

Article

Protein Hydrolysates Modulate Quality Traits of Tomato Fruit Under Salt Stress by Regulating the Expression Patterns of Genes Related to Sugar Metabolism

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Abstract

Salinity is a major stress factor that limits tomato yield and fruit quality. The aim of this study was to evaluate whether vegetal-derived protein hydrolysates (PHs) can alleviate salt stress in tomato plants and how they affect sugar metabolism at the molecular level. A greenhouse experiment was carried out to test three PHs, containing mainly peptides and aminoacids and derived from the enzymatic hydrolysis of protein sources belonging to Leguminosae (PH1), Malvaceae (PH2), and Solanaceae (PH3) plants under non-saline (1 mM NaCl) and saline (50 mM NaCl) conditions. PH1 and PH3 increased marketable yield under non-saline conditions, while no yield improvement was observed under salinity. Nevertheless, all PHs reduced leaf Cl⁻ accumulation and improved fruit nutritional quality by increasing antioxidant activity and total phenol content. Under salt stress, PH1 and PH2 raised the content of total soluble solids, whereas PH3 enhanced titratable acidity. Gene expression analysis revealed that PHs modulated sugar metabolism, shifting it towards starch synthesis and accumulation in fruits, consistent with the observed increase in soluble solids. These results demonstrate that PHs exert family-specific effects on tomato fruit quality and provide molecular evidence of their role in metabolic adjustment under salinity. Practically, vegetal-derived PHs can represent a sustainable agronomic strategy to enhance fruit quality traits and improve tomato marketability in salt-affected cultivation systems.

Keywords: antioxidant activity; biostimulants; gene expression; saline stress; sugar metabolism; tomato quality

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is considered one of the most important crops due to its high economic value [1]. It is renowned for its richness in health-promoting compounds, such as β -carotene, tocopherol (vitamin E), lycopene, ascorbic acid (vitamin C), flavonoids, and phenols [2]; its versatility for use as fresh or dried fruit, juice, and

sauce [3]; and its role as a model crop, especially as a fleshy-fruited plant of the Solanaceae family, for research purposes [4].

Nowadays, agriculture is faced with challenges related to the growing impact of climate change. In particular, salinity is a major factor reducing agricultural yields, affecting more than 20% of available agricultural areas and 33% of irrigated areas, corresponding to about 900 million hectares worldwide [4]. Under saline conditions, soluble salts, especially sodium and chloride, increase soil electrical conductivity (EC), which tends to surpass 4 dS m^{-1} in the rhizosphere, causing a raising osmotic pressure and altering plant–water relationships, which in turn affects plant development, yield, and quality attributes [5,6]. The effects of salinity stress on crops are linked to various factors, such as salt concentration, duration of exposure, and species-specific tolerance to soil salinity [7]. For instance, tomato plants can tolerate electrical conductivity (EC) levels between 2.5 and 2.8 dS m^{-1} , making them relatively sensitive to higher salinity conditions [8]. When these thresholds are exceeded, tomato yield decreases by approximately 10% for each additional unit of EC in the irrigation water [9]. Several research studies have shown that high salt concentrations in their root zone led to reductions in fruit yield [10,11], fruit size [12], and vegetative growth [13] and caused modifications in gene expression [14]. On the other hand, other studies showed that salinity enhances the sensory and nutritional quality of tomato fruits and can positively regulate their metabolism [15]. Salinity can increase the concentration of total soluble solids, including sugar and acids, which are key contributors to taste and flavor [16], and can enhance antioxidant activity and the accumulation of beneficial compounds such as lycopene and vitamin C [17,18]. These antioxidant compounds not only strengthen the plant's defense mechanisms under stress but also play a central role in fruit quality and human health [19]. By protecting plant cells from oxidative damage, antioxidant compounds help preserve fruit shelf life, color, and flavor. Moreover, by neutralizing free radicals, they contribute to reducing inflammation and the risk of chronic diseases such as cardiovascular disorders and certain cancers [20–22]. A high salt content in irrigation water can also stimulate the activity of enzymes such as superoxide dismutase (SOD), catalase, and enzymes of the ascorbate–glutathione cycle in tomato fruits [23–29].

Salinity stress has been shown to promote starch accumulation and increase AGPase (ADP-glucose pyrophosphorylase) activity during tomato fruit development [30]. This enhanced AGPase activity catalyzes the first step of starch biosynthesis, converting glucose-1-phosphate and ATP into ADP-glucose and pyrophosphate [31]. The increased production of ADP-glucose under saline conditions leads to greater starch accumulation in developing fruits. As the fruits mature, this stored starch is broken down into monosaccharides like glucose and fructose, which contribute to a higher sugar content and improved fruit quality upon ripening [30]. These authors also examined the response of AGPase-encoding genes, *AgpL1* and *AgpS1*, in salinity stress conditions and found that these genes promote starch biosynthesis in a manner independent of abscisic acid and osmotic stress, with *AgpL1* playing a more prominent role. Plant responses to salinity run along a broad spectrum of strategies, involving reprogramming of the molecular, cellular, and physiological network [32]. The strategies normally adopted by plants to overcome salinity stress proceed through (i) ion homeostasis and ionic compartmentalization; (ii) signaling mediated by Ca^{2+} ions, reactive oxygen species (ROS), and phytohormones; and (iii) remodulation of primary and secondary metabolism [33–35]. Thus, in addition to osmotic adjustment, ion homeostasis and stimulation of antioxidant defenses constitute the “self” defenses of plants [36]. Nevertheless, these strategies alone are often not able to contrast completely with salt stress.

In this scenario, the use of biostimulants enables plants to respond more effectively to salt stress, supporting innate defenses through the reshaping of cellular, metabolic, and

enzymatic activity [37,38]. Several studies highlighted the ability of vegetal-derived protein hydrolysates (PHs) to counteract salt stress in tomato plants through better regulation of mineral balance [39], enhancement of plants' photosynthetic activity [40], enzymatic metabolism [41], and stimulation of the antioxidant system [42,43]. In addition, Zuzunaga-Rosas and colleagues [44] observed appreciable reductions in malondialdehyde and H₂O₂ values in leaves, as well as in the antioxidant enzyme activity in tomato plants treated with biostimulants derived from rice and oat husk hydrolysates. To the best of our knowledge, no studies have investigated the interactive effects of vegetal-derived PHs and salinity on tomato fruit nutritional quality traits (e.g., sugars, phytochemicals, antioxidant activity, and mineral concentration) and the expression of sugar metabolism-related genes. Therefore, the present greenhouse experiment was designed to evaluate the influence of PHs from different botanical origins on tomato growth, yield, fruit quality, and gene expression under standard (1 mM NaCl) and saline (50 mM NaCl) conditions.

2. Materials and Methods

2.1. Selection and Characterization of Protein Hydrolysates

Three distinct PHs were utilized in the experiments. One of them was 'PH3', which is a commercial product (Trainer®, Hello Nature S.p.A., Rivoli Veronese, Italy), obtained from the enzymatic hydrolysis of proteins derived from species belonging to the Leguminosae family. The other two PHs were obtained from the enzymatic hydrolysis of proteins of different plant matrices belonging to Malvaceae (PH1) and Solanaceae (PH2), as described in detail by Colantoni et al. [45]. Subsequently, the PHs were characterized using liquid chromatography with UHPLC/QTOF-MS to determine the metabolic profile, as reported by Ceccarelli et al. [46].

