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Unraveling the Modulation of Controlled Salinity Stress on Morphometric Traits, Mineral Profile, and Bioactive Metabolome Equilibrium in Hydroponic Basil

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Abstract: Salinity is a major concern in several ecosystems and has a significant impact on global agriculture. To increase the sustainability of horticultural food systems, better management and usage of saline water and soils need to be supported by knowledge of the crop-specific responses to tolerable levels of salinity. The aim of this work was to study the effects of mild salinity on morphological growth and development, leaf color, mineral composition, antioxidant activities, and phenolic profile of sweet basil (*Ocimum basilicum* L.). Plants grew in hydroponics and were exposed to three nutrient solutions (NSs) differing in the NaCl concentration (either 0, 20, or 40 mM). Inhibitory effects on leaf area, fresh yield, and shoot biomass were evident starting from the lowest NaCl concentration, and they became more severe and wide-ranging at 40 mM, also affecting height and root-to-shoot ratio. Salinity increased the nutritional quality in terms of antioxidant activity and polyphenols in leaves, with a reduction in macroelements at 40 mM NaCl. Moreover, the two mild NaCl concentrations specifically modified the concentration of various phenolic acids in leaves. Overall, the use of a slightly saline (20 mM) NS could be tolerated by basil in hydroponics, strongly ameliorating the nutritional profile in the face of relative yield loss. Considering the significantly higher accumulation of bioactive compounds, our work implies that the use of low-salinity water can sustainably increase the nutritional value and the health-promoting features of basil leaves.

Keywords: *Ocimum basilicum*; salt; NaCl; yield; quality; nitrate; minerals; antioxidants; polyphenols



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1. Introduction

Salinity is considered a global limiting factor for agriculture, with around one-fifth of irrigated land suffering from secondary salinization [1]. In plants, the salt stress response encompasses multidimensional changes at molecular, cellular, and physiological levels, which typically result in a yield decline and, in the most severe cases, in stunted growth or death according to the type, intensity, and duration of the stress [2,3]. Salinity is a concern for irrigated and nonirrigated lands and affects freshwater quality, soil health, biodiversity, and ultimately society [4], with climate change predicted to exacerbate the problems of salinity in agriculture [5]. It is, therefore, necessary to investigate and possibly adapt horticultural production systems to more saline conditions.

It is long established that salinity and other suboptimal environmental conditions (e.g., temperature, light intensity, nutrients, and water) have a strong impact on the accumulation of secondary compounds in plants [6–8]. The array of diverse molecules probably represents the most important functional feature of medicinal and aromatic plant (MAP) species [9]. MAPs are becoming increasingly valuable in agriculture, due to their growing use in the medical (including phytotherapy and aromatherapy), pharmaceutical, cosmetic, food, and nutraceutical sectors [9,10]. For these reasons, understanding the response to salt in aromatic plants is important not only to ensure economic returns and sustainability but also to optimize crop quality.

Basil (*Ocimum basilicum* L.) is an herbaceous species mainly cultivated for the aromatic leaves, consumed fresh or processed, and employed (along with the other areal parts) for the extraction of the essential oil [11]. Currently, *O. basilicum* is the most commercially important species of its genus and among the most popular and extensively cultivated herbs around the world [11]. Moreover, basil has the potential to become a model species to study flavor compounds [12]. Although cultivated in several countries, basil is susceptible to chilling [13] and performs better in sunny, warm areas and in light soils [11]. The rising interest in MAPs has created novel commercial opportunities for basil growers [14,15]. In the last decades, the professional basil cultivation has progressively shifted towards soilless systems (mainly hydroponics) in controlled and closed environments, for the intrinsic suitability of this species (in terms of harvest, cycle, and size) and to ensure a more constant quality of the produce by limiting environmental variability [16]. Hydroponic systems also allow easier management of the complementary irrigation with saline water compared to the cultivation in soil [17]. In Italy, for instance, more than half of the basil production comes from hydroponics, and it is mainly employed for the industrial preparation of the pesto sauce, thus requiring standardized productions [18]. Salinity due to nonessential ions such as Na is a primary problem for the recirculation of the nutrient solution in closed-loop systems, deterring the use of irrigation water of inadequate quality especially around the Mediterranean basin [19].

The effects of salinity on basil are various. This species, also at the seedling stage [20], is considered moderately resistant to salt [21], although significant intraspecific variations are reported [22,23]. High levels of NaCl (e.g., 100 mM) can induce significant yield loss [24,25]. Moreover, in basil, salinity can influence secondary metabolites, such as polyphenolics [26,27], volatile organic compounds [28], and carotenoids [24], as well as antioxidant activities [23]. This work aimed to have a wide-ranging view of the response of basil to moderate salt stress, under the premise that the adaptation to mild stress in MAPs has critical and possibly contrasting effects on the quantitative and qualitative features of the plant [29]. Considering that the regular consumption of polyphenols is highly appreciated because of their potential health benefits, also in relation to the prevention of some chronic diseases, we investigated morphological traits, leaf elemental composition, and antioxidant activities and polyphenols (by HPLC) in two saline concentrations (20 and 40 mM NaCl) that are well below the toxicity level for basil. Our specific interest was to understand the interplay between biomass reduction and increase in the antioxidant activity and polyphenolic accumulation, to sustainably optimize yield and nutritional quality in hydroponics. This knowledge is relevant to provide indications for better exploitation of basil according to specific needs and markets and to develop more environmentally sound water resource management strategies.

