

Article

Dynamic Responses of Cherry Tomatoes to Salinity Stress: Growth, SPAD, Sap Ion Regulation and Leachate Chemical Composition Across Developmental Stages

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Abstract

Tomato production is highly affected by salt stress conditions, especially under protected cultivation systems using low-quality irrigation water. This study assessed the effects of increasing electrical conductivity (EC) levels of the nutrient solution (2, 4, 6, and 8 dS m⁻¹) on growth, biomass distribution, RGB-based spectral indices, physiological traits, petiole sap composition, and leachate chemistry in tomato plants (*Solanum lycopersicum* cv. Tiny Tim) cultivated in containers within a bamboo nethouse under tropical conditions. The results obtained reported that increasing salinity significantly reduced fresh and dry biomass (61.72 and 61.55% respectively) across all plant organs, with fruits showing the highest sensitivity. Leaf area index (LAI), SPAD values, leaf nitrogen (N) concentration, leaf relative humidity (LRH), and water uptake also declined progressively under saline conditions. In contrast, root and leaf biomass allocation increased under the highest salinity level. Spectral analyses reported reductions in red and blue RGB components and normalized red and blue index values, while normalized green index increased under higher salinity levels. Petiole sap analysis showed a progressive accumulation of sodium (Na⁺) and chloride (Cl⁻) together with reductions in nitrate (NO₃⁻-N) and potassium (K⁺) concentrations, particularly during the reproductive stage. In contrast, °Brix, calcium (Ca²⁺), and phosphorus (P) concentrations increased under salinity, particularly during the reproductive stage. Leachate analyses confirmed salt accumulation in the substrate and reduced plant water uptake. Overall, petiole sap analysis and RGB-based spectral indices provide a rapid, non-destructive, and cost-effective tool for the early detection of salinity stress in tomato, enabling timely irrigation and fertigation adjustments to improve crop performance and resource-use efficiency under saline conditions.

Keywords: horticultural crops; leachate characterization; NaCl concentration; sap analysis; *Solanum lycopersicum*; water uptake



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1. Introduction

Tomato (*Solanum lycopersicum* L.) is a globally important vegetable crop extensively cultivated under both open field and greenhouse conditions mainly due to its high nutritional value and commercial significance [1–4]. Nevertheless, its cultivation is greatly affected

by environmental stress factors, among which soil and water salinity represents a serious constraint, especially in regions characterized by limited rainfall and intensive irrigation practices [5,6]. In recent years, salinization of agricultural soils has become increasingly severe due to changing climatic conditions, insufficient drainage, inadequate irrigation practices, scarce rainfall, and the growing dependence on low-quality water sources [7,8]. Elevated salt concentrations in the root environment adversely influence plant growth and physiological functions, resulting in declines in fruit yield and quality [9–13].

Saline conditions negatively influence plant water balance by reducing the osmotic potential of the rhizosphere, thereby reducing root water uptake and causing physiological responses similar to drought stress [14,15]. In addition, the excessive uptake and accumulation of toxic ions such as Na^+ and Cl^- interfere with nutrient transport and cellular ionic homeostasis, thereby reducing the photosynthetic capacity and increasing oxidative damage. Consequently, plants tend to reduce vegetative growth, affecting the final reproductive performance [16–20]. Nevertheless, the response to salinity is highly dynamic and depends on multiple factors such as genotype, developmental stage, and severity of stress [21–23]. Therefore, assessing the mechanisms linked to plant adaptation in saline environments is crucial for developing effective management strategies and improving salt tolerance in horticultural crops.

Several methodologies have been used to assess plant tolerance to salt stress, especially those focused on biomass distribution, nutrient behavior, and physiological responses. Parameters such as chlorophyll concentration, ionic composition of plant sap, and leachate chemical composition can provide crucial insights into plant adaptation mechanisms and plant nutritional status under salt stress. Among these methodologies, petiole sap analysis offers a rapid assessment of the nutritional and physiological condition of plants exposed to adverse environments with minimal deterioration of the tested material [24,25]. In addition, digital image processing and RGB-based spectral indices have demonstrated considerable potential for identifying changes in leaf coloration and canopy characteristics associated with stress [26,27]. The combined application of these analytical tools enables a more integrated evaluation of crop performance under saline conditions.