2.2. Experimental Site and Design, Plant Material, and Growth Conditions

The trial was performed in spring–summer 2021 in a protected environment at the Experimental Farm “Nello Lupori”, University of Tuscia, Viterbo (42°25' N; 12°08' E; 310 m above sea level). The plants, arranged in paired rows, were spaced 50 cm along the row, 40 cm between the rows and 1.10 m between two subsequent twin rows, with a density of 3.5 plants m⁻² and a total number of 96 plants. Tomato (*Solanum lycopersicum* L.) cultivar 'Pralyna' (SAIS Sementi, Cesena, Italy) was used in the trial. The 'Pralyna' cultivar is an early cherry tomato F1 hybrid with an indeterminate plant growth, moderate vigor, high resistance to fruit cracking, high resistance to the virus ToMV:0–2 and the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici* races 0,1, and moderate resistance to the virus TYLCV and root-knot nematodes (Ma/Mi/Mj).

The plants of *Solanum lycopersicum* L. cv Pralyna, (SAIS Sementi, Cesena, Italy) were transplanted, at the third true-leaf stage, on the 23 March 2021 into pots (Ø = 24 cm; 9.5 L) containing river sand (Ø 1–3 mm). The average air temperature and relative humidity inside the greenhouse were 24.6 °C and 56%, respectively, maintained by a passive ventilation system through the side panels. The trial was designed according to a completely randomized block factorial design with two factors, the salinity level of the nutrient solution (1 mM NaCl and 50 mM NaCl) and biostimulant treatment (Control, PH1, PH2 and PH3). Each treatment consisted of 4 biological replicates, and each replicate included 3 plants (12 plants per treatment in total) and a total of 32 experimental units.

2.3. Biostimulant Application

The plants were uniformly sprayed (foliar application), using a stainless-steel sprayer (2.5 L), eleven times during the growth cycle of the crop at an interval of ten days, with

concentrated solutions of the biostimulants at 2.5 g L⁻¹ (PH1, PH2, and PH3), adapting the volumes to the development of the crop. The treatments were carried out starting from 7 days after transplanting (DAT) to the end of the trial.

2.4. Nutrient Solution Application

The nutrient solution was applied by fertigation using a drip irrigation system. The composition of the nutrient solution in macronutrients (mM) was 12.0 N-NO₃⁻, 1.8 S, 1.0 P, 7.0 K, 3.0 Ca, 1.8 Mg, and 1.0 N-NH₄⁺, and that in micronutrients (μM) was 20.0 Fe, 9.0 Mn, 0.3 Cu, 1.6 Zn, 20.0 B, and 0.3 Mo [8]. The two nutrient solutions were identical in composition except for the concentration of sodium chloride (NaCl), which was 1.0 mM in the low-NaCl condition and 50.0 mM in the high-NaCl condition, resulting in final electrical conductivity (EC) values of 2.0 and 6.8 dS m⁻¹, respectively. These NaCl concentrations were selected to simulate non-saline and moderate to high salinity stress conditions based on established salinity thresholds for tomato. A previous study [47] demonstrated that the tomato yield begins to decline above an EC threshold of approximately 2.5 dS m⁻¹, with an estimated 10% reduction in yield per unit increase in EC beyond this point. For both solutions, the pH was 6.0 ± 0.2 at 25 °C. Different nutrient solutions were applied through drip lines placed 5 cm away from the plants with one dripper per pot (flow rate 2.4 L h⁻¹). All nutrient solutions were prepared with deionized water.

Low-voltage tensiometers (LT-Irrrometer, Inc., Pullman, WA, USA) were used to monitor the timing of the fertigation process depending on the substrate's matric potential [48]. The tensiometers were randomly arranged, for each treatment, in different pots and were connected to an electronic programmer which managed the start (-5 kPa) and end (-1 kPa) of the fertigation cycle based on the maximum and minimum voltage set points, -5 kPa and -1 kPa, respectively [49]. The duration of the irrigation cycles was adapted to ensure that at least 35% of the nutrient solution was drained from the pots [50].

2.5. Measurements and Harvesting

The Soil-Plant Analysis Development (SPAD) index was assessed on leaves throughout the growth cycle with a SPAD-502 instrument (Konica Minolta Europe, Langenhagen, Germany). The readings were taken 48 DAT on 20 topmost fully expanded leaves per plot. A pool of three leaves per experimental unit was immediately frozen in liquid nitrogen and stored at -80 °C for subsequent analyses. The fruit harvest was conducted once a week and based on the stage of ripening (fully ripened) starting from the 9th of June to the end of the trial (27 July 2021; 123 DAT).

During the harvest period, the marketable yield, number of fruits, and fruit weight average per plant were registered for each treatment. The malformed fruits were rejected because they were of unmarketable quality and therefore considered unmarketable yields. The marketable yield was expressed in kg plant⁻¹. A sample of tomato fruits was selected from each experimental unit at the middle of the harvesting period (1st July), for each treatment, for the evaluation of the qualitative parameters of the fruits. The fruits not used for the aforementioned determinations were immediately frozen in liquid nitrogen inside a falcon tube with a volume of 50 mL and stored at -80 °C for subsequent analyses.

At the end of the growing cycle (123 DAT), coinciding with the last harvest, the dry shoot biomass was determined with a forced-air oven at 65 °C until the plant biomass reached a non-variant weight. Even the root systems, after washing in a stream of water to remove substrate residues, were dried in the same conditions for the determination of root dry biomass.

2.6. Analysis of Fruit Quality

The firmness of the fruits was determined by a portable penetrometer (FT 40; Wagner Instruments, Greenwich, CT, USA) using a special cylindrical plunger with a stainless-steel tip with a diameter of 6 mm. A single measurement was taken along the equatorial line on each fruit. The results were expressed in N mm^{-1} .

The tomato fruits were then cut and blended with a Waring® blender (model HGB140, Waring Commercial, Torrington, CT, USA) for one minute. A portion of the homogenate was passed through two layers of gauze to determine the total soluble solids (TSS) content, which was expressed in °Brix.

The titratable acidity was determined, on an aliquot equal to 20 mL of the filtered juice, by titration with a 0.1 M NaOH solution up to pH 8.1 and expressed as g of citric acid monohydrate per 100 g of weight fresh (FW). The remaining part of the homogenate, unfiltered, was used for the determination of the percentage of dry substance of the fruits, expressed as a percentage of the fresh substance (%), after drying in a forced air oven at 65 °C until a constant weight was reached. The dried samples were preserved for mineral analysis.

The total phenolic content was assessed via the Folin–Ciocâlteu procedure [51], with gallic acid (GAE) (Sigma Aldrich Inc, St. Louis, MO, USA) used as the standard. The results were expressed per 100 g FW.

The antioxidant activity of tomato fruits was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay through the method described by Brand-Williams et al. [52]. As for the ferric reducing antioxidant power (FRAP) assay, it was performed on ethanolic extracts following the method reported by Benzie and Strain [53]. The results were expressed in mM Fe^{2+} 100 g^{-1} FW.

With slight adjustments, the colorimetric test reported by Zou et al. [54] was utilized to measure the total flavonoid concentration, with quercetin (QE) (Sigma Aldrich Inc, St. Louis, MO, USA) serving as the standard. The ethanolic extracts (700 μL) were mixed with 150 μL of 5% (*w/v*) NaNO_2 and 150 μL of 10% (*w/v*) AlCl_3 and incubated for five minutes after the addition of each reagent. After carefully mixing the resulting solution, 1 mL of 1 M NaOH was added, and it was given time to settle for one minute. The absorbance of the supernatant was measured at 510 nm, and the results were expressed as mM QE 100 g^{-1} fresh weight (FW). All determinations were performed in triplicate.