2. Materials and Methods

2.1. Plant Material, Growing Conditions, and Experimental Design

Plants grew in a nonheated glasshouse at “Azienda Agraria e Zootecnica Torre Lama” (Bellizzi, SA, Italy), Department of Agricultural Sciences, University of Naples Federico II, from October to December 2014. Seeds of the basil (*Ocimum basilicum* L.) variety ‘Gecom’ (S.A.I.S. S.p.a., Cesena, FC, Italy), a Genovese type, were sown in vermiculite. On 27 October, at the three true leaves stage, plants were transplanted to plastic pots filled with approximately 1.3 L of a Brill 3 substrate (Brill Substrate, Georgsdorf, Germany) with a density of 23 plants per square meter. A fresh nutrient solution (NS) was employed at each irrigation treatment with a drip system at a 2 L/h flow rate. We tested three levels of NaCl concentration in a randomized block design. Each experimental unit was made of 15 plants and was replicated three times, for a total of 135 plants. The base NS (0 mM NaCl) was prepared with deionized water and had the following concentrations of mineral nutrients: 13.0 mM N-NO₃⁻, 1.0 mM N-NH₄⁺, 1.75 mM S, 1.5 mM P, 5.0 mM K, 4.5 mM Ca, 2.0 mM Mg, 20 μM Fe, 9.0 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20.0 μM B, and 0.3 μM Mo. The two saline treatments were applied using the same base NS supplemented with either

20 mM NaCl or 40 mM NaCl. The pH of the NSs was 6.0. The electrical conductivity (EC) was 2.1 dS/m for the base NS, 3.9 dS/m for the 20 mM NaCl, and 5.8 dS/m for the 40 mM NaCl. Saline treatment started eight days after transplant (DAT) and the growing cycle ended 44 DAT, at the preflowering stage (i.e., commercial maturity).

2.2. Biometric Measurements and Antioxidant Activity

Leaves, stems, and roots from ten plants per block (i.e., replicate) were harvested 39 DAT (4 December) and weighed (fresh weight (fw)) with an analytical balance (Denver Instruments, Denver, CO, USA). Samples were bagged and then dried at 70 °C until constant weight (dry weight (dw)). The total leaf area was measured from ten plants per block using a portable leaf area meter (Li-Cor3000, Li-Cor, Lincoln, NE, USA). The SPAD index was measured on 20 fully expanded leaves per experimental unit with a portable chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan), while color was measured with a CR-300 Chroma Meter (Minolta, Tokyo, Japan) as previously reported [30]. The pairwise difference in color (ΔE^*_{ab}) between treatments was calculated as described [31]. Nitrate content in leaves was evaluated with previously described procedures [30]. The mineral profile (P, K, Ca, Mg, and Na) of the dried leaves was analyzed with an inductively coupled plasma (ICP) emission spectrophotometer (Iris, Thermo Optek, Milan, Italy) as reported [32]. Fresh sweet basil leaves from three plants per experimental unit were immediately frozen in liquid nitrogen after harvest and lyophilized in an Alpha 1–4 LSC plus (Osterode, Germany) freeze drier. The hydrophilic antioxidant activity (HAA) was assessed also as described previously [30].

2.3. Determination of Phenolic Compounds

For the biochemical analyses, we assayed leaves from three plants per experimental block. Phenolics were extracted essentially as described [33]. Briefly, 500 mg of dried leaves was added to 5 mL of methanol:water 30:70 (*v:v*). Samples were thoroughly mixed for 1 min, sonicated for 30 min, centrifuged at 14,800 rpm for 10 min, and then the supernatant was filtered on paper (Whatman, Little Chalfont, UK). The concentrations of caffeoyltartaric acid (CTA), caffeic acid (CA), *p*-coumaric acid (CUA), ferulic acid (FA), chicoric acid (CHA), quercetin rutinoside acid (QRT), and rosmarinic acid (RA) were assessed on the clear supernatant by LC/MS/MS analysis (injection volume: 20 μ L). Each sample was extracted and analyzed in triplicate. Chromatographic separation was performed in an HPLC apparatus equipped with two micropumps, Series 200 (Perkin-Elmer Italia, Milan, Italy) and with a Prodigy ODS3 100 Å column (250 mm \times 4.6 mm, 5 μ m) (Phenomenex, Torrance, CA, USA). The eluents were 0.2% formic acid in water (A) and acetonitrile:methanol 60:40 (*v:v*) (B). The gradient program was 20–30% B (6 min), 30–40% B (10 min), 40–50% B (8 min), 50–90% B (8 min), 90–90% B (3 min), and 90–20% B (3 min) at a constant flow of 0.8 mL/min. MS and MS/MS analyses were carried out on a triple quadrupole mass spectrometer (API 3000, Applied Biosystems Italia, Milan, Italy) equipped with a Turbo Ion Spray electrospray ion source working in the negative ion mode. Compounds were identified by information-dependent acquisition considering the molecular weight, the fragmentation pattern, the retention time, and UV absorption in comparison with those of commercial standards purchased from Sigma-Aldrich (Milan, Italy). The precursor ion and the MS/MS product ions of the phenolic compounds retrieved in the extracts are reported in Table S1. Reagents and solvents (all HPLC grade) were obtained from Merck KGaA (Darmstadt, Germany).