Despite the extensive research conducted on the effects of salinity in tomato production at the worldwide level [28–31], the information available concerning the integrated evaluation of petiole sap chemistry, RGB-based spectral indices, and leachate composition under tropical climate conditions is rather limited. In addition, the use of compact tomato cultivars adapted to container cultivation may serve as suitable experimental models for assessing physiological responses to salt stress, while also enabling accurate assessment of water consumption and nutrient translocation within the plant.

Accordingly, this study was designed to assess the influence of increasing salinity on plant growth, biomass distribution, RGB-based spectral indices, physiological behavior, petiole sap characteristics, and leachate composition in tomato plants grown under protected cultivation conditions in a tropical climate. Furthermore, the identification of the principal nutritional and physiological modifications induced by salt stress, as well as the evaluation of the potential of rapid diagnostic techniques as tools for monitoring crop performance under saline irrigation conditions, may optimize management strategies aimed at improving salinity tolerance and optimizing water and nutrient use efficiency in tomato cultivation systems.

2. Materials and Methods

2.1. Growth Conditions and Plant Material

The study was carried out at the experimental facilities of the Catholic University of Santiago of Guayaquil, located in Ecuador ($2^{\circ}11' \text{ S}$, $79^{\circ}54' \text{ W}$). Seedlings of the tomato culti-

var Tiny Tim were sourced from a commercial nursery and established in 5 L polyethylene containers containing a growth medium consisting of 60% blonde peat, 30% black peat, and 10% perlite (10%), adjusted to a pH of 5.5–6.0. The selection of this cultivar was based on its dwarf growth characteristics and its proven suitability to overcome higher saline concentrations up to 12 dS m^{-1} [32]. In addition, its use is widely recommended by local growers under protected cultivation conditions. The experimental period extended from May 16 to 1 August 2025. The four phenological stages assessed during this experimental period were: vegetative growth, flowering, fruit development, and harvest.

During this time, plants were cultivated in a 30 m^2 bamboo nethouse. Microclimatic conditions within the structure were continuously recorded using data loggers (model TempU 03, HOBO, Bourne, MA, USA). The recorded average temperature and relative humidity during the experiment were 24.96°C and 77.75%, respectively, with maximum values reaching 40.00°C and 94.10%, and minimum values of 19.40°C and 47.00% (Figure 1).

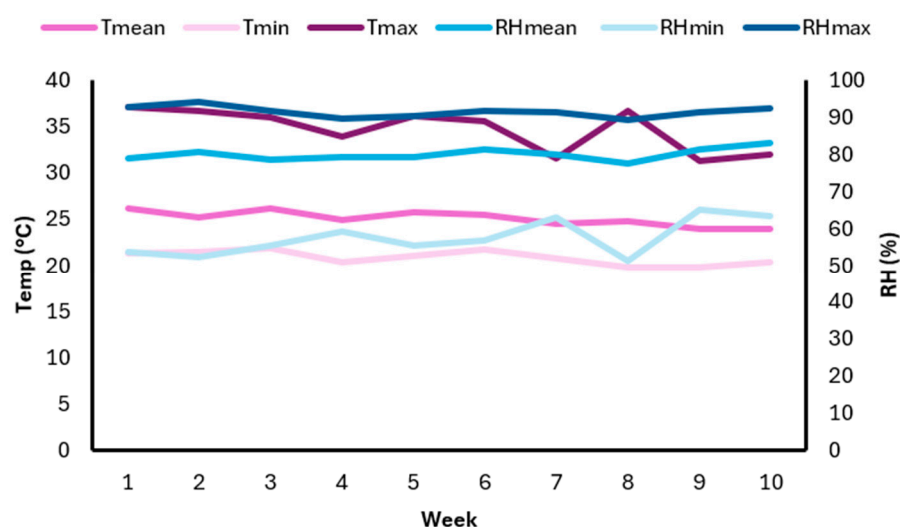


Figure 1. Weekly mean, minimum, and maximum air temperature and relative humidity during the first 10 weeks after transplanting.

