horticulturae

MDPI

Article

Can Biostimulants Increase Resilience of Hydroponically-Grown Tomato to Combined Water and Nutrient Stress?

Panagiotis Kalozoumis, Christos Vourdas, Georgia Ntatsi and Dimitrios Savvas *

Laboratory of Vegetable Production, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece; kalozoumis@aua.gr (P.K.); chrisvourdas@hotmail.com (C.V.); ntatsi@aua.gr (G.N.)
* Correspondence: dsavvas@aua.gr; Tel.: +30-210-529-4510

Abstract: In the current experiment, tomato (*Solanum lycopersicum cv.* Nostymi F1) was cultivated in an open hydroponic system under optimal or stress conditions caused by reducing the supply of nutrient solution by 35–40% and treated with biostimulants to test whether their application can increase crop resilience to combined shortage of nutrients and water. The four different biostimulant treatments were: (i) no biostimulant application, (ii) treatment with the protein-based biostimulants COUPÉ REGENERACIÓN Plus and PROCUAJE RADICULAR provided by EDYPRO, (iii) treatment with a novel biostimulant based on strigolactones, provided by STRIGOLAB and (iv) treatment with MAXICROP, a commercial product consisting of seaweed extracts. Combined stress significantly reduced NO_3^-, P, and K in the root zone of tomato plants. However, the application of the strigolactone-based biostimulant to stressed plants maintained NO_3^- in the root zone to similar levels with non-stressed plants during the first and third months of cultivation. The biostimulants did not increase the vegetative plant biomass at 70 and 120 days after transplanting (DAT). The strigolactone-based biostimulant increased early leaf area development (70 DAT) and early fruit production compared to untreated plants but had no effect on total tomato yield (120 DAT). Maxicrop also increased early fruit yield, while Edypro decreased early and total yield compared to the control plants, an effect ascribed to overdosing, as the application rate was that suggested for soil-grown crops, while the plants were cultivated on an inert substrate. Strigolactone-based biostimulant and Maxicrop could be further studied by testing multiple applications during the cropping period.

Keywords: drought; abiotic stress; seaweed extract; protein hydrolysates; strigolactones; Maxicrop; water use efficiency; nutrient use efficiency

Citation: Kalozoumis, P.; Vourdas, C.; Ntatsi, G.; Savvas, D. Can Biostimulants Increase Resilience of Hydroponically-Grown Tomato to Combined Water and Nutrient Stress?. *Horticulturae* **2021**, *7*, 297. https://doi.org/10.3390/horticulturae7090297

Academic Editors: Anastasios Siomos and Pavlos Tsouvaltzis

Received: 3 August 2021
Accepted: 4 September 2021
Published: 8 September 2021

1. Introduction

Soilless cultivation in greenhouses is becoming increasingly popular in Mediterranean countries [1]. One of the major problems faced by horticulture in many regions of these countries is the low precipitation which restricts the available irrigation water [2]. Furthermore, irrigation water often contains high amounts of Na^+ and Cl^-, which impose salinity stress and concomitantly decrease total yields [3]. In most Mediterranean greenhouses, the soilless cultivation systems are open because these require fewer installation costs, entail an easier crop control (less frequent analysis of the drainage solution, lower automation level, etc.) [4], and avoid the need of drainage solution disinfection to control pathogen infections [5] compared to closed systems. The discharge of the fertigation effluents exacerbates the existing water scarcity conditions since, in open hydroponic systems, the nutrient solution runoff commonly exceeds 30% [6,7] of the total nutrient solution applied.

Avoiding the waste of fertilizers is similarly important. A recent study of Sanjuan-Delmás [8] concluded that tomato grown in an open hydroponic system with 30 to 40% discharge of drainage solution results in 48% and 28% losses of nitrogen and phosphorus, respectively. Production of nitrogen fertilizers through industrial N_2 fixation and phosphorus fertilizers from phosphate rock mining is extremely energy demanding, thereby contributing to global climate change [9,10], while their leaching is primarily responsible for eutrophication [9]. Hence, open soilless cultivation systems, which currently prevail in Mediterranean countries, have to decrease N and P inputs considerably while maintaining similar production levels in order to contribute to an environmentally and socio-economically sustainable greenhouse production system [11].

Biostimulants have shown a potential in many recent studies to mitigate abiotic stress, such as drought and nutrient deficiency, mainly by stimulating natural processes and fostering nutrient uptake, thereby increasing nutrient use efficiency (www.biostimulant.com; 4 September 2021) [12–14]. Consequently, biostimulants may decrease fertilizer consumption without compromising yield [15]. The definition of plant biostimulants proposed by du Jardin [16] is '*any substance or microorganism applied to plants with the aim to enhance nutrition efficiency abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content*'. The main categories as also assigned by du Jardin [16] are (i) humic substances, (ii) substances including proteins and protein hydrolysates, (iii) seaweed extracts, (iv) chitin and chitosan derivatives, (v) inorganic compounds, (vi) beneficial fungi, and (vii) beneficial bacteria. A lot of researchers have already proven that biostimulants can have multiple effects on plants growth [17–20]. However, the large diversity of substances with biostimulant action on plants complicates our understanding of the exact mode of action [21,22], as a significant number of molecules are poorly characterized [23].

Seaweed extracts (SWE) and substances including proteins and protein hydrolysates are two widely studied categories with commercial products already available [24]. SWE are mostly manufactured from *Ascophyllum nodosum* [25] and have been previously described as substances enhancing plant growth and increasing tolerance to biotic and abiotic stress, such as drought, nutrition deficiency, salinity, and thermal stress [26]. The commercial products can be applied by foliar spraying, soil drenching, or dilution in the hydroponic nutrient solution [16,26]. Substances with proteins or protein hydrolysates (PHs) constitute a category of plant biostimulants (PBs) which include 'mixtures of amino acids, polypeptides and oligopeptides that are manufactured from animal or plant protein sources using partial hydrolysis' [19]. However, in the EU, according to Regulation (EU) No. 354/2014, no animal protein hydrolysates can be applied to edible parts in organic crops, while proteins derived from plant sources are gaining increased attention lately due to their superior agronomic value [19]. Drought management has already been studied by Petrozza et al. [27], who applied the commercial biostimulant Megafol®, a mixture of amino acids (proline and tryptophan), glycosides, polysaccharides, organic nitrogen, and organic carbon, on tomato exposed to drought stress, and found that biomass production increased, and plants could recover more quickly when water was again accessible. Other effects of protein mixtures are resistance to nutrient deficiency, thermal stress, salinity, and alkalinity stress [28–32].

Strigolactones (SLs) have also been reported as mediators for the acclimatization of plants to water deprivation [33] and nutrient deficit conditions [34,35]. SL and the phytohormone ABA share the same biosynthetic precursor, both being carotenoid-derived terpenoid lactones [36]. ABA is quickly accumulated under water deficit and is responsible for stomatal closure [37]. Until now, there is a contradiction regarding SL and ABA interactions. In some plants, such as tomato, López-Ráez et al. [38,39] reported a positive correlation between ABA and strigolactones, while, in other species, such as Arabidopsis, the exogenous application of the synthetic strigolactone GR24 reduced ABA levels [40]. SL-depleted *Lotus japonicus* was also characterized as hypersensitive to drought, mainly due to the hyposensitivity of their stomata to ABA produced by plants or applied exogenously [41,42]. SLs are also participating in the plant responses and adaptation under low phosphorus conditions [43], can affect root and shoot architecture [35,43] and induce AMF and bacterial symbiosis [34,44–46]. However, SL products need to be extensively evaluated before they can be used as biostimulants [47], while there are several obstacles that need to be surpassed for SL products to become broadly commercial [33,47]

Taking this framework into consideration, the current study was aimed at comparably evaluating the effects of two commercial biostimulants and a novel strigolactone-based biostimulant on tomato grown under limited water and nutrient supply conditions, and test if their use could minimize the runoff in open soilless culture systems.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, and Treatments

The tomato experiment was carried out from September 2019 to January 2020 in a heated glasshouse located in the facilities of the Agricultural University of Athens (37°59′10′′ N, 23°42′29′′ E, altitude 24 m). Plants were grown hydroponically onto rock-wool slabs placed into bags, using two separate compartments of the greenhouse. The selected tomato cultivar used as a scion was the commercial hybrid 'Nostymi F1', grafted onto a backcross inbred line (BIL-6335) from the genotypes produced and described by Ofner et al. [48]. The selected cultivar is certified by GSPP (Good Seeds and Plant Practices Certificate) and is characterized by a large-sized plum-shaped fruit, its high percentage of extra class fruits, and its long shelf life. Plants were transplanted in the greenhouse on 10 September 2019, with a plant density of 2.25 plants m^{-2}, and the experiment lasted for 4 months (120 days). Before planting, the substrate was fertigated up to the point of saturation to ensure a complete filling of its pores with nutrient solution. Subsequently, the slabs were left to drain freely by slitting the bag at the bottom, as suggested by Savvas et al. [49]. A bumblebee hive was placed into the glasshouse on 12 October 2018 (32 days after transplanting, DAT) to secure optimal pollination. Harvest commenced on 13 November (64 DAT) and continued for two months (Figure A1, see Appendix A). Temperature, relative humidity, and solar radiation in the two greenhouse compartments were recorded every 15 min. Average daily data are presented in Supplementary Figures S1–S3. Plants were continuously monitored for pest management. "Delta" traps against the tomato leaf miner *Tuta absoluta* were installed in the greenhouse, along with black sticky traps in every plant row, as shown in Figure 1. The beneficial insect *Trichogramma brassicae* was also applied for the control of the 'tomato leaf miner' 20 DAT. Blue and yellow sticky traps were also installed to monitor the *Aleyrodidae* and *Thripidae* populations, respectively.