2.4. Statistical Analysis

Measurements of the morphological traits were carried out on ten basil plants per experimental block (made of 15 plants), each replicated three times. The effect of the categorical independent variable “Salt” (three levels: 0, 20 and 40 mM NaCl) on each of the continuous dependant variables was examined using the within-subjects one-way analysis of variance (ANOVA), considering a $p < 0.05$ threshold for statistical significance. Tukey’s

honestly significant difference (HSD) post hoc test was employed to determine which groups in the ANOVA differ from each other. These calculations were performed with the SPSS 26 software package (IBM, Akron, NY, USA). Data are reported as mean \pm standard error of the mean (s.e.). Principal component analysis (PCA) was carried out on all the measured variables by using XLSTAT (Addinsoft, Paris, France).

3. Results

3.1. Effect on the Plant Morphology

The impact of the mild salinity treatment on the morphological traits of basil was prominent, affecting most of the measured parameters (8 out of 11; Table S2). Moreover, statistical differences between the two NaCl concentrations (i.e., 20 and 40 mM) were not always present. The NaCl salt lowered the plant height at 40 mM (−12%), with a nonsignificant reduction at 20 mM (Figure 1A). Nonetheless, the number of leaves per plant was not affected (Figure 1B), suggesting that plants were stunted rather than less developed. This is also supported by the fact that the two saline treatments strongly restricted the leaf area, which, at the highest NaCl concentration employed, was around one-third of the control condition (Figure 1C). Therefore, the fresh yield was reduced in a similar proportion of the leaf area (around 31% at 40 mM NaCl) (Figure 1D). Interestingly, the effect of the salt on the total shoot biomass was also similar in quantitative terms, with the two saline conditions not significantly different (Figure 1E). The analysis of plant growth in terms of dry biomass indicated the overall inhibitory effect of the NaCl, with a reduction (−19%) from 0 to 20 mM NaCl and a limited (−6%) and nonsignificant difference between 20 and 40 mM NaCl (Figure 1F). This was due to an effect on leaves rather than on stem dry weight (Table S2). The edaphic stress impacted the ratio between roots and shoots, with plants progressively safeguarding the production of root biomass with increasing salt concentration (+23% and +46% for 20 and 40 mM NaCl, respectively) (Figure 1G), as indicated by the fact that root biomass was not reduced by NaCl (Table S2). The stress imposed by the salt also affected the percentage of dry mass of the leaves, which significantly increased (+8%) only at 40 mM NaCl (Figure 1H). Overall, the NaCl had an impact on plant height only at the highest saline concentration, and this reduction did not affect the number of leaves. In saline conditions, the yield decrease is due to smaller leaves with a reduced water content, a likely consequence of the inhibitory effect on shoot growth (in terms of dry biomass) and the resulting increase in the root-to-shoot biomass.

3.2. Effect on SPAD Index and Color Coordinates

The salt treatment did not influence the SPAD units, a leaf index related to the chlorophyll content, but did influence the lightness and the chromatic component a^* (Table S3). Post hoc analysis indicated that only the 40 mM NaCl treatment significantly differed from the other two experimental conditions. Specifically, at the higher NaCl concentration, leaf lightness moderately rose, and the CIELAB parameter a^* increased (e.g., it was less negative), indicating a diminished greenness of the leaves (Figure 2). The overall change in visual perception induced by the salt over the leaf color was evaluated considering the ΔE^*_{ab} . As expected, this value reached the maximum (2.51) comparing the 0 and 40 mM conditions; however, this discrepancy is only slightly above the limit (i.e., 2.0) of a color difference that is perceptible through close observation.