2.2. Experimental Design and Treatments

The experiment was conducted using four salinity treatments, established by supplementing a standard Hoagland and Arnon [33] nutrient solution with NaCl to obtain irrigation water electrical conductivity (EC) values of 2.0 dS m^{-1} (T0, control), 4 dS m^{-1} (T1), 6 dS m^{-1} (T2), and 8 dS m^{-1} (T3). The nutrient solution supplied the following macronutrients (mmol L^{-1}): 14 NO_3^- , 1 NH_4^+ , $1 \text{ H}_2\text{PO}_4^-$, 2 SO_4^{2-} , 4 Ca^{2+} , and 2 Mg^{2+} ; together with the following micronutrients (mg L^{-1}): 1.0 Fe, 0.5 Mn, 0.05 Zn, 0.02 Cu, 0.5 B, and 0.01 Mo. The pH of all the saline treatments was adjusted and maintained at 6 throughout the experiment. The salinity treatments were initiated one week after transplanting in order to ensure proper seedling establishment. Prior to salinity application, all the plants received only the standard Hoagland and Arnon solution to reduce osmotic stress during early vegetative development. Irrigation was applied manually on a daily basis, and both EC and pH were monitored daily using a portable conductivity meter and a pH meter (Horiba Laquatwin-EC-11 and Laquatwin-pH-11, Horiba Ltd., Kyoto, Japan, respectively). This study was arranged in a randomized block design consisting of four salinity treatments and four blocks. Each experimental unit comprised four individual plants grown separately in pots, resulting in a total experimental population of 80 plants.

2.3. Biomass Parameters

Following 10 weeks of exposure to salinity conditions, the plants were collected for morphological and biomass analyses. The substrate adhering to the roots was carefully removed from four plants per treatment and the harvested material was immediately stored at 4 °C to prevent tissue degradation. Leaf area index (LAI) was estimated from digital images acquired immediately after harvest using a high-resolution scanner (Epson L3110, Seiko Epson Corp., Suwa, Japan). Image analysis was performed with the ImageJ software (National Institutes of Health, Bethesda, MD, USA) according to the methodology proposed by Vaesen et al. [34]. To assess biomass distribution, the plants were separated into roots, stems, leaves, and fruits. Fresh weight (FW) of each organ was recorded, and total fresh weight (TFW) was calculated as the combined weight of all the organs. The plant materials were then dried in a forced-air oven at 60 °C until constant weight was reached, enabling determination of dry weight (DW). Total dry weight (TDW) corresponded to the sum of the dry weights of all the plant fractions. Dry biomass data were used to derive allocation indices according to García-Caparrós et al. [35], including leaf weight ratio, shoot weight ratio, root weight ratio, and fruit weight ratio, each expressed relative to total plant dry mass.

2.4. Vegetation Spectral Characterization

Leaf images obtained for the determination of leaf area index were saved in JPG format with a spatial resolution of 1200 dpi. From the complete image collection, twenty fully expanded young leaves per treatment were selected, comprising five leaves from each of four plants. Digital color analysis was then conducted using Adobe Photoshop 2020 (Adobe Systems Software, Dublin, Ireland) to quantify the intensity values of the red (R), green (G), and blue (B) and the respective normalized indexes, following the procedure described by Zhao et al. [36].

2.5. Petiole Sap, Leachate Analysis and Leaf Physiological Traits

Leaf material for sap analysis was collected from the most recently fully expanded leaf during several developmental stages (vegetative growth, flowering, fruit development, and harvest). Following each evaluation period, leaves previously used for spectral vegetation index analysis were immediately processed for petiole sap extraction and subsequent chemical characterization. Prior to extraction, samples were gently cleaned with a moist cloth, sectioned into fragments of approximately 0.5 cm, and stored at −16 °C for 24 h.