Figure 1. Tomato plants 17 DAT.

Tomato plants were irrigated and fertigated either optimally or with a deficit supply of nutrient solution. Combined stress was applied by reducing the nutrient solution supply to 33–40% of the standard supply in the non-stress treatment. More specifically, non-stressed treatments were fertigated using a solar meter to control irrigation frequency aiming at obtaining a drainage fraction of 20% of the total NS amount supplied to plants. The irrigation frequency in stressed plants was similar to that applied to the non-stress plants, but the amount of NS provided at each irrigation event was 33% less compared to the non-stressed plants for the first 45 days after transplanting (DAT). However, analysis of the nutrient solution of samples obtained from the root zone of the plants revealed that the electrical conductivity of stressed tomato plants was higher than in the control plants, with potassium having already been significantly increased compared to the control plants (Table 1). Therefore, an adjusted nutrient solution for the reproductive stage with yet reduced nutrient concentrations was applied to the stressed plants at 45 DAT, and at a dose 40% reduced compared to non-stressed plants. After the second month, the root zone analysis, a final adjustment to the nutrient solution was applied. The new nutrient solution was supplied at 66 DAT, and its application continued until the end of the cultivation. Nutrient concentrations in the NS are provided in Supplementary Table S1.

Table 1. Effects of combined restriction of water and nutrients (No stress, NS; Combined stress, CS), and biostimulant (BS) application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant and Maxicrop) at K concentration (mmol L^{-1}) on tomato root zone 30, 60, 90, and 120 days after transplanting (DAT).

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
No stress	11.5 b	6.3 a	6.9 a	6.2 a
Combined Stress	12.8 a	3.9 b	4.8 b	4.6 b
Control	12.3	4.7	6.0	5.2
Edypro	11.8	5.1	6.1	5.3
Strigolactone-based BS	11.9	4.9	5.1	5.4
Maxicrop	12.6	5.6	6.2	5.6
Statistical interactions				
NS × Control	13.3 ab	6.6	7.6	6.4
NS × Edypro	10.7 cd	6.7	7.4	6.1
NS × Strigolactone-based BS	9.7 d	5.0	5.6	5.9
NS × Maxicrop	12.3 abc	6.9	6.9	6.4
CS × Control	11.3 bcd	2.8	4.6	4.1
CS × Edypro	12.9 abc	3.5	4.6	4.6
CS × Strigolactone-based BS	14.2 a	4.8	4.5	4.9
CS × Maxicrop	12.9 abc	4.4	5.6	4.9
Statistical interactions				
Stress	*	***	***	***
BS	ns	ns	ns	ns
Stress × BS	**	ns	ns	ns

Means (n = 4) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$), at $p < 0.05$, $p < 0.01$, and $p < 0.001$, denoted by *, **, and ***, respectively; ns = not significant.

Treatment with Edypro's biostimulant included two different products according to the standard commercial practice suggested by the company: (i) COUPÉ REGENERACIÓN Plus and (ii) PROCUAJE RADICULAR. COUPÉ REGENERACIÓN Plus contains proteins and regenerating plant extracts. The biostimulant has been then elaborated with a special combination of amino acids obtained by enzymatic hydrolysis of vegetable proteins and by fermentation by lactobacillus. PROCUAJE RADICULAR contains micronutrients, penetrating agents, and fertilization precursors. COUPÉ REGENERACIÓN was applied on the date of transplanting and 14 days later, where 3.5 mL of biostimulant was provided in each plant through the nutrient solution. PROCUAJE RADICULAR was applied at the beginning of flowering and every 7 days, where a dose of 0.69 mL was supplied to each plant again through the nutrient solution at one irrigation cycle.

The strigolactone-based biostimulant is a natural strigolactone-enriched solution that is produced by STRIGOLAB. This company has developed an efficient procedure to obtain root exudates enriched in natural strigolactones from aeroponically grown tomato plants.

The strigolactone-based biostimulant was applied by spraying the leaves to drip off before plants set flowers, more specifically on 25 September 2019 (Figure 2). For the preparation of the spraying solution, 3 mL of the strigolactone-based biostimulant was mixed together with 3 mL of acetone. The 6 mL produced solution was then diluted on 20 L of water.

Figure 2. Application of strigolactone-based biostimulant by spraying the leaves 15 DAT.

Maxicrop contains mainly seaweed extracts from *Ascophyllum nodosum* and can be applied in soilless systems either as foliar spray or as soil drench [50]. An experiment with Maxicrop performed by Steveni et al. [51] on hydroponic barley showed that the best growth response (leaf area, shoot, and root dry weight) was obtained when Maxicrop was added to the hydroponic nutrient solution compared to the foliar spray application. Therefore, in the current experiment, the selected method of applying Maxicrop was by diluting 1 mL of the biostimulant for each plant into the nutrient solution at one irrigation event (Figure A2). Each plant received 0.5 mL of the biostimulant only during the first application at transplanting. The standard application dose of Maxicrop was repeated every 14 days. The experiment was set up as a 2-factorial (2 stress applications $\times$ 4 biostimulant treatments) completely randomized block design with 4 replicates per treatment and 9 plants per replicate. The two compartments of the greenhouse hosted all treatments combinations but with 2 replicates. Border rows were also planted in each compartment, which were not included in the experimental design and the measurements.

2.2. Root Zone and Drainage Solution Measurements

Drainage samples were obtained every two days for EC, pH direct measurements, and for the estimation of the percentage of drainage solution. To assess the effect of biostimulants on tomato growth, samples of nutrient solution from the root zone were collected every 30 days using a 10 mL syringe to suck nutrient solution from the rockwool. NO_3^- was determined by applying cadmium reduction to NO_2^-, while phosphorus and potassium were determined as phosphomolybdate blue complex and by using the flame photometer, respectively. In the non-stressed plants, the EC of the drainage solution was maintained between 3.5 and 4.0 dS m^{-1} for improved fruit quality [52] throughout the experiment. Furthermore, the irrigation frequency in the non-stressed treatments was controlled through a pyranometer according to the solar radiation intensity to levels resulting in a drainage percentage that ranged between 20 and 30% of the total supply. The percentage of drainage solution was zero for most of the time during the cultivation period in stressed plants.

2.3. Biomass and Leaf Area Determination

Seventy days after transplanting (DAT), one plant per replicate was sampled for the determination of leaf area and aboveground biomass. Leaf area was measured by a LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA). Fresh biomass and leaf area were again determined at the end of the cultivation (4 months period). When the samples were collected, fresh weight was recorded, and then samples were oven-dried at 65 °C for at least 72 h, until a constant weight was achieved. The dried samples were used to determine their dry weight.

2.4. Total Yield Determination

The impact of the experimental treatments on fruit yield was assessed by manually harvesting three times a week, all commercially ripe fruits. Fruit harvest commenced on 13 November 2019 (64 DAT) and was terminated on 10 January 2020 (120 DAT). According to the EU regulation (543/2011), harvested fruits were classified into 4 classes (Extra class, Class I, Class II, non-marketable). Non-marketable fruits are not presented in the results.

2.5. Leaves and Fruits Nutrient Analyses, Fruit Quality Characteristics

Dried samples of tomato leaves and fruit were collected at the end of cultivation (120 DAT) and were powdered using a blade mill. The samples were then used for chemical analyses to determine the total N, P, K, and Zn in tomato leaves and P, K concentrations in fruit, respectively. Total N of tomato leaves was determined by applying the Kjeldahl method [53] and more specifically by mineralizing the dried and powdered plant tissue samples with sulfuric acid in the presence of potassium sulfate and copper sulfate as catalysts at a temperature of 440 °C. For the estimation of P, K, and Zn, the samples were dry ashed at 550 °C for 5 h, and the ash was dissolved in 1 M HCl. Phosphorus was measured photometrically as phosphomolybdate blue complex at 880 nm using a 96-position microplate spectrophotometer (Anthos Zenyth 200; Biochrom, Cambridge, United Kindom). Potassium was determined using a flame photometer (Sherwood Model 410, Cambridge, UK) and zinc using an atomic absorption spectrophotometer (AA-7000, Shimadzu Co., Tokyo, Japan). Fruit acidity was determined in 10 g of juice by potentiometric titration with 0.02 M NaOH to pH 8.1. Total soluble–solid content (TSSC) was determined by squeezing tomato juice directly onto the refractometer (Schmidt & Haensch HR32B, Berlin, Germany), and values were expressed in °Brix units against a refractive index.