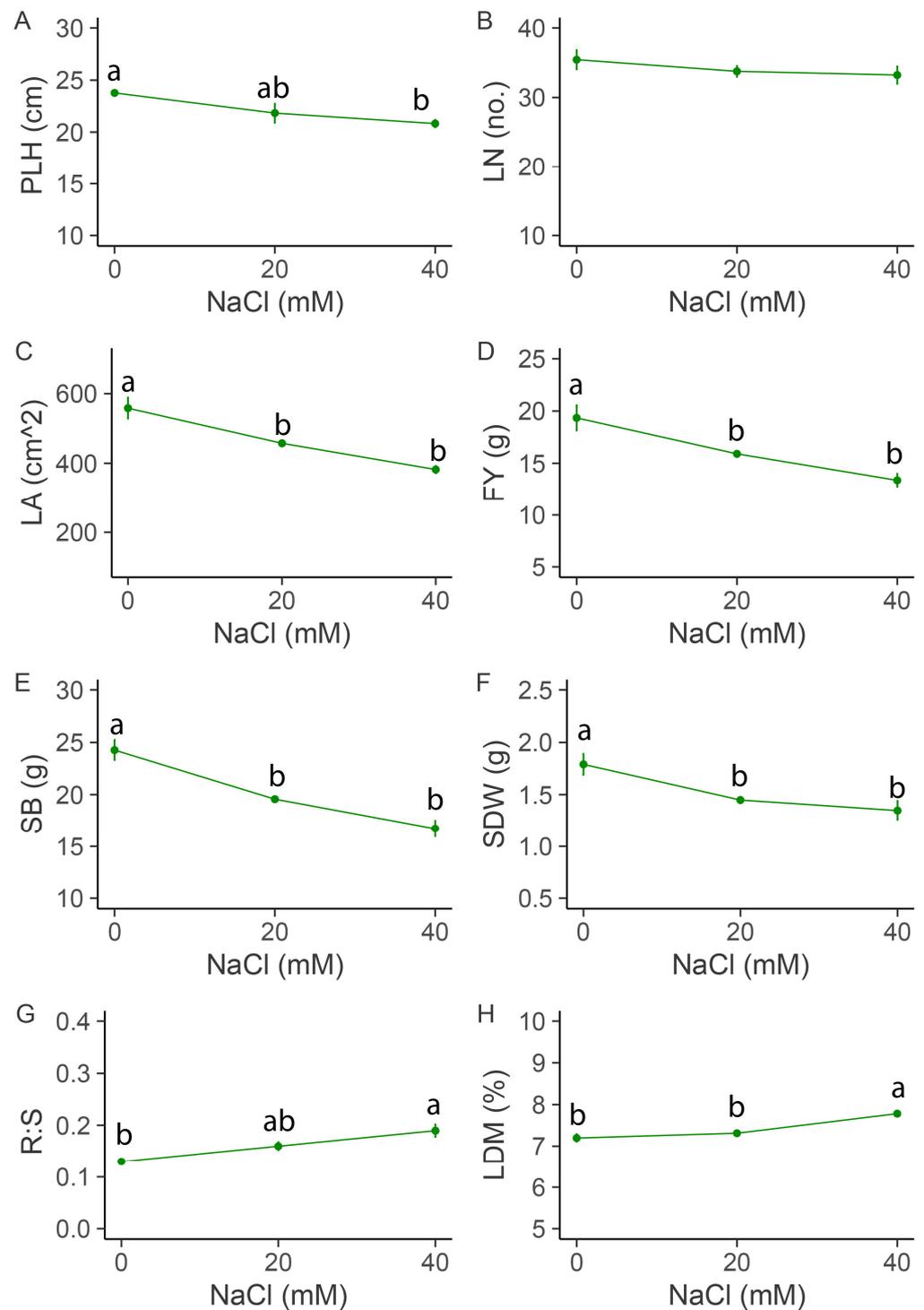


Figure 1. Effects of the NaCl in the nutrient solution on the morphometric traits of basil: (A) plant height (PLH); (B) leaf number (LN); (C) leaf area (LA); (D) fresh yield (FY); (E) shoot biomass (SB); (F) shoot dry weight (SDW); (G) root-to-shoot ratio (R:S); (H) leaf dry matter (LDM). Data are mean \pm standard error of the mean. Different letters indicate significantly different means according to the Tukey HSD test ($p < 0.05$).

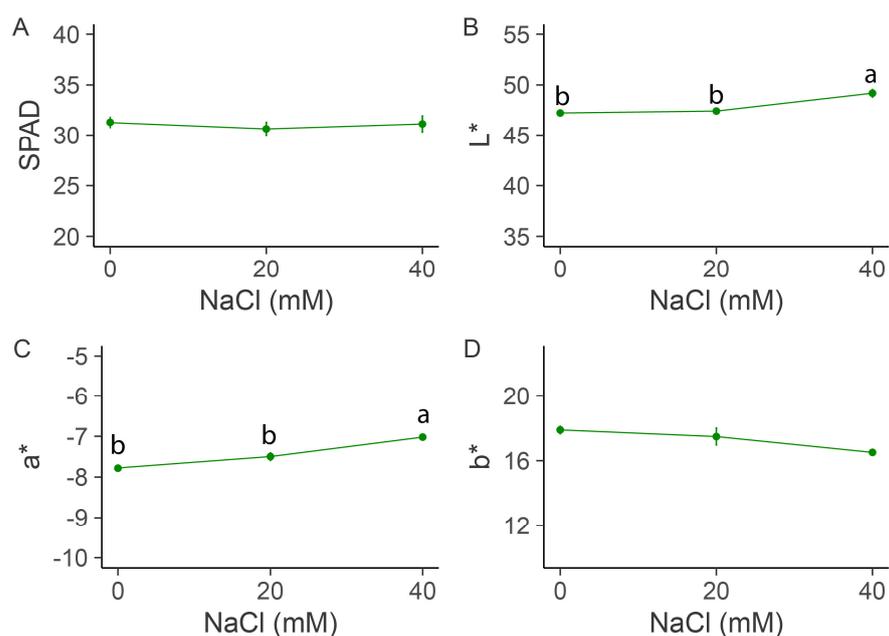


Figure 2. Effects of the NaCl in the nutrient solution on SPAD index and the L*, a*, and b* color coordinates according to the CIELAB standard: (A) SPAD; (B) L*; (C) a*; (D) b*. Data are mean \pm standard error of the mean. Different letters indicate significantly different means according to the Tukey HSD test ($p < 0.05$).

3.3. Effects on the Elemental Composition of the Leaves

We measured the nitrate content in basil leaves because this parameter influences the nutritional quality of this species and, more generally, of leafy greens. Both the 20 and 40 mM NaCl treatments significantly reduced the nitrates in leaves (Table S4), on average, by 23% compared to the control conditions (Figure 3A). NaCl also affected the amount of all the mineral elements analyzed (Table S4). Specifically, the strongest reduction was observed for Ca (−38% and −54% for the 20 and 40 mM NaCl, respectively) (Figure 3D). For P, K, and Mg, only the 40 mM dose caused a statistically significant reduction compared to the control condition, although a declining trend according to increasing NaCl concentration could be inferred (Figure 3B,C,E). K was the element that little changed relative to the control condition (−16% and −32% for the 20 and 40 mM NaCl, respectively). As expected, the amount of Na in leaves rose upon application of increasingly saline NS (Figure 3F). Na was the only analyzed element that accumulated in statistically different amounts moving from the 20 mM to the 40 mM NaCl treatment.