Petiole sap, containing xylem and phloem fluids as well as apoplastic, cytosolic, and vacuolar fractions, was extracted using a hydraulic press according to the methodology reported by Cadahía [37]. The extracted solution was analyzed for pH as well as NO_3^- -N, P, Cl^- , K^+ , Ca^{2+} , and Na^+ concentrations. Sap pH was determined using a portable pH meter (Horiba Laquatwin-pH-11, Horiba Ltd., Kyoto, Japan). Nitrate (NO_3^- -N), potassium (K^+), calcium (Ca^{2+}) and sodium (Na^+) concentrations (mg L^{-1}) were quantified using ion-selective electrodes (Horiba Laquatwin series, Horiba Ltd., Kyoto, Japan). Phosphorus (P) concentration was analyzed with a digital photometer (Hanna HI 706, Hanna Instruments, Langnau bei Reiden, Switzerland), and chloride (Cl^-) concentration was determined using a digital colorimeter (Hanna HI 753, Hanna Instruments, Switzerland). Total soluble solids in the petiole sap were additionally determined as °Brix using a digital refractometer (model Flagfront, range 0–55% °Brix, accuracy $\pm 0.1\%$, with automatic temperature compensation).

Physiological parameters were also recorded on recently matured leaves through non-destructive measurements. Chlorophyll content, nitrogen status, leaf temperature (LTemp), and leaf relative humidity (LRH) were simultaneously assessed using a portable chlorophyll

meter (model GYJ-D, GOYOJO, Shenzhen, China), enabling rapid and non-destructive evaluation of plant physiological status.

During the course of the experiment, both the volume of nutrient solution applied and the leachate collected were recorded weekly for each treatment. Nutrient solutions and leachates were chemically characterized on a weekly basis. EC and pH were monitored using portable meters (Horiba Laquatwin-EC-11 and Laquatwin-pH-11, Horiba Ltd., Kyoto, Japan; respectively). The ionic composition of the leachates was determined following the same analytical procedures described for petiole sap analysis.

2.6. Statistical Analysis

The effects of the treatments were analyzed by a one-way analysis of variance (ANOVA). When significant differences were detected, mean comparisons were performed using Fisher's least significant difference (LSD) test at a significance level of $p < 0.05$. Prior to statistical analysis, ANOVA assumptions, including normality of residuals and homogeneity of variances, were verified. Correlation analyses were additionally performed to assess the relationships among petiole sap parameters. All the statistical analyses were conducted using Statgraphics Plus for Windows (Statpoint Technologies Inc., Warrenton, VA, USA).

3. Results

3.1. Biomass Parameters

A progressive increase in salinity levels within the nutrient solution led to a significant decline in both fresh and dry biomass across all the plant organs (roots, stems, leaves, and fruits), which was reflected in a substantial reduction in total plant biomass. This decrease followed a clear gradient with increasing EC, with the most pronounced effects observed at 8 dS m^{-1} (T3). Among the evaluated organs, fruit biomass exhibited the greatest sensitivity to salinity conditions, showing the highest reduction in both fresh and dry weight, decreasing from 1637.00 and 125.89 g in T0 to 589.32 and 45.32 g in T3, respectively (Table 1).

In contrast, biomass partitioning responses to salinity were less uniform across different plant organs. Root weight ratio increased significantly to 7.60% in T3, while leaf weight ratio reached 17.97% compared to the control treatment (T0). In contrast, fruit weight ratio decreased significantly to 66.97% in T3 compared to the control treatment (T0). Stem weight ratio did not show a consistent response among the treatments (Table 1).

3.2. Vegetation Spectral Characterization

The increasing salinity levels in the nutrient solution resulted in a significant reduction in leaf area index (LAI), with a decrease of 56.92% in T3 compared to T0. In contrast, RGB color components showed a differential response to salinity. The red component (R) decreased by 27.84% in T3, while the green component (G) remained unaffected across the treatments. Conversely, the blue component (B) showed significant differences, with a reduction of 8.84% in T3 compared to T0.

Regarding vegetation indices, no significant differences were observed in NBI among the treatments. However, NRI values decreased significantly as salinity increased (15.38% in T3), whereas NGI showed a significant increase (10.64%) under moderate to high EC levels (Table 2).

3.3. Petiole Sap Parameters

Salinity effects on petiole sap composition varied across phenological stages, with the most pronounced responses observed during the reproductive phase (Table 3).