2.6. Statistical Analysis

Analysis of variance (ANOVA) of the experimental data was performed using the software package Statistica for Windows 12.0 (Tulsa, OK, USA). To separate treatment means within each measured parameter, Duncan's Multiple Range Test was performed at $p \leq 0.05$.

3. Results

3.1. pH in Drainage Solution and Concentration of N, P, K in Tomato Root Zone

The pH in the drainage solution was maintained between 5.5 and 6.5 in all treatments during the whole cropping period (Figure 3) except for Edypro plants at the first 20 DAT. The application of Edypro's COUPÉ REGENERACIÓN Plus at transplanting and 14 DAT increased pH in the drainage solution to levels above 7. COUPÉ REGENERACIÓN Plus was not applied again, and the PROCUAJE RADICULAR, which was applied 15 DAT and every week ever after, did not affect pH in the drainage solution until the end of cultivation.

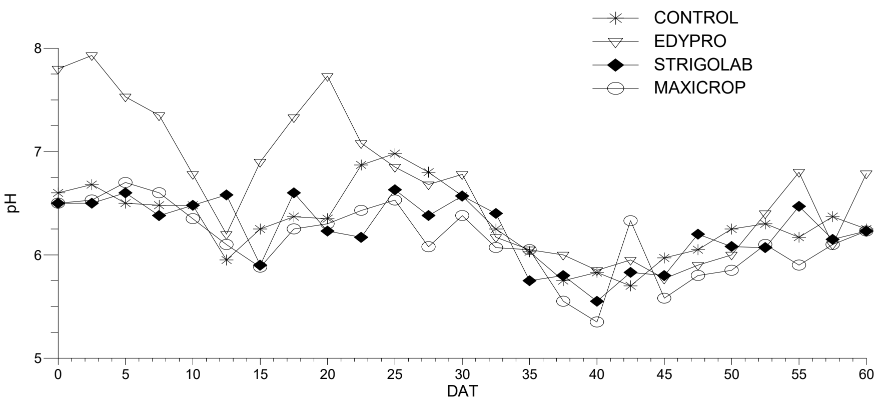

Figure 3. Effects of biostimulant application on pH at the drainage solution.

The impact of combined stress application on the K concentration in the root zone of tomato was significant during the entire cultivation period. For the first month of cultivation, K was 11% more accumulated in the root zone of combined stressed plants compared to non-stressed plants (Table 1). In the following months, though, the lower concentration of K supplied in combined stressed plants resulted in decreased concentration of K in the root zone of stressed tomatoes compared to the plants supplied with optimal levels of water and nutrients. Biostimulants did not have a significant impact on K concentration in the root zone of plants for most of the period of tomato cultivation. The strigolactone-based biostimulant had a significant impact on K compared to the control treatment, but only at the first month of cultivation. More specifically, under non-stressed conditions, K decreased when the strigolactone-based biostimulant was applied to plants compared to the control plants, while under combined stressed conditions, K increased in the root zone of plants treated with the strigolactone-based biostimulant compared to control plants. Edypro also decreased K 30 DAT at non-stressed plants compared to control, but non-significant differences were observed in stressed plants. Finally, Maxicrop had the least impact on K concentration resulting only in increased K compared to strigolactone-based biostimulant in non-stressed plants at 30 DAT.

Nitrate concentration in the root zone of tomato plants was at similar levels in plants cultivated under non-stressed and plants cultivated under combined stress conditions during the first 30 days of cultivation (Table 2). From that time point onwards and until the end of the cultivation, the concentration of nitrates was reduced in the nutrient solution supplied to stressed plants, and this reduction resulted in a reduced nitrate concentration in the root zone of stressed plants compared to non-stressed plants. The biostimulants had a clear impact on nitrates in the root zone, but the impact was mainly present in tomatoes grown under combined stress. Under non-stressed conditions, differences between biostimulant treatments were observed, with Maxicrop increasing nitrates significantly compared to the control and the strigolactone-based biostimulant treatments, but only in the first 30 days of the experiment. In contrast, under combined stress conditions, the strigolactone-based biostimulant increased nitrates in the root zone of tomatoes during the first 30 days of cultivation, but only compared to Edypro. At 60 DAT, the biostimulants did not impose any significant differences, but at 90 DAT, nitrates in the root zone of control plants cultivated under combined stress were significantly reduced compared to non-stressed plants, while plants treated with strigolactone-based biostimulant and Maxicrop biostimulant maintained nitrate concentration to a level comparable to most non-stressed plants.

Table 2. Effects of combined restriction of water and nutrients (No stress, NS; Combined stress, CS), and biostimulant (BS) application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant and Maxicrop) on NO_3^- concentration (mmol L^{-1}) in the root zone of tomato 30, 60, 90, and 120 days after transplanting (DAT).

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
No stress	10.3	10.7 a	10.5 a	10.2 a
Combined Stress	10.3	8.0 b	6.8 b	5.3 b
Control	9.9	9.4	7.9	7.8
Edypro	9.8	8.2	8.1	8.2
Strigolactone-based BS	10.4	10.1	9.7	7.9
Maxicrop	11.4	9.6	9.0	7.3
Statistical interactions				
NS × Control	9.8 bc	11.1	11.9 a	9.7 a
NS × Edypro	10.2 abc	10.6	10.4 ab	9.6 a
NS × Strigolactone-based BS	9.4 c	9.8	9.4 ab	10.9 a
NS × Maxicrop	11.7 a	11.5	10.5 ab	10.7 a
CS × Control	10.0 abc	7.7	3.9 d	5.8 bc
CS × Edypro	9.4 c	5.9	5.8 cd	6.8 b
CS × Strigolactone-based BS	11.4 ab	10.5	9.9 ab	4.8 cd
CS × Maxicrop	10.5 abc	7.7	7.5 bc	3.9 d
Statistical interactions				
Stress	ns	*	***	***
BS	ns	ns	ns	ns
Stress × BS	*	ns	*	**

Means (n = 4) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$), at $p < 0.05$, $p < 0.01$, and $p < 0.001$, denoted by *, **, and ***, respectively; ns = not significant.

Nevertheless, this impact was reversed in combined stressed plants at the end of cultivation. The strigolactone-based biostimulant maintained lower nitrate concentrations in the root zone compared to Edypro. In the meantime, Maxicrop had lower nitrates compared to Edypro and the control treatment.

The P concentration in the root zone was also reduced in plants exposed to combined stress from the second month of cultivation and until the end of the experiment, compared to non-stressed plants (Table 3). Regarding the application of biostimulants, Maxicrop showed increased P concentration compared to the control and Edypro 30 DAT and only under optimal water and nutrient supply. On the other hand, the biostimulants of Edypro had an impact on P concentration at the third month of cultivation, resulting in increasing P by 33% compared to control and 40% compared to strigolactone-based biostimulant and Maxicrop.

Table 3. Effects of combined restriction of water and nutrients (No stress, NS; Combined stress, CS), and biostimulants application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant and Maxicrop) at P concentration (mmol L^{-1}) on tomato root zone 30, 60, 90, and 120 days after transplanting (DAT).

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
No stress	1.7	1.9 a	2.1 a	1.4 a
Combined Stress	1.7	0.9 b	1.0 b	0.9 b
Control	1.5	1.6	1.5 b	1.2
Edypro	1.7	1.4	2.0 a	1.2
Strigolactone-based biostimulant	1.8	1.3	1.4 b	1.2
Maxicrop	1.9	1.3	1.4 b	1.0

Table 3. *Cont.*

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
Statistical interactions				
NS × Control	1.5 b	2.0	2.2 b	1.4
NS × Edypro	1.4 b	2.2	2.8 a	1.5
NS × Strigolactone-based biostimulant	1.9 ab	1.6	1.8 b	1.4
NS × Maxicrop	2.1 a	1.6	1.7 b	1.3
CS × Control	1.5 b	1.1	0.9 c	0.9
CS × Edypro	2.0 ab	0.7	1.2 c	0.8
CS × Strigolactone-based biostimulant	1.8 ab	1.0	1.0 c	1.0
CS × Maxicrop	1.6 ab	1.0	1.1 c	0.7
Statistical interactions				
Stress	ns	*	***	***
BS	ns	ns	**	ns
Stress × BS	*	ns	*	ns

Means (n = 4) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$), at $p < 0.05$, $p < 0.01$, and $p < 0.001$, denoted by *, **, and ***, respectively; ns = not significant.