2.7. Analysis of Total Nitrogen, Minerals, and Organic Acids

Analyses of the total leaf nitrogen content were carried out on dried and ground samples according to the Kjeldahl method [55] by implementing 1 g of dry leaf tissue. As reported in detail by Rouphael et al. [50], 250 mg of dry ground sample was used for the analysis of cations and anions using an ion chromatograph (ICS-3000; Dionex, Sunnyvale, CA, USA). Organic acids were quantified as described by El-Nakhel et al. [56]. The results were expressed in mg g^{-1} dry weight (DW).

2.8. Determination of Chlorophyll and Carotenoids

The determination of total chlorophylls (chlorophyll a and chlorophyll b) and total carotenoids was performed according to the method described by Lichtenthaler and Wellburn [57]. Briefly, chlorophyll a, chlorophyll b, and carotenoids were extracted by homogenization of fresh leaf tissues (0.5 g) in acetone (80% *v/v*) for 15 min in the dark. Subsequently, the obtained extracts were centrifuged (3000 rpm for 5 min), and the concentration of each of the pigments was determined by UV-Vis spectrophotometry (Beckman DU-50 UV-visible; Beckman Instruments, Inc., Fullerton, CA, USA), 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 chlorophyll b. All pigments were expressed in mg g^{-1} fresh weight (FW).

2.9. Determination of Proline, Total Proteins and Malondialdehyde

Free proline (Pro) content was determined according to the method of Bates et al. [58] using L-proline (Sigma Aldrich Inc, St. Louis, MO, USA) as a standard. Approximately 0.5 g of leaf tissue was homogenized in 10 mL of sulfosalicylic acid (30 g L^{-1}) (Sigma Aldrich Inc, St. Louis, MO, USA); the homogenate was filtered through Whatman No. 2 filter paper. Then, 2 mL of filtrate was reacted with 2 mL of acid-ninhydrin (Sigma Aldrich Inc, St. Louis, MO, USA) (1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of phosphoric acid 6 mol L^{-1}) and 2 mL of glacial acetic acid in a test tube at 100°C for 1 h. The reaction was stopped in an ice bath, 4 mL of toluene (Sigma Aldrich Inc, St. Louis, MO, USA) was added, and the reaction product (supernatant) was recovered after vortex mixing and subsequent sedimentation. The absorbance of the supernatant was determined at 520 nm using toluene as a blank. The concentration of proline was determined on a fresh weight (FW) basis and expressed as $\text{mM } 100 \text{ g}^{-1} \text{ FW}$.

Total protein content was determined using the Bradford assay, with bovine serum albumin used as a standard [59]. Briefly, 100 μL of the extraction mixture (Bio-Rad Laboratories, Inc., Louisville, KY, USA) was added to an equal volume of sample appropriately diluted in water. Absorbance was read at 595 nm after 30 min of incubation with the blank containing 100 μL of extraction buffer and reaction mixture.

The extraction of malondialdehyde (MDA) from leaf tissues was performed, as reported by Heath and Packer [60], in 0.1% (*w/v*) trichloroacetic acid (TCA) (Sigma Aldrich Inc, St. Louis, MO, USA). The extract was centrifuged at $15,000\times g$ for 10 min, and 0.5 mL of the resulting supernatant was added to 1.5 mL of 0.5% (*w/v*) TBA (Sigma Aldrich Inc, St. Louis, MO, USA) in 20% (*w/v*) TCA. The mixture was incubated, under shaking, at 90°C in a bain-marie for 20 min and then stopped in an ice bath. Afterwards, the samples were centrifuged at $10,000\times g$ for 5 min and absorbance was read at 532 nm. The nonspecific absorbance value at 600 nm was subtracted. The concentration of MDA was determined by extinction coefficient correction ($155 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as $\mu\text{M } 100 \text{ g}^{-1} \text{ FW}$.

2.10. RNA Extraction and Gene Expression Analysis

A Spectrum™ Plant Total RNA (Sigma-Aldrich, St. Louis, MO, USA) kit was used to extract RNA from tomato fruits. Samples of tomatoes were homogenized in liquid N_2 , and 100 mg of the fruit powder was extracted according to the manufacturer's instructions. Following the user handbook, 10 U of DNaseRQ1 (Promega Corporation, Madison, WI, USA) was added to the extracted total RNA, and the ImProm-II Reverse Transcription System (Promega) was used to synthesize cDNA. Three independent biological replicates were subjected to quantitative real-time RT-PCR utilizing the CFX96 Touch Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and SsoFast™ EvaGreen R Supermix (Bio-Rad). The target genes were amplified using gene-specific primers, as reported in Supplementary Materials Table S1. The expression levels of target genes were normalized with those of the housekeeping gene Elongation Factor 1- α [43]. The normalized expression value calculation was based on the housekeeping transcript [61]. The $2^{-\Delta\Delta\text{CT}}$ method was used for the calculation of the relative expression ratio values [62].

2.11. Statistical Analysis

To evaluate the significance of the effects induced by the individual factors and of the interactions between the pairs of factors, salinity level (NaCl), and biostimulant treatment (B), as well as the interaction among these factors (NaCl $\times$ B), a two-way analysis of

variance (ANOVA) was performed. Before performing the ANOVA, the experimental data were checked for normal distribution and homogeneity of variance using Levene's test. All data are presented as the mean $\pm$ standard error (SE). Tukey's post hoc test ($p = 0.05$) was adopted for the comparison between the means of each measured variable. All statistical analyses were performed using SPSS 10 software for Windows (SAS Inc., Cary, NC, USA).

3. Results

3.1. Effect of Salinity and PH on Plant Biomass and Its Partitioning

The effects of the two doses of NaCl and the application of three biostimulants (PH1, PH2, and PH3) (B) on the dry weight (DW) of leaves, stems, and roots are presented in Table S2 and Figure 1. Regardless of the biostimulant treatment, the dry weight of leaves and stem components showed a reduction due to the increase in the concentration of NaCl in the nutrient solution from 1 to 50 mM (Table S2). Biostimulant treatment affected the leaves' dry weight, with no significant differences regarding the interaction between the biostimulant and NaCl dosage. Significantly, the leaves' dry weight had the highest value in plants treated with PH1 compared to those treated with PH2 and the control plants, with no significant differences among them. Moreover, no significant differences were recorded between the dry weight of the leaves of plants treated with PH1 and PH3. In addition, no significant differences were recorded in the stem dry weight for neither the effect of biostimulants nor its interaction with the NaCl dosage (Table S2). The dry weight of roots was significantly influenced by each single treatment, as well as by the treatment interactions (Figure 1). As for the root dry weight, it was enhanced by PH2 and PH3 in comparison with the control treatment only under non-saline conditions (Figure 1).