3.4. Effects on HAA and Total Polyphenols

Considering the importance of the antioxidants for leafy greens and, specifically, the phytotherapeutic use of basil, we measured the antioxidant activity of the aqueous extract, and total polyphenols because they are the most abundant phytochemicals in the human diet that can remove oxidizing agents in cells [34]. Salinity affected both parameters (Table S5). It was noteworthy that NaCl increased both traits, on average +77% for the HAA and +201% for the polyphenols (Figure 4A,B, respectively). Moreover, this increase in the nutritional quality of the basil leaf was not different when the two saline conditions were compared.

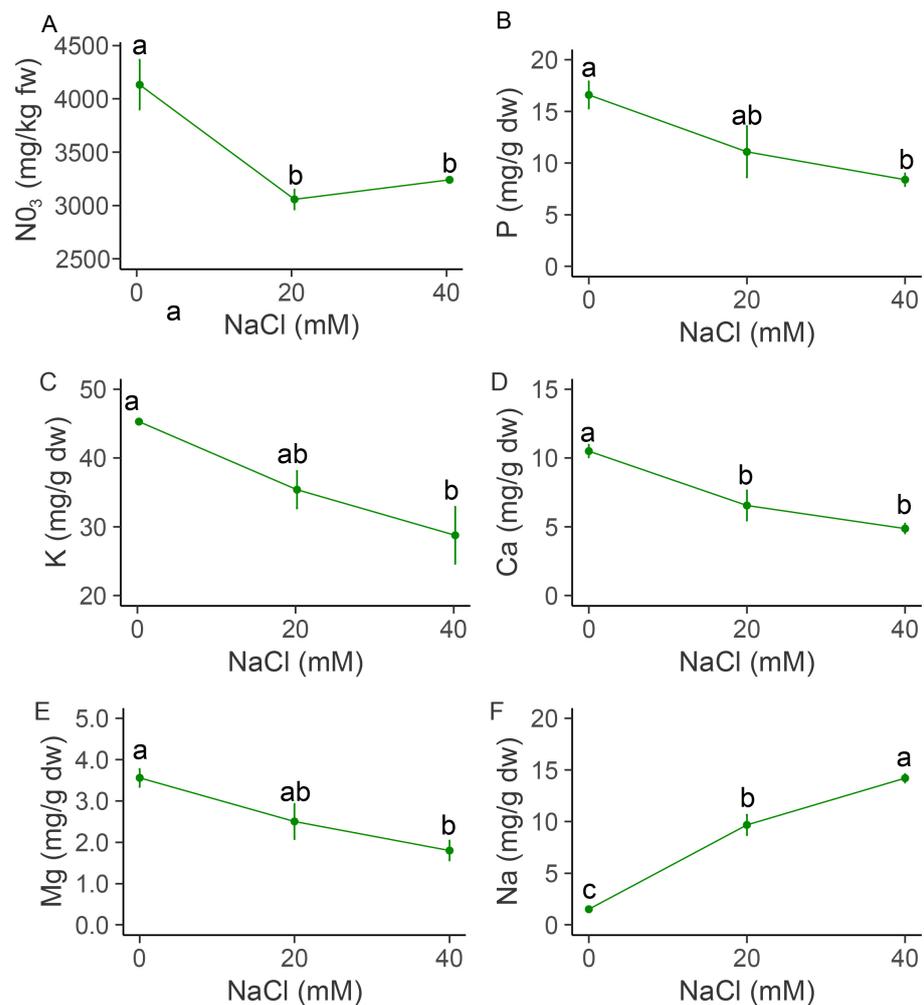


Figure 3. Effects of the NaCl in the nutrient solution on the elemental composition of the basil leaves: (A) nitrate; (B) P; (C) K; (D) Ca; (E) Mg; (F) Na. Data are mean \pm standard error of the mean. Different letters indicate significantly different means according to the Tukey HSD test ($p < 0.05$).

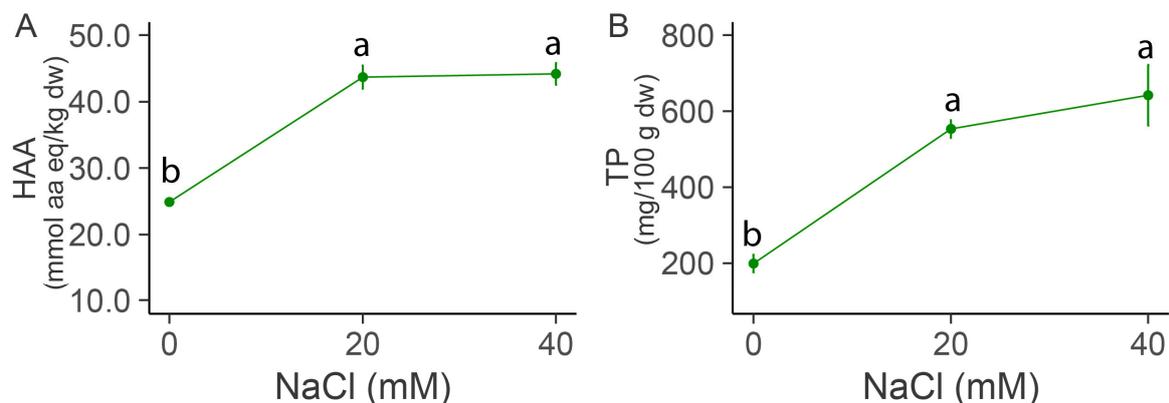


Figure 4. Effects of the NaCl in the nutrient solution on the hydrophilic antioxidant activity (HAA) and the total polyphenols (TP) in leaves: (A) HAA; (B) TP. Data are mean \pm standard error of the mean. Different letters indicate significantly different means according to the Tukey HSD test ($p < 0.05$).