3.2. Fresh Biomass and Leaf Area of Plants

Fresh biomass, as determined at 70 and 120 days after transplanting, was clearly reduced when plants were cultivated under combined water and nutrient stress compared to the control treatment (Figure 4). The application of biostimulants had no significant impact on plant biomass at optimal or combined stress conditions.

On the other hand, at 70 DAT, the leaf area of plants cultivated under optimal water and nutrient supply was reduced when biostimulants were applied to plants (Figure 5i). In contrast, under combined stress conditions, the strigolactone-based biostimulant increased leaf area compared to untreated plants and plants treated with Edypro biostimulants. In fact, the increase in leaf area in plants cultivated under combined stressed conditions and treated with strigolactone-based biostimulant was comparable with the control plants cultivated under optimal water and nutrient supply. At the end of the cultivation, however, biostimulants did not have any significant impact on the tomato leaf area (Figure 5ii).

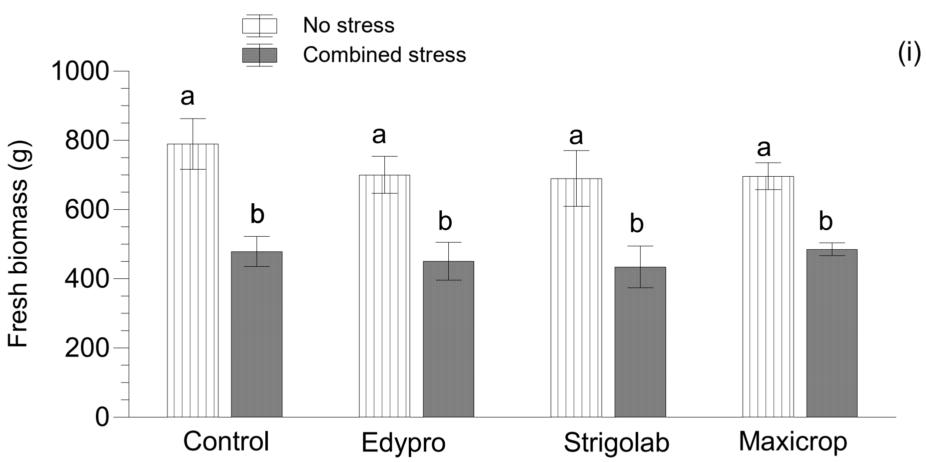

Figure 4. *Cont.*

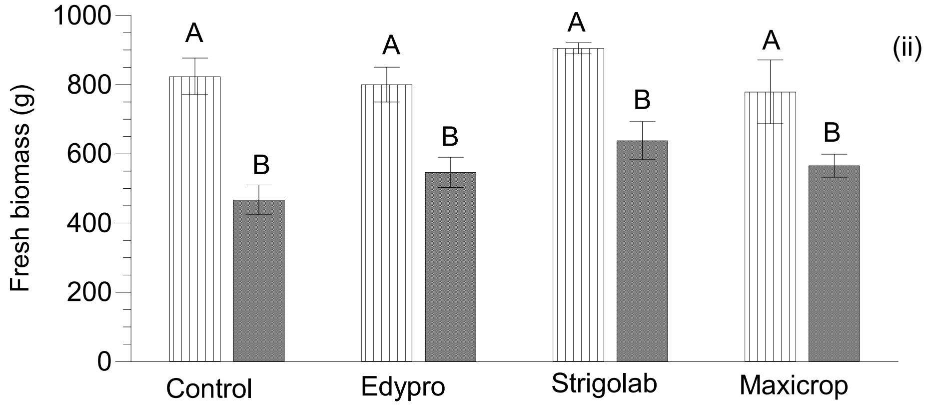

Figure 4. Effects of combined water and nutrient stress and biostimulant application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant, and Maxicrop) on tomato aboveground biomass (g) (**i**) 70 days after transplanting, and (**ii**) at the end of the cultivation period. Means ($n = 4$) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

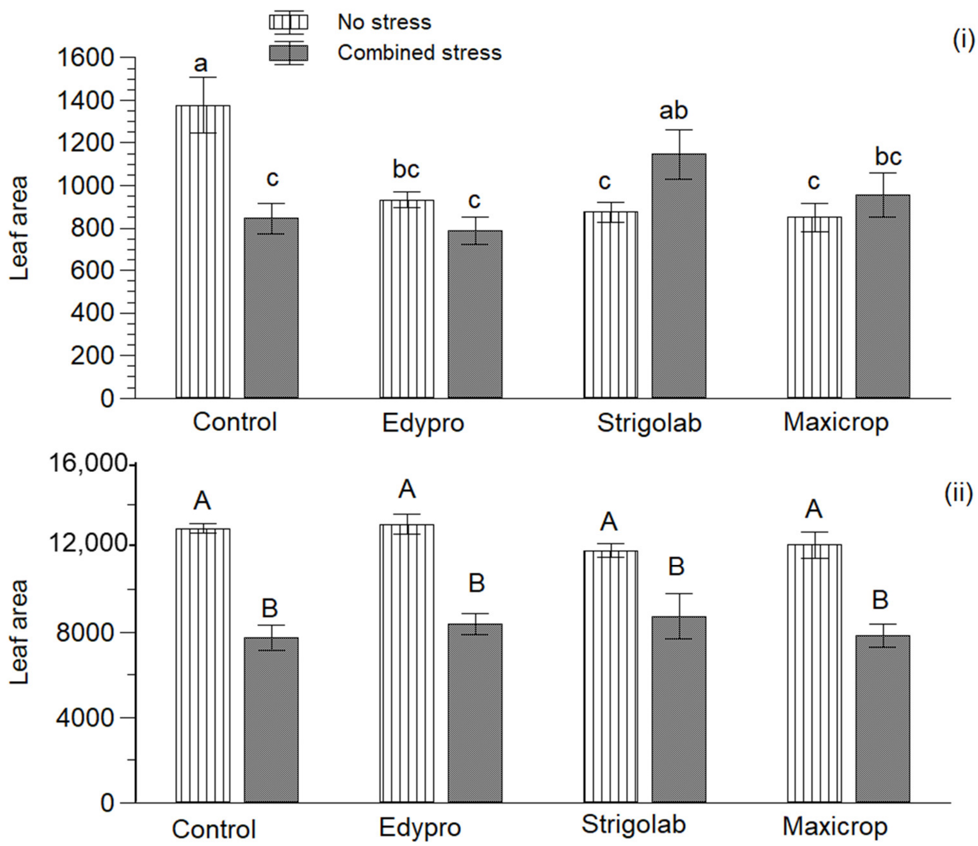

Figure 5. Effects of combined water and nutrient stress and biostimulant application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant, and Maxicrop) on tomato leaf area (cm^2) (**i**) 70 days after transplanting, and (**ii**) at the end of the cultivation period. Means ($n = 4$) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

3.3. Tomato Yield Components

The exposure of tomato to combined water and nutrient stress had no impact on tomatoes' early fruit yield compared with non-stressed plants (Table 4). However, at the end of cultivation, plants exposed to combined stressed had 19.7% less fruit production compared to non-stressed conditions. Yield reduction in stressed plants was observed due to both a lower number of fruits and a lower mean fruit weight compared to the non-stressed plants. Fruits graded Extra class were also 24% reduced under combined stressed conditions compared to optimal conditions, while fruits graded Class I, Class II, and non-marketable did not exhibit any statistical differences (data not shown). The strigolactone-based biostimulant increased early fruit yield compared to the control treatment, Maxicrop, and Edypro. However, at the end of the cultivation, none of the biostimulants applied in this experiment were able to increase fruit yield compared to the control treatment. In contrast, Edypro biostimulant showed lower early fruit yield and total yield compared to the control treatment. Reduced yield in Edypro plants was the result of a smaller number of fruits per plant, despite the fact that plants treated with Edypro biostimulant produced fruits with similar mean fresh weight compared to the control plants and plants treated with the other two biostimulants. Furthermore, the application of the Edypro biostimulants reduced the number of fruit graded Extra Class compared to the non-treated plants and plants treated with strigolactone-based biostimulant, while fruit graded Class I, Class II, and non-marketable did not show any statistical differences between the biostimulant treatments (data not shown).

Table 4. Effects of combined restriction of water and nutrients and biostimulant (BS) application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant, and Maxicrop) on tomato early and total fruit production, fruit number, fruit mean weight, and weight of fruits graded at Extra Class.