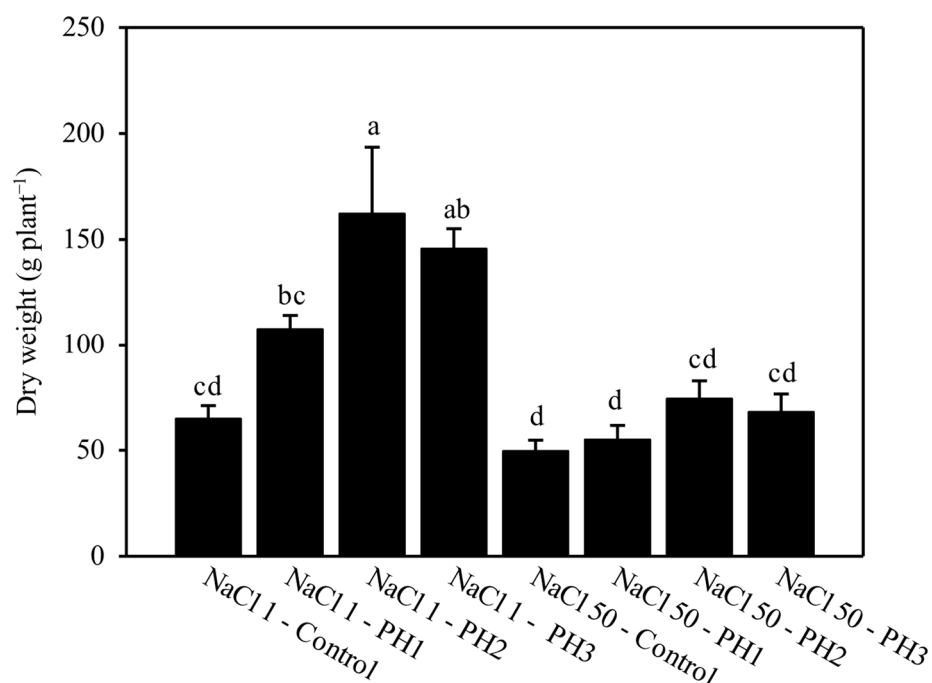


Figure 1. Effect of salinity and biostimulant application on dry weight of roots in tomato plants. Vertical bars indicate means $\pm$ standard error; different letters indicate significant difference according to Tukey's post hoc test ($p = 0.05$), $n = 4$. NaCl 1:1 mM of NaCl in nutrient solution (NS); NaCl 50:50 mM of NaCl in NS. PH1, PH2, and PH3 are plant hydrolysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively.

3.2. Effect of Salinity and PH on Fruit Yield and Yield Components

The results of total and marketable yield, marketable fruit number, and marketable fruit mean weight are reported in Table 1. Salinity significantly reduced all yield traits. The total and marketable yields and number of marketable fruits were significantly affected by biostimulant application, while no significant effects of biostimulant application were recorded for the marketable fruit mean weight. The biostimulant $\times$ NaCl interaction was significant only for marketable yield, which was enhanced by PH application only under non-saline conditions, with the highest values in PH1 and PH3 treatments compared to the untreated control. Plants treated with the PH1 biostimulant also had the highest value for total yield in comparison with all other PH treatments and the control (Table 1), and it had the highest marketable fruit number, but it was not significantly different from the PH3 treatment.

Table 1. Effect of salinity and biostimulant application on total and marketable yields, number, and mean weight of marketable tomato fruit plants.

Source of Variance	Yield (kg Plant ⁻¹)		Marketable Fruit Number (n. Plant ⁻¹)	Marketable Fruit Mean Weight (g Fruit ⁻¹)
	Total	Marketable		
NaCl level (mM)				
1	2.66 $\pm$ 0.06 a	2.11 $\pm$ 0.06 a	149.18 $\pm$ 4.01 a	14.15 $\pm$ 0.32 a
50	1.34 $\pm$ 0.03 b	1.18 $\pm$ 0.03 b	107.17 $\pm$ 2.21 b	11.02 $\pm$ 0.27 b
Biostimulant (B)				
Control	1.95 $\pm$ 0.15 b	1.50 $\pm$ 0.09 b	119.89 $\pm$ 4.43 b	12.28 $\pm$ 0.49
PH1	2.22 $\pm$ 0.19 a	1.84 $\pm$ 0.17 a	136.48 $\pm$ 6.96 a	12.98 $\pm$ 0.60
PH2	1.96 $\pm$ 0.17 b	1.50 $\pm$ 0.12 b	119.60 $\pm$ 5.57 b	12.51 $\pm$ 0.56
PH3	1.97 $\pm$ 0.16 b	1.72 $\pm$ 0.14 ab	131.70 $\pm$ 7.77 ab	12.53 $\pm$ 0.60
NaCl $\times$ B				
NaCl 1 $\times$ Control	2.56 $\pm$ 0.09	1.79 $\pm$ 0.08 c	132.67 $\pm$ 5.44	13.53 $\pm$ 0.57
NaCl 1 $\times$ PH1	2.89 $\pm$ 0.10	2.45 $\pm$ 0.10 a	165.70 $\pm$ 5.37	14.76 $\pm$ 0.61
NaCl 1 $\times$ PH2	2.63 $\pm$ 0.12	2.01 $\pm$ 0.04 bc	140.30 $\pm$ 4.66	14.31 $\pm$ 0.31
NaCl 1 $\times$ PH3	2.58 $\pm$ 0.14	2.22 $\pm$ 0.14 ab	157.22 $\pm$ 11.38	14.11 $\pm$ 0.87
NaCl 50 $\times$ Control	1.28 $\pm$ 0.06	1.18 $\pm$ 0.05 d	108.40 $\pm$ 4.46	10.87 $\pm$ 0.45
NaCl 50 $\times$ PH1	1.48 $\pm$ 0.06	1.23 $\pm$ 0.06 d	109.91 $\pm$ 3.65	11.19 $\pm$ 0.53
NaCl 50 $\times$ PH2	1.28 $\pm$ 0.06	1.10 $\pm$ 0.07 d	98.90 $\pm$ 3.75	11.08 $\pm$ 0.70
NaCl 50 $\times$ PH3	1.36 $\pm$ 0.05	1.21 $\pm$ 0.05 d	110.82 $\pm$ 5.19	10.95 $\pm$ 0.44
Significance				
NaCl	***	***	***	***
B	*	***	**	ns
NaCl $\times$ B	ns	**	ns	ns

ns, not significant; *, **, and *** represent significance at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's post hoc test ($p = 0.05$). All data are expressed as mean $\pm$ standard error. (n = 4). PH1, PH2, and PH3 are plant hydrolysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively.

3.3. Effects of Salinity and PH on Leaf Mineral Composition

Table 2 shows the effects of NaCl and the biostimulant treatment (B) on the mineral profile of the leaves (N, P, S, K, Ca, Mg, Na, and Cl). The calcium concentration was not significantly affected by both factors and their interaction. Nitrogen, P, S, K, and Mg were significantly reduced when increasing the concentration of NaCl in the nutrient solution, while Na and Cl showed the opposite trend. The Cl concentration was significantly affected by the interaction of both factors, where all the PH applications reduced the accumulation of Cl in leaves under 50 mM NaCl NS, while under 1 mM NaCl NS, the values

did not change after the different applications (Table 2). The nitrate concentrations in the leaves were significantly affected only by salinity treatment ($p < 0.001$), with higher values observed with the saline treatment ($26.67 \pm 1.10 \text{ mg g}^{-1} \text{ DW}$) in comparison with the non-saline treatment ($8.60 \pm 0.52 \text{ mg g}^{-1} \text{ DW}$)

Table 2. Effect of salinity and biostimulant application on mineral concentration in tomato leaves.