Table 1. Effects of increasing salinity levels in the nutrient solution on biomass production and allocation parameters in tomato plants. Root fresh weight (RFW), stem fresh weight (SFW), leaf fresh weight (LFW), fruit fresh weight (FFW), total fresh weight (TFW), root dry weight (RDW), stem dry weight (SDW), leaf dry weight (LDW), fruit dry weight (FDW), total dry weight (TDW), root weight ratio (RWR), stem weight ratio (SWR), leaf weight ratio (LWR), and fruit weight ratio (FWR).

	RFW (g)	SFW (g)	LFW (g)	FFW (g)	TFW (g)	RDW (g)	SDW (g)	LDW (g)	FDW (g)	TDW (g)	RWR (-)	SWR (-)	LWR (-)	FWR (-)
T0	69.00 ± 4.64 a	70.60 ± 2.30 a	144.40 ± 5.64 a	1637.00 ± 159.65 a	1921.00 ± 190.19 a	6.58 ± 0.44 a	15.37 ± 1.17 a	27.94 ± 1.09 a	125.89 ± 8.58 a	175.77 ± 13.86 a	3.76 ± 0.37 b	8.81 ± 1.25 a	15.98 ± 1.51 b	71.45 ± 2.97 a
T1	37.60 ± 2.61 b	50.60 ± 4.04 b	130.80 ± 4.10 b	1014.94 ± 97.58 b	1233.94 ± 121.78 b	3.90 ± 0.67 b	10.94 ± 1.10 b	17.28 ± 1.86 b	89.31 ± 7.35 b	121.33 ± 12.26 b	3.12 ± 0.32 b	8.99 ± 1.27 a	14.36 ± 1.11 b	73.52 ± 1.36 a
T2	31.20 ± 2.49 c	34.40 ± 2.51 c	79.80 ± 5.12 c	802.13 ± 92.93 c	947.53 ± 93.16 c	3.06 ± 0.67 c	6.63 ± 1.57 c	15.04 ± 1.97 c	69.45 ± 6.83 c	94.18 ± 7.60 c	3.23 ± 0.58 b	7.03 ± 1.46 b	16.08 ± 1.68 a	73.66 ± 1.77 a
T3	28.60 ± 1.82 c	31.00 ± 2.74 c	73.40 ± 4.93 c	589.32 ± 68.27 d	735.32 ± 65.31 d	2.08 ± 0.45 d	5.00 ± 0.75 c	12.10 ± 1.63 c	45.32 ± 5.25 b	67.50 ± 3.70 d	7.60 ± 2.47 a	7.46 ± 1.39 b	17.97 ± 1.97 a	66.97 ± 1.53 b

* Values are means ± standard error (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$), while “ns” denotes non-significant differences.

Table 2. Effects of increasing salinity levels in the nutrient solution on leaf area index and leaf vegetation indices (–: dimensionless). R, red; G, green; B, blue; NRI (Normalized Red Index) = $R/(R + G + B)$; NGI (Normalized Green Index) = $G/(R + G + B)$; NBI (Normalized Blue Index) = $B/(R + G + B)$.

	LAI	R (-)	G (-)	B (-)	NBI (-)	NRI (-)	NGI (-)
T0	9831.74 ± 1196.17 a	26.47 ± 2.30 a	32.13 ± 2.10 a	9.50 ± 0.42 a	0.14 ± 0.01 a	0.39 ± 0.02 a	0.47 ± 0.03 b
T1	5907.928 ± 430.90 b	21.30 ± 2.59 b	29.44 ± 1.99 a	9.53 ± 0.34 a	0.16 ± 0.01 a	0.36 ± 0.01 b	0.46 ± 0.02 b
T2	4877.84 ± 417.14 c	20.02 ± 2.52 b	30.09 ± 1.92 a	8.60 ± 0.38 b	0.15 ± 0.01 a	0.34 ± 0.02 b	0.51 ± 0.03 a
T3	4235.96 ± 119.33 d	19.10 ± 1.55 b	30.31 ± 1.93 a	8.66 ± 0.47 b	0.15 ± 0.01 a	0.33 ± 0.02 b	0.52 ± 0.02 a

* Values are means ± standard error (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$), while “ns” denotes non-significant differences.