Treatment	Early Fruit Yield (kg m^{-2})	Total Fruit Yield (kg m^{-2})	Number of Fruit m^{-2}	Mean Fruit Weight (g)	Extra Class (kg m^{-2})
No stress	1.75	3.35 a	13.4 a	125.7 a	0.85 a
Combined Stress	1.68	2.69 b	11.5 b	115.9 b	0.65 b
Control	1.82 b	3.29 a	13.1 a	125.6	0.92 a
Edypro	1.27 c	2.51 b	10.8 b	116.2	0.52 b
Strigolactone-based BS	2.07 a	3.17 a	13.1 a	121.2	0.83 a
Maxicrop	1.71 b	3.10 a	12.9 a	120.3	0.73 ab
Statistical interactions					
Stress	ns	***	**	***	*
BS	***	**	*	ns	*
Stress × BS	ns	ns	ns	ns	ns

Means (n = 4) followed by different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$), at $p < 0.05$, $p < 0.01$, and $p < 0.001$, denoted by *, **, and ***, respectively; ns = not significant.

3.4. Leaf and Fruit Nutrient Concentrations, and Fruit Quality

Water and nutrient restriction decreased the nitrogen concentration in tomato leaves (Table 5). Phosphorus was also decreased by 20% in leaves of tomato cultivated under combined stress, whereas in tomato fruit, the stress had no impact on phosphorus concentration. In contrast, potassium concentration decreased by 12% in tomato fruits, while there were no significant differences in tomato leaves. Regarding the effect of biostimulants on the tomato nutrients concentration, Edypro biostimulant increased potassium in tomato leaves compared to all other treatments and in tomato fruits compared to the control treatment. The biostimulant of Edypro also increased zinc in tomato leaves compared to control and Maxicrop application. Maxicrop biostimulant also increased potassium in tomato fruits compared to the control treatment. The application of strigolactone-based biostimulant had no impact on N, P, K, or Zn in tomato leaves nor on P or K in tomato fruit compared to the control treatment. Finally, the total soluble solids and acidity of tomato fruit were not influenced either by the combined stress conditions or by the application of biostimulants.

Table 5. Effects of combined restriction of water and nutrients and biostimulant (BS) application (Control: No biostimulant applied, Edypro: application of COUPÉ REGENERACIÓN and PROCUAJE RADICULAR, strigolactone-based biostimulant, and Maxicrop) on N, P, K, Zn concentrations of tomato leaves and P, K, total soluble solids (TSS, °Brix) and acidity (g citric acid per 100 g of fresh fruit weight) on tomato fruits.

	Leaves				Fruits			
Treatment	**N ($g\ kg^{-1}$ dw)**	**P ($g\ kg^{-1}$ dw)**	**K ($g\ kg^{-1}$ dw)**	**Zn ($\mu g\ g^{-1}$)**	**P ($g\ kg^{-1}$ dw)**	**K ($g\ kg^{-1}$ dw)**	**TSS (°Brix)**	**Acidity (g Citric Acid per 100 g fw**
No stress	38.6 a	8.6 a	46.8	37.58	5.9	38.1 a	3.45	0.34
Combined Stress	34.9 b	7.0 b	47.0	45.25	6.3	33.4 b	3.74	0.35
Control	37.5	7.7	45.4 b	31.75 b	6.3	31.6 b	3.68	0.34
Edypro	34.7	6.8	50.3 a	58.50 a	6.1	38.9 a	3.66	0.35
Strigolactone-based BS	37.0	9.1	45.9 b	44.50 ab	5.9	34.9 ab	3.59	0.35
Maxicrop	37.6	7.7	45.9 b	30.92 b	6.2	37.6 a	3.46	0.33
Statistical interactions								
Stress	**	*	ns	ns	ns	*	ns	ns
BS	ns	ns	*	**	ns	*	ns	ns
Stress × BS	ns	ns	ns	ns	ns	ns	ns	ns

Means (n = 4) followed by different letters indicate significant differences according to the Duncan's multiple range test ($p < 0.05$), at $p < 0.05$ and $p < 0.01$, denoted by *, **, respectively; ns = not significant.

4. Discussion

4.1. Effects of Combined Stress on Tomato Growth, Nutrition, and Yield

The mean concentration of NO_3 in the root zone of tomato plants was lower compared to that suggested in the review of Savvas and Gruda [6] for open hydroponic systems. Savvas and Gruda [6] recommended 18.00 mmol L^{-1} and 17.20 mmol L^{-1} NO_3 in the root zone of tomato plants for the vegetative and reproductive stages, respectively. However, Savvas and Gruda [6] also recommended a higher supply of NO_3 compared to that supplied in the present experiment. Phosphorus concentration in the root zone was generally maintained above 1 mmol L^{-1} even in plants exposed to combined nutrient and water stress, so there was not any phosphorus deficiency in this experiment as P levels were close to those recommended by Savvas and Gruda [6]. K concentration was high at the first month of cultivation, both in non-stressed and stressed plants. This outcome was possibly present due to the low runoff of non-stressed plants and the absence of runoff in stressed plants. As previously mentioned, this result suggested an adjustment in the composition of the nutrient solution supplied to both non-stressed and stressed plants 45 DAT. K concentration was reduced in the nutrient solution supplied to both stress treatments during the rest of the cultivation period, with non-stressed plants having optimal K in the root zone, whereas combined stress plants had low levels for tomato needs [6]. The application of combined water and nutrient stress is a severe stress for tomato plants [54] and consequently reduced N and P in tomato leaves and thus significantly restricted total yield and yield components. In contrast, though, combined water and nutrient stress are known to increase TSS and acidity in tomato fruits [55]. However, as Koleska et al. [14] reported, TSS and TA are variety dependent, and 60% nutrient reduction could decrease TSS and TA with biostimulants not being able to prevent this effect. So, NOSTYMY F1 is not so sensitive to changes in fruit quality parameters due to the application of the combined stress and the biostimulants.

4.2. Biostimulants of Edypro

The pH in the drainage solution was maintained between the optimal thresholds of 5.5 and 6.5 [56] except for Edypro plants at the beginning of cultivation. Edypro plants showed increased pH at the beginning of the experiment, and the two high peaks were observed exactly at the days of the application of Edypro biostimulant COUPÉ REGENERACIÓN Plus (0 and 14 DAT). This biostimulant was not applied again throughout the experiment, and increased pH was not observed again. Increased pH above 7 can lead to growth restrictions [57] as P, Fe, Mn, and Zn deficiencies are anticipated. Regarding N, P, K in the root zone of tomato, plant-derived hydrolysates can affect N concentration in the root zone of hydroponic tomato, especially when they are applied as substrate drench and not as foliar spray [58]. However, the Edypro biostimulants did not increase the N concentration significantly, especially compared to the control treatment. Edypro only increased the P levels 90 DAT in the root zone of non-stressed plants compared to untreated plants and plants treated with Maxicrop and strigolactone-based biostimulant. EDYPRO's PROCUAJE RADICULAR contained only micronutrients and not N or P. However, the increased P in the root zone of plants treated with the Edypro biostimulants could be related to the increased Zn supplied by Edypro biostimulant, as an increased level of Zn in the nutrient solution decreases the levels of P in the tomato leaves and roots [59]. Hence, increased P in the root zone is possibly related to the lower P uptake by plants. Indeed, Zn concentration in tomato leaves increased compared to control plants, while P slightly decreased, but not significantly. Nevertheless, P and Zn were between the optimal thresholds for tomato leaves [52]. Increased K concentration in soil-grown tomato leaves and fruits due to the application of protein-based biostimulants has already been reported by Rouphael et al. [19], and it could be explained not only by the increased root growth of plants but also as a result of the higher expression of nutrient transporters in cell membranes, resulting in higher nutrient accumulation in tomato shoots [60]. The nutritional disorders in the leaves of plants treated with the Edypro biostimulant are possibly responsible for the decrease in early and total fruit production compared to the control plants and plants treated with strigolactone-based biostimulant and Maxicrop. In the current study, the application rates of the two Edypro biostimulants were similar to those suggested for soil-grown crops. However, in soilless culture, the volume of the rooting medium per plant is much lower than in crops grown in the soil. Furthermore, in soilless crops grown on inert substrates, such as rockwool, there is no ion exchange capacity in the root zone. Thus, the same application rate per plant may be much more effective in soilless culture compared to soil-grown crops. Hence, the excessive increase in pH after application of COUPÉ REGENERACIÓN Plus and the leaf toxicity symptoms in plants treated with PROCUAJE RADICULAR (Figure 6) are most likely ascribed to overdosage. The Edypro biostimulants COUPÉ REGENERACIÓN Plus and PROCUAJE RADICULAR have not been previously tested under soilless tomato crop, and future studies with substantially reduced biostimulant doses could possibly result in different yield outcomes.

Figure 6. Leaf toxicity symptoms at plants treated with Edypro biostimulants (photo at 89 DAT).