Source of Vari- ance	Mineral Elements ($\text{mg g}^{-1} \text{ DW}$)							
	N	P	S	K	Ca	Mg	Na	Cl
NaCl level (mM)								
1	$25.31 \pm 0.40 \text{ a}$	$4.62 \pm 0.23 \text{ a}$	$12.07 \pm 0.38 \text{ a}$	$41.90 \pm 1.01 \text{ a}$	34.14 ± 0.94	$5.42 \pm 0.17 \text{ a}$	$4.25 \pm 0.32 \text{ b}$	$8.56 \pm 0.41 \text{ b}$
50	$22.62 \pm 0.25 \text{ b}$	$3.68 \pm 0.16 \text{ b}$	$6.99 \pm 0.30 \text{ b}$	$15.59 \pm 0.71 \text{ b}$	32.32 ± 0.90	$3.35 \pm 0.14 \text{ b}$	$27.86 \pm 1.29 \text{ a}$	$72.06 \pm 2.17 \text{ a}$
Biostimulant (B)								
Control	23.56 ± 0.87	4.21 ± 0.48	9.48 ± 1.00	29.67 ± 5.00	35.00 ± 1.21	4.52 ± 0.42	17.87 ± 4.93	$41.64 \pm 14.98 \text{ a}$
PH1	23.89 ± 0.66	4.22 ± 0.26	10.11 ± 1.08	29.61 ± 5.65	32.36 ± 1.06	4.62 ± 0.36	14.78 ± 4.48	$40.48 \pm 12.02 \text{ ab}$
PH2	24.33 ± 0.59	3.97 ± 0.24	8.82 ± 0.97	27.06 ± 4.73	31.52 ± 1.44	4.22 ± 0.58	15.71 ± 4.40	$44.15 \pm 13.23 \text{ a}$
PH3	24.10 ± 0.65	4.20 ± 0.32	9.73 ± 1.22	28.64 ± 5.06	34.04 ± 1.42	4.17 ± 0.38	15.86 ± 4.77	$35.61 \pm 10.45 \text{ b}$
NaCl $\times$ B								
NaCl 1 $\times$ Control	24.85 ± 1.41	4.87 ± 0.79	11.89 ± 0.61	42.23 ± 2.94	36.46 ± 2.21	5.54 ± 0.34	4.92 ± 0.71	$9.87 \pm 0.84 \text{ d}$
NaCl 1 $\times$ PH1	25.41 ± 0.68	4.69 ± 0.17	12.84 ± 0.61	44.42 ± 0.82	31.06 ± 1.44	5.47 ± 0.25	3.07 ± 0.29	$8.72 \pm 0.36 \text{ d}$
NaCl 1 $\times$ PH2	25.59 ± 0.52	4.36 ± 0.37	10.91 ± 1.09	39.16 ± 1.75	33.14 ± 1.78	5.57 ± 0.55	4.19 ± 0.42	$7.28 \pm 1.35 \text{ d}$
NaCl 1 $\times$ PH3	25.41 ± 0.60	4.56 ± 0.49	12.64 ± 0.46	41.78 ± 1.86	35.89 ± 1.17	5.09 ± 0.19	4.83 ± 0.72	$8.03 \pm 0.32 \text{ d}$
NaCl 50 $\times$ Control	22.26 ± 0.64	3.54 ± 0.41	7.07 ± 0.62	17.11 ± 1.66	33.54 ± 0.75	3.51 ± 0.18	30.82 ± 1.05	$83.99 \pm 0.30 \text{ a}$
NaCl 50 $\times$ PH1	22.37 ± 0.14	3.74 ± 0.37	7.38 ± 0.24	14.81 ± 1.39	33.65 ± 1.45	3.77 ± 0.25	26.48 ± 1.44	$72.24 \pm 1.37 \text{ b}$
NaCl 50 $\times$ PH2	23.37 ± 0.53	3.58 ± 0.15	6.73 ± 0.51	14.95 ± 1.88	29.90 ± 2.19	2.87 ± 0.28	27.24 ± 1.31	$71.81 \pm 4.10 \text{ bc}$
NaCl 50 $\times$ PH3	22.79 ± 0.66	3.84 ± 0.39	6.81 ± 1.02	15.50 ± 0.96	32.18 ± 2.40	3.25 ± 0.27	26.90 ± 4.95	$63.18 \pm 1.45 \text{ c}$
Significance								
NaCl	***	**	***	***	ns	***	***	***
B	ns	ns	ns	ns	ns	ns	ns	***
NaCl $\times$ B	ns	ns	ns	ns	ns	ns	ns	***

ns, not significant; **, and *** represent significance at $p \leq 0.05$, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's post hoc test ($p = 0.05$). All data are expressed as mean $\pm$ standard error. ($n = 4$). PH1, PH2, and PH3 are plant hydrol-ysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively.

3.4. Effect of Salinity and PH on Fruit Quality

The effect of the NaCl concentration and biostimulants on fruit quality are presented in Table 3. Dry matter was significantly affected only by the NaCl treatment, with the highest value observed under saline conditions. Fruit firmness, total soluble solids, titrat-able acidity, and oxalic acid were significantly affected by the NaCl $\times$ biostimulant inter-action (Table 3). With 1 mM of NaCl, fruit firmness was increased only by the PH3 treat-ment in comparison with the untreated control, while all PH treatments increased fruit firmness under 50 mM of NaCl. No significant differences were recorded among biostim-ulant treatments for total soluble solids under non-saline conditions, while PH1 and PH2 increased the content of total soluble solids compared to the untreated control under sa-line conditions. Compared to the untreated control, PH3 enhanced the titratable acidity of fruits only at 50 mM of NaCl, while there were no significant differences among biostim-ulant treatments under non-saline conditions (Table 3). Concerning the organic acid con-centration, citric acid was not significantly affected by NaCl and biostimulant treatments, while malic acid was significantly reduced by salinity. As for oxalic acid, it was signifi-cantly affected by the NaCl $\times$ biostimulant interaction, with lower values observed in all PH treatments compared to the untreated control only under non-saline conditions.

Table 3. Effect of salinity and biostimulant application on tomato fruit quality traits.