Table 3. Effects of increasing salinity levels in the nutrient solution on petiole sap composition at different phenological stages in tomato plants. pH (–), nitrate (NO_3^- -N), potassium (K^+), calcium (Ca^{2+}), sodium (Na^+), phosphorus (P), and chloride (Cl^-) concentrations (mg L^{-1}), and total soluble solids ($^\circ\text{Brix}$).

Growth Stage		pH	NO_3^- -N	K^+	Ca^{2+}	Na^+	P	Cl^-	$^\circ\text{Brix}$
T0	Vegetative growth	5.48 ± 0.08 a	826.48 ± 94.13 a	3340.15 ± 185.06 a	151.82 ± 7.49 a	52.03 ± 4.52 a	512.21 ± 70.48 a	536.55 ± 45.60 d	3.08 ± 0.25 b
T1		5.54 ± 0.11 a	876.20 ± 101.73 a	3460.99 ± 186.08 a	153.23 ± 8.31 a	61.12 ± 4.18 a	512.35 ± 37.01 a	682.76 ± 48.16 c	3.12 ± 0.31 b
T2		5.56 ± 0.11 a	825.12 ± 71.41 a	3520.85 ± 358.12 a	153.28 ± 14.82 a	50.19 ± 7.90 a	536.36 ± 41.59 a	780.36 ± 63.24 b	3.21 ± 0.31 b
T3		5.50 ± 0.18 a	822.23 ± 83.79 a	3610.52 ± 288.09 a	165.52 ± 7.90 a	56.25 ± 5.93 a	552.25 ± 44.28 a	852.14 ± 87.86 a	4.21 ± 0.31 a
T0	Flowering	5.40 ± 0.08 a	1341.76 ± 118.92 a	3740.25 ± 250.99 b	153.41 ± 12.72 b	70.61 ± 7.58 d	550.21 ± 15.81 d	1376.25 ± 150.43 d	3.24 ± 0.18 b
T1		5.30 ± 0.11 a	1157.12 ± 83.65 a	3860.31 ± 254.95 b	148.00 ± 13.03 b	194.02 ± 11.25 c	600.35 ± 17.52 c	2243.75 ± 100.58 c	3.16 ± 0.20 b
T2		5.40 ± 0.07 a	781.85 ± 78.10 b	4315.25 ± 147.90 a	192.42 ± 19.78 a	269.34 ± 32.40 b	650.36 ± 13.25 b	3506.25 ± 108.07 b	4.20 ± 0.21 a
T3		5.22 ± 0.19 a	626.7 ± 96.68 b	4220.34 ± 281.95 a	187.50 ± 18.15 a	384.17 ± 43.58 a	700.12 ± 12.64 a	4275.09 ± 200.90 a	4.35 ± 0.25 a
T0	Fruit development	5.34 ± 0.18 a	1004.44 ± 58.32 a	3960.11 ± 148.32 a	234.23 ± 21.40 c	68.62 ± 8.17 d	570.45 ± 14.14 b	1623.32 ± 108.65 d	3.28 ± 0.20 b
T1		5.28 ± 0.11 a	950.45 ± 65.52 ab	3910.28 ± 172.36 a	238.21 ± 23.54 c	198.81 ± 15.58 c	580.58 ± 17.23 b	3579.15 ± 98.26 c	3.68 ± 0.24 b
T2		4.96 ± 0.10 b	905.51 ± 48.65 b	3580.35 ± 155.23 b	275.76 ± 21.18 b	472.56 ± 51.65 b	620.47 ± 18.45 a	5325.18 ± 92.02 b	4.23 ± 0.28 a
T3		5.00 ± 0.12 b	589.75 ± 56.54 c	3360.36 ± 154.85 b	330.29 ± 31.62 a	580.19 ± 53.02 a	640.35 ± 12.25 a	7100.36 ± 110.12 a	4.43 ± 0.32 a
T0	Harvest	5.48 ± 0.14 a	603.20 ± 50.68 a	3720.10 ± 220.36 a	274.67 ± 31.30 c	98.81 ± 20.93 d	395.31 ± 14.52 d	3374.97 ± 105.12 d	4.18 ± 0.39 b
T1		5.40 ± 0.15 a	463.24 ± 62.52 b	3220.92 ± 201.36 b	360.14 ± 54.31 b	467.12 ± 25.88 c	444.02 ± 18.23 c	7602.52 ± 253.01 c	4.14 ± 0.34 b
T2		5.06 ± 0.19 b	375.16 ± 20.21 c	2910.75 ± 180.36 c	682.11 ± 66.10 a	850.55 ± 68.55 b	512.02 ± 13.36 b	8475.65 ± 183.32 b	5.74 ± 0.33 a
T3		5.02 ± 0.11 b	345.44 ± 52.91 c	2460.19 ± 185.36 d	736.39 ± 77.30 a	1016.85 ± 85.32 a	551.02 ± 15.60 a	9687.54 ± 352.36 a	5.78 ± 0.28 a