4.3. Strigolactone-Based Biostimulant

It is known that when synthetic strigolactones are applied to the leaves of tomato, enhancement of stomatal closure is observed [61,62], and this action is mostly independent of ABA [33] as strigolactones act as prominent regulators of stomatal closure [61]. So, plants cultivated under combined stress application and treated with strigolactone-based biostimulant probably showed improved stress acclimatization due to higher sensitivity of stomatal closure and decreased stomatal apertures [61], which enhanced water use efficiency of plants. Nutrient use efficiency can also be enhanced by other SL-related mechanisms. Based on the current knowledge, under limited nutrient conditions, especially when plants are grown in phosphate-limiting media, application of SLs can increase lateral root formation and elongate root hairs, a result not observed under optimal growth conditions [35], and thus later increase their nutrient uptake capability [46]. It is also known that significant differences in nutrient uptake and nutrient translocation occur when SL-treated plants are also combined with the application of AMF because SLs are mainly connected with the stimulation of AMF and N-fixing rhizobial bacteria symbiosis [34,44–46]. Nevertheless, strigolactone-induced differences in N and K in the root zone of tomato cannot be fully justified and have to be further studied.

The differences in the levels of N and K in the root zone of plants, especially at the 1st month of cultivation, did not significantly affect plants biomass 70 and 120 DAT, whereas leaf area 70 DAT of strigolactone-based biostimulant plants which were cultivated under combined stress was comparable to control plants cultivated under optimal water and nutrient supply. However, the increased leaf area and fruit production of strigolactone-based biostimulant plants led to a greater depletion of NO_3 at the root zone towards the end of the experiment, and this resulted in finally neither a significant increase in total leaf area and plants biomass nor differences in tomato leaf nutrient content. Increased leaf area in plants increases light interception and concomitantly the whole-plant photosynthesis, thereby increasing tomato fruit production [63]. Hence, it seems that the strigolactone-based biostimulant managed to increase early fruit production by increasing the whole plant photosynthesis. However, an exogenous application of strigolactones at the beginning of the cropping period is incapable of maintaining high strigolactone levels throughout the cropping period, or even for a long part of the cropping period. Hence, any beneficial effects of their application on the fruit yield of greenhouse tomato may disappear in the long term. Consequently, to obtain a benefit in terms of fruit yield, strigolactone-based biostimulants should be applied not once but repeated several times during the cropping period. The intervals between applications during the cropping period, taking into consideration the trade-off between yield benefits and application costs, has to be defined based on future experimental work.

4.4. Maxicrop

Biostimulants based on SWE have not been studied yet regarding their impact on N, P, or K in the root zone. The biostimulant Maxicrop contains trace amounts of macro- and micronutrients [51], but the 14-day intervals between applications cannot justify the increased NO_3^- and P compared to control plants under non-stressed conditions. Application of seaweed extracts, though, has already been reported to increase tomato biomass and leaf area of tomato cultivated under high and low nitrogen supply [64–66], but Maxicrop did not manage to increase both parameters in the present study both under optimal and combined stressed conditions. Regarding the impact of Maxicrop on early yield, Colla et al. [18] stated that SWE are capable of increasing early yield in soil-grown tomato compared to the control plants. As Colla et al. [18] concluded, increased productivity can be related to the presence of polysaccharides in *Ascophyllum nodosum*, which can stimulate endogenous hormone homeostasis [67] and that the SWE 'Kelpak' in their experiment also contained a high auxin:cytokinin ratio, which may have contributed to increased early fruit set, especially under high temperatures where pollen viability problems are present in tomato [68]. In contrast to early yield, total yield was not increased compared to control plants, although a number of studies have reported that seaweed extracts can increase tomato yield [17,18,64] under field conditions. In soil-grown corps, enhancement of nutrient uptake is mainly due to improvement in soil structure by solubilizing micronutrients, and by increasing the colonization by arbuscular mycorrhizal fungi [25]. Zodape et al. [17] also reported that SWE could be superior to chemical fertilizers because their organic matter not only holds the moisture more effectively but also minerals contained in the organic matter can remain in the upper soil level and be easily accessible to the roots. However, most of these mechanisms are irrelevant with soilless grown crops. When SWE were applied at soilless grown rocket (*Eruca sativa*) and sweet pepper (*Capsicum annuum*), there was no impact on their total yield [69,70]. Finally, Maxicrop increased K concentration in tomato fruit, a result already reported by Zodape et al. [17] and Ali et al. [64] in soil-grown tomatoes, while Colla et al. [18] found no significant differences in K concentration between SWE and the control. The increase in K concentration due to Maxicrop application was not a result of enhanced macro-element supply due to their presence in the biostimulant [51], as both the application rates and the frequency of application do not justify such an effect. However, there is currently no convincing explanation as most mechanisms of enhanced nutrient uptake are strongly related to the improvement in soil properties [64], and in the current study, the application was in a soilless crop.

5. Conclusions

Reduction in water and nutrient supply by 33–40% in the nutrient solution supplied to tomato plants compared to optimal fertigation resulted in a significant reduction in NO_3^-, P, and K levels in the root zone of tomato grown in an open hydroponic system. The inadequate concentrations of essential elements affect plant growth (biomass, leaf area), nutrient status, and finally, total fruit production of plants. Many biostimulants have already been tested in soil-grown tomato and have resulted in increased growth and enhanced fruit production accompanied by enhanced water and nutrient use efficiency. This efficiency is mainly the outcome of different mechanisms, such as increased root development and improvement in soil properties. However, biostimulant application has not yet been widely studied in the soilless cultivation of tomato, and more studies with different biostimulant treatments are needed in order to clarify whether biostimulants could benefit the development and production of tomato plants. In the current study, the application of biostimulants containing strigolactone and seawater extracts increased the early yield, while the strigolactone-based biostimulant showed also increased early leaf area development. These two biostimulants maintained NO_3^- at an adequate concentration in the root zone of tomato plants even until 90 days after the beginning of the application of reduced nutrients. It is known that $NO_{3,}$ P, and K could be completely withdrawn in recirculated hydroponic systems 2 to 3 weeks prior to the termination of the crop without adverse effects on the yield and fruit quality of tomato plants [71]. However, stressed plants in the present study were continuously suffering from lower nutrient inputs, especially after the first 30 DAT. An important issue regarding the application of biostimulants in soilless grown crops is the determination of the amount and frequency of applications. As Vasquez-Hernandez et al. [72] clearly stated, the dose of substances separates positive- from negative-resulting stress. In agreement with this consideration, the Edypro biostimulants were probably applied at excessive doses, while a single application of the strigolactone-based biostimulant or the application of Maxicrop at 14-day intervals was maybe insufficient for obtaining clearly positive results.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7090297/s1. Table S1: Nutrient concentrations in the nutrient solution supplied to non-stressed plants and plants exposed to combined water and nutrient stress (35–40% reduction in water, N, and P supply) during the vegetative (0–45 DAT) and reproductive (reproductive stage I, 46–66 DAT; reproductive stage II, 67–120 DAT) stage. Figure S1: Daily mean air temperature (°C) in the two compartments of the greenhouse. Figure S2: Daily mean relative humidity (%) in the two compartments of the greenhouse. Figure S3: Daily mean solar radiation (W m^{-2}) in the two compartments of the greenhouse.

Author Contributions: Conceptualization, G.N. and D.S.; Data curation, P.K., G.N. and D.S.; Formal analysis, P.K., C.V. and D.S.; Funding acquisition, G.N. and D.S.; Investigation, P.K., G.N. and D.S.; Methodology, P.K., C.V., G.N. and D.S.; Project administration, G.N. and D.S.; Resources, G.N. and D.S.; Software, P.K. and D.S.; Supervision, G.N. and D.S.; Validation, P.K., C.V., G.N. and D.S.; Visualization, P.K., C.V. and D.S.; Writing—original draft, P.K., C.V., G.N. and D.S.; Writing—review & editing, P.K., C.V., G.N. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Commission within the HORIZON2020 project "TOMRES—A novel and integrated approach to increase multiple combined stress tolerance in plants using tomato as a model" (Grant Agreement 727929).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Acknowledgments: We thank Edypro and strigolactone-based biostimulant companies for providing us their biostimulant. We also thank Ellagret S.A. for donating us the biostimulant Maxicrop. We also thank DKG Group for donating the rockwool cubes and slabs.

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

Appendix A

Figure A1. Tomato plants with ripe fruits at 97 DAT.

Figure A2. Application of Maxicrop as substrate drench at 5 DAT concomitantly at one irrigation event.