Source of Variance	Dry Matter (%)	Firmness (N mm ⁻¹)	Total Soluble Solids (°Brix)	Titrateable Acidity (g of Citric Acid 100 g ⁻¹ FW)	Citric Acid (mg g ⁻¹ DW)	Malic Acid (mg g ⁻¹ DW)	Oxalic Acid (mg g ⁻¹ DW)
NaCl level (mM)							
1	9.40 ± 0.11 b	1.41 ± 0.02 b	7.88 ± 0.09 b	0.62 ± 0.02 b	29.18 ± 0.63	4.44 ± 0.15 a	0.41 ± 0.03 a
50	10.97 ± 0.11 a	1.61 ± 0.03 a	9.38 ± 0.22 a	0.79 ± 0.02 a	29.56 ± 0.68	3.68 ± 0.07 b	0.30 ± 0.01 b
Biostimulant (B)							
Control	10.22 ± 0.26	1.32 ± 0.03 b	8.78 ± 0.22 b	0.67 ± 0.02	29.98 ± 0.79	4.17 ± 0.23	0.43 ± 0.05 a
PH1	10.11 ± 0.36	1.38 ± 0.04 b	9.33 ± 0.51 a	0.71 ± 0.03	27.88 ± 0.92	3.97 ± 0.21	0.34 ± 0.03 b
PH2	10.24 ± 0.26	1.66 ± 0.04 a	8.14 ± 0.15 c	0.72 ± 0.03	30.32 ± 1.03	4.15 ± 0.30	0.33 ± 0.02 b
PH3	10.17 ± 0.44	1.68 ± 0.04 a	8.29 ± 0.33 c	0.70 ± 0.07	29.30 ± 0.82	3.97 ± 0.09	0.31 ± 0.01 b
NaCl × B							
NaCl 1 × Control	9.63 ± 0.12	1.33 ± 0.04 def	8.23 ± 0.06 de	0.62 ± 0.01 cd	28.83 ± 0.72	4.65 ± 0.26	0.54 ± 0.04 a
NaCl 1 × PH1	9.32 ± 0.30	1.25 ± 0.04 f	8.05 ± 0.19 de	0.66 ± 0.02 c	28.92 ± 0.92	4.44 ± 0.18	0.41 ± 0.03 b
NaCl 1 × PH2	9.59 ± 0.17	1.44 ± 0.03 cde	7.80 ± 0.06 de	0.66 ± 0.02 c	31.13 ± 1.73	4.61 ± 0.53	0.36 ± 0.02 bc
NaCl 1 × PH3	9.08 ± 0.16	1.60 ± 0.04 bc	7.45 ± 0.09 e	0.54 ± 0.03 d	27.86 ± 1.26	4.07 ± 0.17	0.31 ± 0.02 bc
NaCl 50 × Control	10.81 ± 0.24	1.31 ± 0.04 ef	9.33 ± 0.11 b	0.72 ± 0.01 bc	31.12 ± 1.24	3.68 ± 0.16	0.31 ± 0.04 bc
NaCl 50 × PH1	10.89 ± 0.31	1.51 ± 0.05 cd	10.60 ± 0.29 a	0.77 ± 0.03 b	26.84 ± 1.54	3.51 ± 0.18	0.27 ± 0.01 c
NaCl 50 × PH2	10.89 ± 0.03	1.89 ± 0.04 a	8.48 ± 0.15 cd	0.78 ± 0.02 ab	29.51 ± 1.25	3.68 ± 0.06	0.30 ± 0.01 bc
NaCl 50 × PH3	11.26 ± 0.25	1.75 ± 0.06 ab	9.13 ± 0.21 bc	0.87 ± 0.02 a	30.74 ± 0.38	3.86 ± 0.06	0.31 ± 0.02 bc
Significance							
NaCl	***	***	***	***	ns	***	***
B	ns	***	***	ns	ns	ns	***
NaCl × B	ns	***	***	***	ns	ns	**

ns, not significant; ** and *** represent significance at $p \leq 0.01$ and 0.001 , respectively. Different letters within each column indicate significant differences according to Tukey's post hoc test ($p = 0.05$). All data are expressed as mean ± standard error. ($n = 4$). PH1, PH2, and PH3 are plant hydrolysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively.

The effects of salinity and biostimulant application on antioxidant activity (FRAP and DPPH) are shown in Table 4. FRAP and DPPH were significantly influenced by salinity and biostimulant application without any significant NaCl × B interaction. FRAP and DPPH were significantly increased by salinity level and PH applications, especially the PH3 treatment. Similarly, the proline content was significantly affected by salinity and biostimulant application without any significant NaCl × B interaction. The proline content was increased by the salinity level and by PH2 and PH3 treatments in comparison with the untreated control (Table 4). Total phenols and flavonoids were significantly affected by NaCl × B interaction. Under non-saline conditions, PH treatments, especially the PH3 treatment, significantly enhanced the total phenols and flavonoids in comparison to the untreated control. With 50 mM NaCl NS, the total phenols were not significantly different among biostimulant treatments, while flavonoids were reduced by PH2 compared to the untreated control.

Table 4. Effect of salinity and biostimulant application on ferric reducing antioxidant power assay (FRAP), 1,1-Diphenyl-2-picrylhydrazyl assay (DPPH), total phenols, flavonoids, and proline in tomato fruits.

Source of Variance	FRAP (mM Fe ²⁺ 100 g ⁻¹ FW)	DPPH (mM Trolox 100 g ⁻¹ FW)	Total Phenols (mM GAE 100 g ⁻¹ FW)	Flavonoids (mM QE 100 g ⁻¹ FW)	Proline (mM Pro 100 g ⁻¹ FW)
NaCl level (mM)					
1	1.09 ± 0.06 b	1.70 ± 0.06 b	416.01 ± 15.53 a	40.83 ± 1.68	672.87 ± 52.76 b
50	2.27 ± 0.08 a	2.42 ± 0.05 a	366.00 ± 7.48 b	41.79 ± 1.25	2127.33 ± 43.40 a
Biostimulant (B)					
Control	1.23 ± 0.14 c	1.76 ± 0.11 c	327.48 ± 9.11 c	38.31 ± 2.41 b	1257.93 ± 64.04 b
PH1	1.67 ± 0.17 b	2.00 ± 0.12 b	391.60 ± 13.83 b	39.30 ± 1.33 b	1315.24 ± 61.37 b
PH2	1.76 ± 0.21 b	2.17 ± 0.11 ab	390.89 ± 11.71 b	37.91 ± 1.01 b	1491.76 ± 72.62 a
PH3	2.06 ± 0.15 a	2.34 ± 0.09 a	460.35 ± 19.75 a	50.17 ± 1.60 a	1535.47 ± 74.33 a
NaCl × B					
NaCl 1 × Control	0.75 ± 0.07	1.41 ± 0.08	299.40 ± 9.77 e	30.29 ± 1.61 e	494.89 ± 96.83
NaCl 1 × PH1	1.11 ± 0.09	1.58 ± 0.08	417.96 ± 14.47 bc	40.13 ± 1.56 bcd	624.04 ± 83.85
NaCl 1 × PH2	1.04 ± 0.08	1.84 ± 0.09	429.97 ± 6.52 b	39.58 ± 0.69 cd	790.00 ± 118.59
NaCl 1 × PH3	1.46 ± 0.06	1.99 ± 0.06	500.06 ± 23.62 a	51.92 ± 2.89 a	782.55 ± 118.59
NaCl 50 × Control	1.78 ± 0.06	2.10 ± 0.10	348.54 ± 8.41 de	45.19 ± 1.65 abc	2020.96 ± 83.85
NaCl 50 × PH1	2.17 ± 0.16	2.42 ± 0.09	368.53 ± 19.94 bcd	37.98 ± 2.53 cde	2006.44 ± 89.64
NaCl 50 × PH2	2.48 ± 0.08	2.50 ± 0.09	356.70 ± 11.14 cde	36.45 ± 1.68 de	2193.51 ± 83.85
NaCl 50 × PH3	2.59 ± 0.08	2.65 ± 0.05	404.76 ± 8.83 bcd	48.12 ± 0.23 ab	2288.39 ± 89.64
Significance					
NaCl	***	***	***	ns	***
B	***	***	***	***	*
NaCl × B	ns	ns	***	***	ns

ns, not significant; *, and *** represent significance at $p \leq 0.05$, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's post hoc test ($p = 0.05$). All data are expressed as mean ± standard error. (n = 4). PH1, PH2, and PH3 are plant hydrolysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively.