3.5. Effect on the Polyphenolic Profile of Basil Leaves

Considering the known relation between plant stress-response and the accumulation of secondary compounds, we tested if, also under mild saline conditions, different NaCl concentrations specifically alter the accumulation of polyphenols. The saline treatment varied the accumulation of five out of the seven detected compounds, irrespective of the absolute amount of the various polyphenols (Table S5). It was interesting that different and, in some cases, divergent responses were recorded, despite the overall positive effect of the salt stress on their total amount. For instance, the quercetin rutinoside acid was the only polyphenol that constantly increased with higher saline conditions, reaching a 2× higher concentration at 40 mM NaCl (Figure 5F). At this experimental condition, the concentrations of *p*-coumaric acid, chicoric acid, and rosmarinic acid (the major caffeic acid ester) were also significantly higher than the control condition (Figure 5B,D,G). Among them, there was a statistical difference between the two saline conditions only for the *p*-coumaric acid. The highest relative increase was observed for chicoric acid (just above 10× higher at 40 mM than the control condition). Ferulic acid was the only compound that peaked at 20 mM (5× higher than in the control leaves), while its concentration at 40 mM did not differ from the no salt treatment (Figure 5E). At 20 mM, ferulic acid became the predominant phenolic acid in basil leaves. Overall, this analysis indicates that while any applied salt stress increased the sum of polyphenols, the two different mild NaCl concentrations employed specifically modified the phenolic acid composition in basil leaves.

To summarize the information content of the different analyses, we performed a principal component analysis (PCA) on all the measured variables. The first two principal components were held to graphically visualize the relation between the experimental conditions. The first two principal components were associated with eigenvalues higher than 1 and explained 100% of the cumulative variance, with the first and second principal components accounting for 81.7% and 18.3%, respectively. The multivariate analysis indicated that the different treatments are well separated, with the 0 mM NaCl condition more distant from the other two salt concentrations (Figure 6). The control treatment (0 mM NaCl) was distinguished for morphometric traits (fresh yield, shoot biomass, shoot dry weight, leaf area and plant height) and for cations (K, Ca and Mg) and nitrate content (Figure 6). Interestingly, sweet basil treated with 20 or 40 mM NaCl delivered leaves with high concentration of total polyphenols and hydrophilic antioxidant activity (Figure 6). Finally, sweet basil irrigated with 40 mM NaCl was positioned on the negative side of the first principal component in the lower left quadrant of the PCA score plot, characterized overall by higher target polyphenols (chicoric acid, *p*-coumaric acid, quercetin rutinoside acid) and color coordinates (L^* and a^*).