References

1. Katsoulas, N.; Savvas, D.; Kitta, E.; Bartzanas, T.; Kittas, C. Extension and evaluation of a model for automatic drainage solution management in tomato crops grown in semi-closed hydroponic systems. *Comput. Electron. Agric.* **2015**, *113*, 61–71. [CrossRef]
2. Saadi, S.; Todorovic, M.; Tanasijevic, L.; Pereira, L.S.; Pizzigalli, C.; Lionello, P. Climate change and Mediterranean agriculture: Impacts on winter wheat and tomato crop evapotranspiration, irrigation requirements and yield. *Agric. Water Manag.* **2015**, *147*, 103–115. [CrossRef]
3. Adams, P. Effects of increasing the salinity of the nutrient solution with major nutrients or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool. *J. Hortic. Sci.* **2015**, *66*, 201–207. [CrossRef]
4. Muñoz, P.; Paranjpe, A.; Montero, J.I.; Antón, A. Cascade crops: An alternative solution for increasing sustainability of greenhouse tomato crops in Mediterranean zone. In Proceedings of the XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 927, Lisbon, Portugal, 22 August 2010; pp. 801–805.
5. Wohanka, W. Nutrient solution disinfection. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embryo Publishing: Athens, Greece, 2002; pp. 345–372.
6. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
7. Meric, M.K.; Tuzel, I.H.; Tuzel, Y.; Oztekin, G.B. Effects of nutrition systems and irrigation programs on tomato in soilless culture. *Agric. Water Manag.* **2011**, *99*, 19–25. [CrossRef]
8. Sanjuan-Delmás, D.; Josa, A.; Muñoz, P.; Gassó, S.; Rieradevall, J.; Gabarrell, X. Applying nutrient dynamics to adjust the nutrient-water balance in hydroponic crops. A case study with open hydroponic tomato crops from Barcelona. *Sci. Hortic.* **2020**, *261*, 108908. [CrossRef]
9. Chen, J.G.; Crooks, R.M.; Seefeldt, L.C.; Bren, K.L.; Bullock, R.M.; Darensbourg, M.Y.; Holland, P.L.; Hoffman, B.; Janik, M.J.; Jones, A.K.; et al. Beyond fossil fuel–driven nitrogen transformations. *Science* **2018**, *360*, 6391. [CrossRef]
10. Cordell, D.; White, S. Tracking phosphorus security: Indicators of phosphorus vulnerability in the global food system. *Food Secur.* **2015**, *7*, 337–350. [CrossRef]
11. Yang, L.; Huang, B.; Mao, M.; Yao, L.; Niedermann, S.; Hu, W.; Chen, Y. Sustainability assessment of greenhouse vegetable farming practices from environmental, economic, and socio-institutional perspectives in China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17287–17297. [CrossRef]
12. Craigie, J. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* **2011**, *23*, 371–393. [CrossRef]
13. Sangha, J.S.; Kelloway, S.; Critchley, A.T.; Prithiviraj, B. Seaweeds (macroalgae) and their extracts as contributors of plant productivity and quality: The current status of our understanding. *Sea Plants* **2014**, *71*, 189–219.
14. Koleška, I.; Hasanagić, D.; Todorović, V.; Murtić, S.; Klokić, I.; Parađiković, N.; Kukavica, B. Biostimulant prevents yield loss and reduces oxidative damage in tomato plants grown on reduced NPK nutrition. *J. Plant Interact.* **2017**, *12*, 209–218. [CrossRef]
15. Kunicki, E.; Grabowska, A.; Sękara, A.; Wojciechowska, R. The effect of cultivar type, time of cultivation, and biostimulant treatment on the yield of spinach (*Spinacia oleracea* L.). *Folia Hortic.* **2010**, *22*, 9–13. [CrossRef]
16. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
17. Zodape, S.T.; Gupta, A.; Bhandari, S.C.; Rawat, U.S.; Chaudhary, D.R.; Eswaran, K.; Chikara, J. Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato (*Lycopersicon esculentum* Mill). *J. Sci. Ind. Res.* **2011**, *70*, 215–219.
18. Colla, G.; Cardarelli, M.; Bonini, P.; Rouphael, Y. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. *HortScience* **2017**, *52*, 1214–1220. [CrossRef]

19. Rouphael, Y.; Colla, G.; Giordano, M.; El-Nakhel, C.; Kyriacou, M.C.; De Pascale, S. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Sci. Hortic.* **2017**, *226*, 353–360. [CrossRef]
20. Francesca, S.; Cirillo, V.; Raimondi, G.; Maggio, A.; Barone, A.; Rigano, M.M. A Novel Protein Hydrolysate-Based Biostimulant Improves Tomato Performances under Drought Stress. *Plants* **2021**, *10*, 783. [CrossRef]
21. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [CrossRef]
22. Rose, M.T.; Patti, A.F.; Little, K.R.; Brown, A.L.; Jackson, W.R.; Cavagnaro, T.R. A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. *Adv. Agron.* **2014**, *124*, 37–89.
23. Brown, P.; Saa, S. Biostimulants in agriculture. *Front. Plant Sci.* **2015**, *6*, 671. [CrossRef] [PubMed]
24. Sharma, H.S.; Fleming, C.; Selby, C.; Rao, J.R.; Martin, T. Plant biostimulants: A review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *J. Appl. Phycol.* **2014**, *26*, 465–490. [CrossRef]
25. Halpern, M.; Bar-Tal, A.; Ofek, M.; Minz, D.; Muller, T.; Yermiyahu, U. The use of biostimulants for enhancing nutrient uptake. *Adv. Agron.* **2015**, *130*, 141–174. [CrossRef]
26. Xu, C.; Leskovar, D.I. Effects of *A. nodosum* seaweed extracts on spinach growth, physiology and nutrition value under drought stress. *Sci. Hortic.* **2015**, *183*, 39–47. [CrossRef]
27. Petrozza, A.; Santaniello, A.; Summerer, S.; Di Tommaso, G.; Di Tommaso, D.; Paparelli, E.; Piaggesi, A.; Perata, P.; Cellini, F. Physiological responses to Megafol® treatments in tomato plants under drought stress: A phenomic and molecular approach. *Sci. Hortic.* **2014**, *174*, 185–192. [CrossRef]
28. Botta, A. Enhancing plant tolerance to temperature stress with amino acids: An approach to their mode of action. *Acta Hortic.* **2013**, *1009*, 29–35. [CrossRef]
29. Cerdán, M.; Sánchez-Sánchez, A.; Jordá, D.J.; Juárez, M.; Andreu, J.S. Effect of commercial amino acids on iron nutrition of tomato plants grown underlime-induced iron deficiency. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 1–8. [CrossRef]
30. Ertani, A.; Schiavon, M.; Muscolo, A.; Nardi, S. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant Soil* **2013**, *364*, 145–158. [CrossRef]
31. Lucini, L.; Rouphael, Y.; Cardarelli, M.; Canguier, R.; Kumar, P.; Colla, G. The effect of a plant-derived biostimulant on metabolic profiling and cropperformance of lettuce grown under saline conditions. *Sci. Hortic.* **2015**, *182*, 124–133. [CrossRef]
32. Visconti, F.; de Paz, J.M.; Bonet, L.; Jordà, M.; Quinones, A.; Intrigliolo, D.S. Effects of a commercial calcium protein hydrolysate on the salt tolerance of *Diospyros kaki* L. cv. Rojo Brillante grafted on *Diospyros lotus* L. *Sci. Hortic.* **2015**, *185*, 129–138. [CrossRef]
33. Cardinale, F.; Korwin Krukowski, P.; Schubert, A.; Visentin, I. Strigolactones: Mediators of osmotic stress responses with a potential for agrochemical manipulation of crop resilience. *J. Exp. Bot.* **2018**, *69*, 2291–2303. [CrossRef]
34. Marzec, M.; Muszynska, A.; Gruszka, D. The role of strigolactones in nutrient-stress responses in plants. *Int. J. Mol. Sci.* **2013**, *14*, 9286–9304. [CrossRef] [PubMed]
35. Chesterfield, R.J.; Vickers, C.E.; Beveridge, C.A. Translation of strigolactones from plant hormone to agriculture: Achievements, future perspectives, and challenges. *Trends Plant Sci.* **2020**, *25*, 1087–1106. [CrossRef]
36. Matusova, R.; Rani, K.; Verstappen, F.W.; Franssen, M.C.; Beale, M.H.; Bouwmeester, H.J. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiol.* **2005**, *139*, 920–934. [CrossRef]
37. Zhu, J.K. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* **2002**, *53*, 247–273. [CrossRef]
38. López-Ráez, J.A.; Bouwmeester, H. Fine-tuning regulation of strigolactone biosynthesis under phosphate starvation. *Plant Signal. Behav.* **2008**, *3*, 963–965. [CrossRef] [PubMed]
39. López-Ráez, J.A.; Kohlen, W.; Charnikhova, T.; Mulder, P.; Undas, A.K.; Sergeant, M.J.; Verstappen, F.; Bugg, T.D.H.; Thompson, A.J.; Ruyter-Spira, C.; et al. Does abscisic acid affect strigolactone biosynthesis? *New Phytol.* **2010**, *187*, 343–354. [CrossRef]
40. Toh, S.; Kamiya, Y.; Kawakami, N.; Nambara, E.; McCourt, P.; Tsuchiya, Y. Thermoinhibition uncovers a role for strigolactones in *Arabidopsis* seed germination. *Plant Cell Physiol.* **2012**, *53*, 107–117. [CrossRef]
41. Liu, J.; Novero, M.; Charnikhova, T.; Ferrandino, A.; Schubert, A.; Ruyter-Spira, C.; Bonfante, P.; Lovisolo, C.; Bouwmeester, H.J.; Cardinale, F. Carotenoid cleavage dioxygenase 7 modulates plant growth, reproduction, senescence, and determinate nodulation in the model legume Lotus japonicus. *J. Exp. Bot.* **2013**, *64*, 1967–1981. [CrossRef]
42. Liu, J.; He, H.; Vitali, M.; Visentin, I.; Charnikhova, T.; Haider, I.; Schubert, A.; Ruyter-Spira, C.; Bouwmeester, H.J.; Lovisolo, C.; et al. Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: Exploring the interaction between strigolactones and ABA under abiotic stress. *Planta* **2015**, *241*, 1435–1451. [CrossRef]
43. Koltai, H. Strigolactones activate different hormonal pathways for regulation of root development in response to phosphate growth conditions. *Ann. Bot.* **2013**, *112*, 409–415. [CrossRef]
44. Umehara, M. Strigolactone, a key regulator of nutrient allocation in plants. *Plant Biotechnol.* **2011**, *28*, 429–437. [CrossRef]
45. Ruiz-Lozano, J.M.; Aroca, R.; Zamarreño, Á.M.; Molina, S.; Andreo-Jiménez, B.; Porcel, R.; García-Mina, G.M.; Ruyter-Spira, C.; López-Ráez, J.A. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant Cell Environ.* **2016**, *39*, 441–452. [CrossRef] [PubMed]