3.5. Effect of Salinity and PH on Modulation of Sugar Metabolism-Related Gene Expression

Figure 2 shows the relative expression levels of six genes (*SlAgpL3*, *SlAgpS1*, *SlSus3*, *SlSps1*, *SlVIN2*, and *SlCIN3*) under high and low-saline conditions across four treatments: control, PH1, PH2, and PH3. The expression levels of these genes were differentially regulated by the interaction of NaCl × B. The gene *SlAgpL3* was significantly highly expressed under PH1 and PH3 treatments with respect to the untreated plants and the PH2 treatment, especially under low-saline conditions (Figure 2A). The results of gene expression analysis showed that under high-saline conditions, *SlAgpS1* is highly significantly expressed by PH1, followed by PH3, PH2, and untreated plants, with no significant differences between PH2 and PH3. In the case of 1 mM NaCl NS, *SlAgpS1* did not show any significant variation in expression levels (Figure 2B). The gene *SlSus3* showed the highest expression level in untreated plants, followed by PH1, PH2, and PH3, with no significant difference between the latter in the case of high-salinity conditions (50 mM NaCl NS). On the other hand, treatments with PH2 and PH3 induced the strongest upregulation of *SlSus3*, followed by PH1 and untreated plants, in the case of low-saline conditions (Figure 2C). *SlSps1* was significantly upregulated in untreated plants exposed to high-saline conditions, followed by PH1 and PH3 treatments, with no significant difference between the latter (Figure 2D). On the other hand, PH2-treated plants showed the lowest expression level of *SlSps1*. Similarly, in low-salt conditions, *SlSps1* showed the lowest expression level

in PH2-treated plants with respect to the untreated plants, PH1, and PH3 (Figure 2D). The *SIVIN2* gene was significantly upregulated by the PH2 treatment in high-salinity conditions, followed by untreated plants and both PH1 and PH3, with no significant difference between the latter. In the case of low-salt conditions, *SIVIN2* exhibited a significantly higher expression level in PH1-treated plants, followed by PH2 and PH3, with no significant difference between the latter and untreated plants (Figure 2E). As far as *SICIN3* is concerned, in high-salinity conditions, treatment with biostimulants induced a progressive downregulation in its expression, following the trend PH1 > PH2 > PH3. Differently, in low-saline conditions, treatment with biostimulants induced an increase in the expression of *SICIN3*, albeit the differences detected are not statistically significant (Figure 2F).

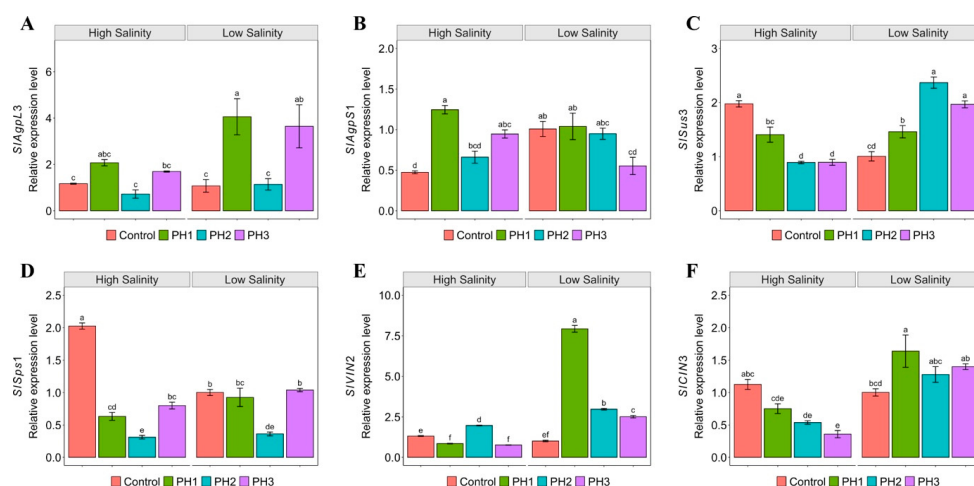


Figure 2. Gene expression analysis carried out in tomato fruits grown in either high or low-saline conditions and subjected to different biostimulant treatments. The expression levels of *SIAppL3* (A), *SIAppS1* (B), *SIsus3* (C), *SIsps1* (D), *SIVIN2* (E), and *SICIN3* (F) were assessed by qRT-PCR in tomato fruits produced by plants subjected to different levels of salt and to different biostimulant treatments. Data are normalized to one internal control, the translation elongation factor isoform 1-alpha (EF1-alpha), whereas the relative expression levels were calculated using an untreated control (i.e., control in low-saline conditions), which were set with a value of 1. Data are shown as the means $\pm$ standard errors. Different letters indicate significantly different values between the different samples using a one-way ANOVA with Tukey's post hoc tests ($p = 0.05$). PH1, PH2, and PH3 are plant hydrolysates from Malvaceae, Solanaceae, and Leguminosae family matrices, respectively. Low salinity: 1 mM NaCl; high salinity: 50 mM NaCl.

4. Discussion

4.1. Influence of Salinity and PH on Biomass Partitioning: Interpretation

The results showed that salt stress reduced plant biomass. In general terms, it is demonstrated that elevated salinity levels in plant tissues hinder the growth of roots and leaves in many crops [63]. Regardless of the NaCl concentration, biostimulant treatments augmented the leaves' and roots' dry weights with respect to the untreated control plants, and these results are in line with previous studies that showed the ability of PHs application to increase leaf dry weight [64] and root dry weight [65,66]. PHs act on plants differently according to their origin, composition, dose and method of application, and level of abiotic stress [41,43,67,68]. This can explain the differential responses of the plants' dry weight observed in this study to the PHs' application, notably the PH2 and PH3-derived protein hydrolysates, which had a more pronounced effect on increasing the root dry weight in the case of non-saline conditions. It has been reported that PH3 contains a root hair-stimulating peptide that can promote rooting in tomato cuttings [69].

4.2. Implications of Salinity and PH on Fruit Production

Plant yield was also negatively affected by induced salinity stress, whereas the total and marketable yields and marketable fruits were enhanced by the application of PH1. This result can be attributed to the PH components' effect on photosynthetic activity, which increased the photosynthates translocation from source to sink organs [70]. Although PHs have been shown to induce various physiological and metabolic processes in response to salinity [71,72], the results of this work show that applying PHs did not have a significant effect in terms of mitigating salinity stress on crop yield. This result may be due to the high magnitude of salinity stress applied in this study (50 mM NaCl), which may have restrained the effect of PHs.

4.3. Implications of Salinity and PH on Leaf Mineral Nutrition

The induced salinity stress in this study reduced the contents of total N and several mineral elements in tomato leaves. This reduction may be attributed to the competitive inhibition of nutrient uptake by excessive Na^+ and Cl^- ions, which compete with the uptake of useful nutrients such as K^+ , Ca^{2+} , Mg^{2+} , and NO_3^- [73]. In addition, salinity stress suppresses transpiration and root uptake of water and decreases nutrient transport to the aerial part of the plant [74,75]. On the other hand, the application of PH1, PH2, and especially PH3 decreased the leaf Cl concentration under saline conditions compared to the untreated control plants. Zhang et al. [39] found that using a vegetal-derived PH and a seaweed extract with a high amino acid content reduced the Cl concentration by about 4 folds in tomato leaves under high-saline conditions (150 mM NaCl). Different research has shown that PHs maintain ion homeostasis under stress conditions [76], including the regulation of Cl ions, and accordingly, this might influence the activity of specific ion channels and transporters in plant cells, which could regulate the uptake and transport of chlorine ions.