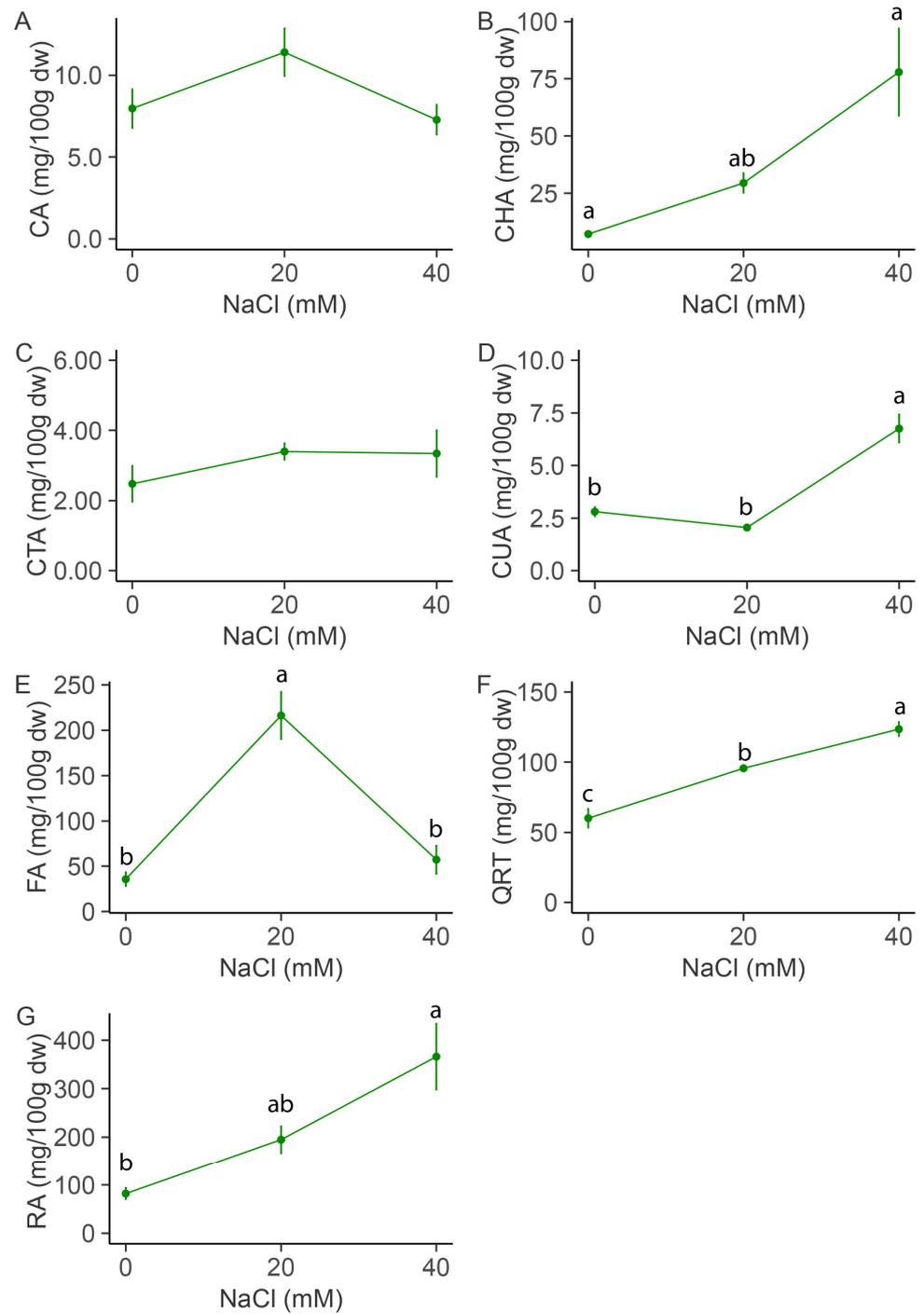


Figure 5. Effects of the NaCl in the nutrient solution on the polyphenolic compounds in leaves: (A) caffeic acid (CA); (B) chicoric acid (CHA); (C) caffeoyltartaric acid (CTA); (D) *p*-coumaric acid (CUA); (E) ferulic acid (FA); (F) quercetin rutinoside acid (QRT); (G) rosmarinic acid (RA). Data are mean \pm standard error of the mean. Different letters indicate significantly different means according to the Tukey HSD test ($p < 0.05$).

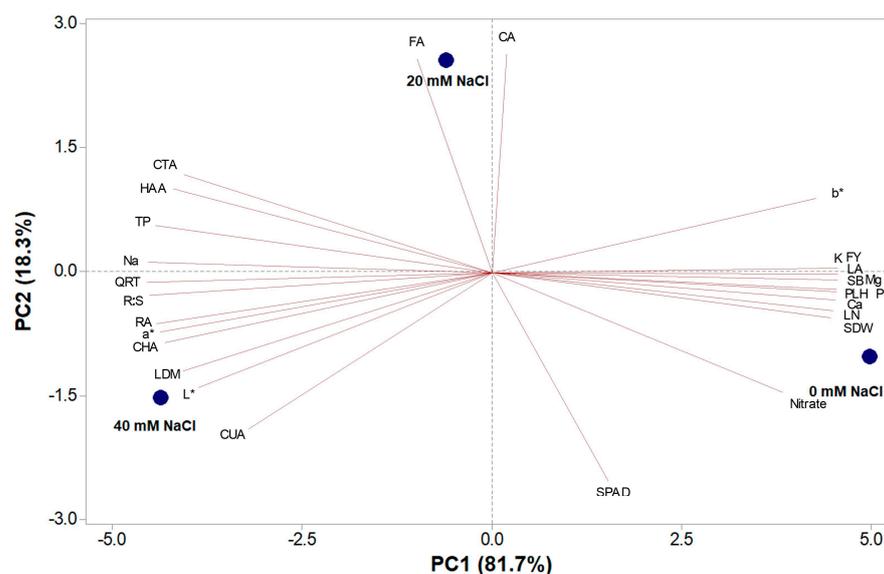


Figure 6. Principal component analysis (PCA) of the basil response to the three experimental conditions, 0, 20, and 40 mM NaCl. Plant height (PLH), leaf number (LN), leaf area (LA), fresh yield (FY), shoot biomass (SB), shoot dry weight (SDW), root-to-shoot ratio (R:S), leaf dry matter (LDM), hydrophilic antioxidant activity (HAA), total polyphenols (TP), caffeic acid (CA), chicoric acid (CHA), caffeoyltartaric acid (CTA), *p*-coumaric acid (CUA), ferulic acid (FA), quercetin rutinoside acid (QRT), rosmarinic acid (RA).

4. Discussion

In addition to its historical position in the national gastronomy, the professional cultivation of basil in Italy is economically sustained by the food industry, with large-scale production occurring mainly in hydroponics. Soilless systems offer the possibility to tailor the quality of the edible fraction by modulating preharvest factors, including the electric conductivity of the nutrient solution. This is relevant also because, in recent years, the growing concerns about the availability and quality of freshwater have expanded the need for a more comprehensive evaluation of the effects of a mild NaCl stress on the yield and quality of basil, as well as other MAP species [35,36].

The vegetative growth of the basil plants was negatively affected by the salinity, while development (measured by the number of leaves per plant) was not influenced. The saline treatment decreased fresh yield because of smaller leaves with reduced water content. Salinity is a known inhibitor of plant growth, as also reported in other aromatic Lamiaceae such as sage [37], spearmint [38], and marjoram [39]. Specifically, the reduction in the leaf area is one of the first and most recorded symptoms of the salinity in stress in plants [40], and our data added that in basil, this parameter is the most highly responsive in mild NaCl concentrations. In basil and other Lamiaceae, the effects of salt stress can be more severe and also include a significantly lower number of leaves, in addition to the reduction in the leaf area and leaf biomass [24,41]. Nonetheless, in basil the number of internodes was little reduced only from 75 mM NaCl [24]. On the other hand, in peppermint, the leaf fresh weight and area were reduced at EC values of 2.8 and 5.6 dS/m, respectively [41]. The inhibitory effect of salt on shoot dry biomass along with the limited effect on roots resulted in elevated root-to-shoot biomass. In a previous study, only a concentration of seawater higher than 40% limited root growth in basil, while an increased root-to-shoot ratio was observed starting from 5% [42]. Finally, salinity had a small effect on leaf color, confirming that the saline conditions were not close to a toxicity level [27].

