh production achieved is attributable to the bioactive molecules in Trainer®. Bioactive compounds such as readily absorbed amino acids and PH signaling peptides provide a plethora of beneficial effects that influence the growth and upregulation of N and C metabolism [52]. Specifically, the main effects of PHs would seem to be attributable to the "hair growth-promoting peptide", which can modify the root architecture (increased length, density, and the number of lateral roots), leading to increased nutrient uptake [53,54]. Furthermore, biostimulants would also act as physiological primers that can promote indol-3-acetic acid (IAA) and abscisic acid (ABA) biosynthesis and enhance photosynthetic activity [16,55,56].

Similar to the findings of Cristofano et al. [52] on two *Lactuca sativa* L. cultivars (Ballerina and Canasta) grown hydroponically, the use of biostimulant in nutrient solution at dose BT_2 significantly increased ACO_2 in both cultivars, justifying the increase in yield (Tables 1 and 2). As hypothesized by Rouphael et al. [21], the improvement in net CO_2 assimilation rate could be reasoned to be the beneficial effect of the biostimulant on stomatal conductance. Furthermore, the improved photosynthetic performance and the higher value of water use efficiency (WUEi) obtained at the dose of BT_2, compared with the control condition, were accompanied by an increase in K (Table 3). This element plays a crucial role in osmotic balance and turgor-dependent processes such as stomatal opening and thus CO_2 diffusion; its increase could have induced better stomatal reactivity by stimulating growth and yield [57,58]. The improved functioning of the photosynthetic machinery after the application of PHs in the nutrient solution could have been related to a higher amount of pigment and a higher Fv/Fm, compared with the control (Tables 2 and 4). Indeed, as suggested by Yakhin and collaborators [59], the biostimulant would improve light utilization efficiency, dissipate excitation energy in photosystem II antennae, and increase the photosynthetic pigment biosynthesis.

Moreover, the reviewed literature shows a clear effect of biostimulants on reducing the nitrate concentration in leafy vegetables such as arugula, lettuce, chard, spinach, peppermint, spearmint, and pakchoi [4,60]. As suggested by Colla et al. [61] and Calvo et al. [62], PHs would regulate metabolic pathways involved in nitrogen metabolism by overloading the phloem with amino acids, thereby limiting nitrate uptake and storage. However, our results on nitrate storage in basil tissues at different doses of biostimulants in nutrient solution are contradictory. At the BT_1 dose, the nitrate decreased significantly (−6.6%) compared with the control, consistent with the results reported by the authors above. Nitrate reduction was even more evident when plants were grown in NSC_2 (Table 3). On the contrary, the BT_2 dose, regardless of the NSC and CV, caused an increase in nitrate (+8.8%), probably attributable to increased plant transpiration activity (Tables 2 and 3). This discrepancy in the results recorded in our experiment further emphasizes how the effects of the biostimulant on the storage of nitrate in basil are strongly influenced by the dose used.

5. Conclusions

Reducing chemical inputs is vital, especially in hydroponics, which uses nutrient solutions that often exceed the real needs of plants. From this perspective, the integration of biostimulants into nutrient solutions is an environmentally sustainable strategy for horticultural production. Our results showed a different physiological and productive response between Eleonora and Italiano Classico. Particularly, Eleonora provided the highest fresh production but the lowest leaf-to-stem ratio, an essential parameter for transforming leaves into "pesto" sauce. Italiano Classico recorded the lowest nitrate values. Surprisingly, the concentration of the nutrient solution did not affect the fresh production in either cultivar, while nitrate, phosphorus, potassium, and total chlorophyll increased as the concentration of macronutrients increased. Supplementation of Trainer® into the nutrient solution improved physiological parameters (ACO_2, gs, E, Fv/Fm) in a dose-dependent manner, thus increasing fresh production. In particular, the $BT_1 \times NSC_1$ combination compared with the control condition with double the fertilizer concentration in the nutrient solution (NSC_2) showed that a significant yield increase could be achieved in a sustainable manner, and it demonstrated the feasibility of filling in the reduction in fertilizer input, with interesting economic implications to consider. Not least, regardless of cultivar and nutrient solution, using the biostimulant at the BT_1 dose significantly reduced nitrate, the antinutritional compound par excellence in leafy vegetables.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8050409/s1, Table S1: Trainer® Composition.

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