4.4. Understanding the Effects of Salinity and PH on Fruit Quality

In this study, tomato fruit quality was evaluated based on several key parameters, including fruit dry matter, fruit firmness, total soluble solids (TSS), titratable acidity, and organic acids [77], which were significantly influenced by the applied treatments, particularly when under salinity stress. Together, these factors determine the marketability of tomato, consumer acceptance, and suitability for various culinary uses [78]. Salinity stress on tomato crops can improve the quality of the fruit by increasing the TSS, fruit firmness, titratable acidity, and organic acid contents [25,79]. In this study, similar results were obtained after the application of saline stress (50 mM) to tomato plants. PHs can improve tomato fruit quality under salinity stress through a variety of processes like the increase in sugar concentration and antioxidant contents [69]. Results of the application of PHs in this study revealed an increase in TSS, titratable acidity, and fruit firmness when under saline stress conditions. PH2 and PH3 were responsible for increasing tomato fruit firmness, PH1 increased the TSS content, and PH3 augmented the titratable acidity under high-saline conditions. These results can be related to the different compositions of PHs used in this study, which led to different responses in tomato plants [80,81]. The organic acid (citric, malic, and oxalic acids) content, together with soluble sugars (fructose and glucose), contribute to the tomato fruit flavor [9]. PHs influenced the fruit content of oxalic acid, reducing it with respect to the untreated plants in the case of non-saline conditions, while PH1 was the only treatment reducing oxalic acid in saline conditions. According to the literature, oxalic acid can lower the bioavailability of calcium (Ca^{2+}), which makes it an anti-nutrient. An increased risk of kidney stones developing in the human body may result from this combination. Consequently, it would be essential to take into

consideration the reduction in oxalic acid levels brought about by PHs as a positive aspect for human health [82].

In our study, the application of PHs significantly affected the antioxidant profile of tomato fruits (Table 4). These findings are consistent with previous studies, which have reported that biostimulants, including PHs, enhanced the accumulation of antioxidant compounds and activated enzymatic and non-enzymatic defense pathways under stress conditions [2,21,22]. The increase in antioxidant activity observed with PH applications, especially under salinity stress, highlights their potential role in mitigating oxidative damage and maintaining fruit quality under adverse conditions [41,80].

4.5. Salinity and PH Influence on Sugar Metabolism-Related Gene Expression: Insights

To further deepen our understanding of the effects of biostimulants observed in tomato plants exposed to different NaCl levels, the modulation of genes involved in the regulation of sugars metabolism was investigated in tomato fruits. The accumulation of TSS in fruits has been shown to be correlated with the increase in starch content [30]; therefore, the expression of ADP glucose pyrophosphorilase (AGPase), sucrose synthase (SuSy), Sucrose Phosphate Synthase (SPS), and invertase (INV) genes was investigated. The AGPase enzyme is formed by a small subunit (*SlAgpS1*) and a large subunit, which, in the tomato genome, is encoded in three isoforms (*SlAgpL1*, *SlAgpL2*, and *SlAgpL3*) [83]. *SlAgpL1* and *SlAgpL2* did not show detectable expression levels in the tomato samples, whereas *SlAgpL3* displayed significant upregulation following the biostimulant treatment independently from the growing conditions applied (Figure 2A). Although limited to tomato plants grown under saline stress, the same transcriptional modulation was shown by the *SlAgpS1* gene (Figure 2B). A previous study demonstrated that *SlAgpL3* showed poor responsiveness to salinity stress [30], suggesting that the up-regulation of this gene observed in this study may be ascribable to the mode of action of PH1. Indeed, the induction of *SlAgpL3* and *SlAgpS1* genes might indicate a possible metabolic shift toward the starch biosynthesis triggered by PH1 aiming at buffering the osmotic stress induced by salinity. In tomato genome, SuSy genes form a small gene family composed of four isoforms, namely *SlSus1*, *SlSus2*, *SlSus3*, and *SlSus4* [84–87], which are involved in the reversible catabolism of starch. The *SlSus3* expression analysis demonstrated that, depending on the salinity level in the growth substrate, the effect of biostimulant treatments could be different, showing downregulation under high-saline conditions and upregulation under control conditions (Figure 2C). On the other hand, the expression of *SlSus4* was not detectable in our experimental conditions. Sucrose Phosphate Synthase (SPS) is a key enzyme involved in sucrose synthesis, starting from fructose and UDP-glucose, already shown to be differentially modulated in response to abiotic stresses, for instance, drought and heat [13,88,89]. In high-saline conditions, the expression of *SlSps1* was decreased by the treatment with biostimulants, whilst in control conditions, the application of PHs did not show significant effects on transcriptional modulation (Figure 2D). The only exception was presented by PH2, which caused the downregulation of *SlSps1* in both high and low-salt conditions (Figure 2D). The invertase enzymes (INV), based on their characteristics and their subcellular localization, are grouped in Vacuolar (VIN), Cell Wall (CWIN), and Cytosolic (CIN) invertase, and they catalyze the irreversible catabolism of sucrose [74]. Whilst the expression of *CWIN* was not detectable in our experimental conditions, both *SIVIN2* and *SICIN3* displayed upregulation following the biostimulant treatments in low-salt conditions (Figure 2E,F), possibly suggesting an increase in sugar metabolism that might also be in good agreement with the detected content of organic acids. On the other hand, in high-salt conditions, both the invertases analyzed showed a decrease in the expression levels upon biostimulant treatments (Figure 2E,F), except in the case of the PH2 treatment that induced the upregulation of *SIVIN2*. The downregulation of these genes

induced by PHs may suggest a general suppression of sugar transport and catabolism under salinity stress, which is already known to directly inhibit phloem sucrose loading and translocation [90]. Therefore, PHs might promote sugar storage rather than metabolism as a stress tolerance strategy. Interestingly, similar effects on the primary metabolism of sugars have also been observed in lettuce plants exposed to high salinity stress and treated with Graminaceae-derived protein hydrolysates [91].

5. Conclusions

Soil salinity is a major challenge threatening plant productivity, and sustainable solutions are urgently needed. Protein hydrolysates (PHs), as plant biostimulants, can effectively support crop performance by improving plant growth, fruit quality, and stress resilience. In this study, under non-saline conditions, PH1 and PH3 significantly increased tomato marketable fruit yield (PH3: total yield +13.8%, marketable yield +22.6%; PH1: marketable yield +14.6%), demonstrating their potential to enhance productivity. Under saline conditions, while PHs did not significantly increase yield, they reduced toxic ion accumulation (notably Cl^-), improved fruit quality through higher total soluble solids and titratable acidity, and modulated sugar metabolism toward starch synthesis, contributing to plant stress tolerance. These results highlight that the effectiveness of PHs depends on their botanical origin and composition, which can differentially influence growth, yield, and fruit quality. Therefore, PHs can be considered a sustainable tool to enhance crop performances under optimal conditions and mitigate the physiological impacts of salinity stress, supporting resilient agricultural production.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae11091108/s1>, Table S1: Primer sequences used for RT-qPCR analysis; Table S2: Effect of salinity and biostimulant application on dry weights of leaves and stems in tomato plants.

Author Contributions: A.E.C., S.M., Y.P. and M.C. wrote the first draft of the manuscript. A.V.C. and M.C. performed the phenotyping measurements and quality analysis of fruits. S.M. and Y.P. performed the gene expression analysis. C.E.-N. and Y.R. performed the mineral analysis. All authors were involved in data analysis, data interpretation, and revising the manuscript. Y.P., M.C. and G.C. coordinated the whole project, provided the intellectual input, set up the experiment, and corrected the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The dataset generated for this study is available upon request to the corresponding authors.

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