The inhibitory effect of the saline treatments cannot be disconnected from the alteration of nutrient uptake and transport. Our data indicated that the elemental composition of the leaves was highly affected. All the analyzed mineral nutrients (present in a fixed amount in the NSs) were reduced at increasing NaCl concentrations, consistent with the higher uptake

and translocation in leaves of the NaCl. The antagonist ionic relation between Na^+ and Ca^{2+} or K^+ and between Cl^- and NO_3^- is a key factor affecting the mineral composition of the plants under NaCl stress [43–45]. This effect was evident also in mild concentrations, as also occurred in *Mentha pulegium*, where a significant inhibition on K^+ was evident starting from 25 mM NaCl [46]. It was noteworthy that nitrate reduction did not well correlate with Cl concentration in leaves, suggesting the mechanisms other than anion–anion antagonism may account for the observed phenomenon [44,47]. The significant reduction in nitrate at the lowest NaCl concentration also has dietary implications because a limited accumulation of this anion in edible products is desired to avoid potential health hazards.

The NaCl also increased the quality of basil in terms of hydrophilic antioxidant activity and polyphenols, which suggests that the observed effects are due also to oxidative stress, and not only to ion antagonisms and osmotic imbalance [3]. Salt stress disturbs cellular homeostasis in different ways, with consequent oxidative damage associated with an overproduction of reactive oxygen species (ROS) [3]. In our mild salinity concentrations, basil plants can protect themselves by increasing the antioxidant activity and polyphenols, a major biochemical class of antioxidants in plants. From an applied perspective, the data indicated that a positive effect on the quality attributes of the basil leaves can be obtained with the lowest NaCl concentration (20 mM), and no further benefits were obtained by moving to the most severe suboptimal conditions. In rosemary, both antioxidant activities and total phenols at 50 and 100 mM NaCl were higher than the control condition, and they both decreased at 150 mM NaCl [48].

While several reports indicated that plants generally accumulate phenolics during salt stress [49], relatively little is known about the effect on specific compounds. While our analysis confirmed that rosmarinic acid is a predominant phenolic compound in “Genovese” basil [50], the data also indicated that the large quantitative variation due to NaCl is accompanied by different accumulations of specific compounds. For instance, two hydroxycinnamic acid derivatives, caffeic acid and caffeoyltartaric acid, were not affected by the NaCl treatment, but *p*-coumaric acid, present in a similarly low amount, strongly increased at 40 mM. Moreover, a substantial dose-dependent variation was present for two major components, ferulic acid and rosmarinic acid. While the latter showed a constant increase with higher salt concentration, the former peaked at 20 mM. Overall, the impact on salinity on the polyphenol composition appears to be more complex and larger than on other measured parameters, although NaCl treatments never caused a decrease in specific polyphenols. The relative increase in the concentration of major polyphenols in leaves was above the biomass reduction, suggesting an increasing biosynthesis and accumulation of these compounds. The polyphenol accumulation in basil may also vary according to the cultivar or type [27,50], and further studies may verify the importance of the genetic factor in the response of individual compounds to NaCl.

5. Conclusions

The multidimensional evaluation of the response of the plants under mild salinity conditions is a necessary step to increase the environmental sustainability and quality of basil. This work confirmed the complex impact of salinity on hydroponic basil and provided some indications on the factors that should be considered when employing mildly saline irrigation water. Basil can be considered moderately salt-tolerant, with a little growth reduction occurring at 20 mM NaCl and a reduced yield mainly due to smaller leaves. This saline concentration offered the most interesting results based on the magnitude of the morphological alterations and the increase in the leaf quality in terms of nitrate content, antioxidant activity, and polyphenol profile. Considering the expected positive impact on the nutraceutical properties of basil and, potentially, on its market value, our study encourages more detailed analyses of the health-related properties of basil following the implementation of nonconventional (saline or sodic) water sources in cultivation.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7090273/s1>, Table S1: Table S1. LC/MS/MS characteristics of phenolic compounds identified and quantified in the basil extracts. Table S2: One-way ANOVA table of the morphometric traits. The unit of measurement of each variable is reported in brackets. Table S3: One-way ANOVA table of the SPAD index and color parameters. Table S4: One-way ANOVA table of the mineral content in basil leaves. The unit of measurement of each variable is reported in brackets. Table S5: One-way ANOVA table of the biochemical traits investigated. The unit of measurement of each variable is reported in brackets.

Author Contributions: Conceptualization, G.C. and Y.R.; methodology, P.V. and Y.R.; software, G.C. and Y.R.; formal analysis, G.C., P.V., P.C., and Y.R.; investigation, G.C. and Y.R.; resources, Y.R.; data curation G.C. and Y.R.; writing—original draft preparation, G.C.; writing—review and editing, G.C., P.V., P.C., and Y.R.; visualization, G.C., P.V., P.C., and Y.R.; supervision, G.C. and Y.R.; project administration Y.R. All authors have read and agreed to the published version of the manuscript.

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