46. Mostofa, M.G.; Li, W.; Nguyen, K.H.; Fujita, M.; Tran, L.S.P. Strigolactones in plant adaptation to abiotic stresses: An emerging avenue of plant research. *Plant Cell Environ.* **2018**, *41*, 2227–2243. [CrossRef] [PubMed]
47. Vurro, M.; Prandi, C.; Baroccio, F. Strigolactones: How far is their commercial use for agricultural purposes? *Pest Manag. Sci.* **2016**, *72*, 2026–2034. [CrossRef]
48. Ofner, I.; Lashbrooke, J.; Pleban, T.; Aharoni, A.; Zamir, D. *Solanum pennellii* backcross inbred lines (BIL s) link small genomic bins with tomato traits. *Plant J.* **2016**, *87*, 151–160. [CrossRef] [PubMed]
49. Savvas, D.; Gianquinto, G.P.; Tüzel, Y.; Gruda, N. Soilless culture. In *Good Agricultural Practices for Greenhouse Vegetable Crops—Principles for Mediterranean Climate Areas*; FAO: Rome, Italy, 2013; Volume 217, pp. 303–354.
50. Stirk, W.A.; van Staden, J. Seaweed products as biostimulants in agriculture. In *World Seaweed Resources*; ETI Information Services Lts: Amesterdam, The Netherlands, 2006; pp. 80–84.
51. Steveni, C.M.; Norrington-Davies, J.; Hankins, S.D. Effect of seaweed concentrate on hydroponically grown spring barley. J. *Appl. Phycol.* **1992**, *4*, 173–180. [CrossRef]
52. Passam, H.C.; Karapanos, I.C.; Bebeli, P.J.; Savvas, D. A review of recent research on tomato nutrition, breeding and post-harvest technology with reference to fruit quality. *Eur. J. Plant Sci. Biotechnol.* **2007**, *1*, 1–21.
53. Bremner, J.M. Total nitrogen. In *Methods of Soil Analysis*; Black, C.A., Evans, D.D., White, I.L., Ensminger, L.E., Clark, F.E., Eds.; ACSESS: Madison, WI, USA, 1965; pp. 1149–11789.
54. Kalozoumis, P.; Savvas, D.; Aliferis, K.; Ntatsi, G.; Marakis, G.; Simou, E.; Tampakaki, A.; Karapanos, I. Impact of Plant Growth-Promoting Rhizobacteria Inoculation and Grafting on Tolerance of Tomato to Combined Water and Nutrient Stress Assessed via Metabolomics Analysis. *Front. Plant Sci.* **2021**, *12*, 814. [CrossRef]
55. Liu, K.; Zhang, T.Q.; Tan, C.S.; Astatkie, T. Responses of fruit yield and quality of processing tomato to drip-irrigation and fertilizers phosphorus and potassium. *Agronomy* **2011**, *103*, 1339–1345. [CrossRef]
56. Adams, P. Nutritional control in hydroponics. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embryo Publishing: Athens, Greece, 2002; pp. 211–261.
57. Sonneveld, C. Composition of nutrient solutions. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embryo Publishing: Athens, Greece, 2002; pp. 179–210. [CrossRef]
58. Sestili, F.; Rouphael, Y.; Cardarelli, M.; Pucci, A.; Bonini, P.; Canaguier, R.; Colla, G. Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. *Front Plant Sci.* **2018**, *9*, 1233. [CrossRef] [PubMed]
59. Kaya, C.; Higgs, D. Inter-relationships between zinc nutrition, growth parameters, and nutrient physiology in a hydroponically grown tomato cultivar. *J. Plant Nutr.* **2001**, *24*, 1491–1503. [CrossRef]
60. Ertani, A.; Schiavon, M.; Nardi, S. Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicon* L. in response to an alfalfa-protein hydrolysate using microarrays. *Front. Plant Sci.* **2017**, *8*, 1159. [CrossRef]
61. Lv, S.; Zhang, Y.; Li, C.; Liu, Z.; Yang, N.; Pan, L.; Wu, J.; Wang, J.; Yang, J.; Lv, Y.; et al. Strigolactone-triggered stomatal closure requires hydrogen peroxide synthesis and nitric oxide production in an abscisic acid-independent manner. *New Phytol.* **2018**, *217*, 290–304. [CrossRef]
62. Visentin, I.; Vitali, M.; Ferrero, M.; Zhang, Y.; Ruyter-Spira, C.; Novák, O.; Strnad, M.; Lovisolo, C.; Schubert, A.; Cardinale, F. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytol.* **2016**, *212*, 954–963. [CrossRef]
63. Heuvelink, E.; Bakker, M.J.; Elings, A.; Kaarsemaker, R.C.; Marcelis, L.F.M. Effect of leaf area on tomato yield. In Proceedings of the International Conference on Sustainable Greenhouse Systems-Greensys2004, Leuven, Belgium, 12–16 September 2004; Volume 691, pp. 43–50. [CrossRef]
64. Ali, N.; Farrell, A.; Ramsubhag, A.; Jayaraman, J. The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions. *J. Appl. Phycol.* **2016**, *28*, 1353–1362. [CrossRef]
65. Goñi, O.; Quille, P.; O'Connell, S. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Phycol. Biochem.* **2018**, *126*, 63–73. [CrossRef]
66. González-González, M.F.; Ocampo-Alvarez, H.; Santacruz-Ruvalcaba, F.; Sánchez-Hernández, C.V.; Casarrubias-Castillo, K.; Becerril-Espinosa, A.; Castañeda-Nava, J.J.; Hernández-Herrera, R.M. Physiological, ecological, and biochemical implications in tomato plants of two plant biostimulants: Arbuscular mycorrhizal fungi and seaweed extract. *Front. Plant Sci.* **2020**, *11*, 999. [CrossRef]
67. Rolland, F.; Moore, B.; Sheen, J. Sugar sensing and signaling in plants. *Plant Cell* **2002**, *14*, S185–S205. [CrossRef]
68. Singh, I.S.; Shono, M.A. Heat-stress effects on dry matter partitioning, pollen viability and fruit yield in tomato genotypes. *Indian J. Plant Physiol.* **2012**, *17*, 103–112.
69. Vernieri, P.; Borghesi, E.; Tognoni, F.; Serra, G.; Ferrante, A.; Piagessi, A. Use of biostimulants for reducing nutrient solution concentration in floating system. *Acta Hortic.* **2006**, *718*, 477–484. [CrossRef]
70. Parađiković, N.; Vinković, T.; Vinković Vrček, I.; Žuntar, I.; Bojić, M.; Medić-Šarić, M. Effect of natural biostimulants on yield and nutritional quality: An example of sweet yellow pepper (*Capsicum annuum* L.) plants. *J. Sci. Food Agric.* **2011**, *91*, 2146–2152. [CrossRef]

71. Siddiqi, M.Y.; Kronzucker, H.J.; Britto, D.T.; Glass, A.D.M. Growth of a tomato crop at reduced nutrient concentrations as a strategy to limit eutrophication. *J. Plant Nutr.* **1998**, *21*, 1879–1895. [CrossRef]
72. Vázquez-Hernández, M.C.; Parola-Contreras, I.; Montoya-Gómez, L.M.; Torres-Pacheco, I.; Schwarz, D.; Guevara-González, R.G. Eustressors: Chemical and physical stress factors used to enhance vegetables production. *Sci. Hortic.* **2019**, *250*, 223–229. [CrossRef]