* Values are means $\pm$ standard error ($n = 4$). Different letters indicate significant differences among treatments ($p < 0.05$), while “ns” denotes non-significant differences.

During the vegetative stage, all the parameters except Cl^- and $^{\circ}\text{Brix}$ values remained stable across the treatments. The Cl^- concentrations in sap increased progressively in NaCl-treated plants. In addition, $^{\circ}\text{Brix}$ values were significantly higher under higher salinity conditions, especially at the highest EC level.

At the flowering stage, salinity effects became clearer. The values of pH remained without significant differences among the treatments. NO_3^- -N concentration in sap decreased significantly with increasing EC (53.29% in T3 compared to T0), while K^+ and Ca^{2+} concentrations showed a marked increase (12.84 and 22.22%, respectively), especially in the T2 and T3 treatments. The Na^+ , chloride and phosphorus concentrations in sap exhibited a strong and consistent rise under the NaCl treatments. $^{\circ}\text{Brix}$ values also increased significantly under salinity (34.26% in T3 compared to T0), with the highest values recorded in the T2 and T3 treatments.

During fruit development, the effect of salinity on sap nutrient composition was even intensified. NO_3^- -N concentration decreased markedly under higher EC levels (41.29% in T3 compared to T0), while Ca^{2+} showed a strong increase, especially in T3 (41.01%). The Na^+ and Cl^- concentrations in sap continued to accumulate significantly in response to salinity. Potassium concentration and pH values showed a slight reduction at the highest salinity level (15.14 and 6.37%, respectively). $^{\circ}\text{Brix}$ values and P concentration increased significantly in the NaCl-treated plants, reaching their highest levels under severe salinity conditions.

At harvest, salinity effects were most pronounced. The values of pH showed a slight reduction at the highest salinity level (8.39% in T3 compared to T0). NO_3^- -N concentration in sap reached its lowest values under saline conditions (42.73% reduction in T3 compared to T0), whereas Ca^{2+} , Na^+ and P showed their highest accumulation levels, particularly in T3. The Cl^- concentration in sap also increased sharply with salinity (187.04% in T3 compared to T0). Potassium concentration showed a decreasing trend at higher EC levels. $^{\circ}\text{Brix}$ values were significantly higher in the NaCl treatments (38.28% increase in T3 compared to T0), with maximum values observed in the T2 and T3 treatments.

Under vegetative conditions (Figure 2a), correlations among ions were generally moderate, with more evident associations observed among osmotic-related variables. Strong positive correlations were identified between Na^+ and Cl^- ($r = 0.82$), as well as between Cl^- and $^{\circ}\text{Brix}$ ($r = 0.97$). Likewise, Na^+ showed positive correlations with Ca^{2+} ($r = 0.71$) and $^{\circ}\text{Brix}$ ($r = 0.74$). Moderate positive correlations were observed between NO_3^- -N and P ($r = 0.55$), NO_3^- -N and $^{\circ}\text{Brix}$ ($r = 0.60$), and P and $^{\circ}\text{Brix}$ ($r = 0.57$), whereas K^+ remained largely uncorrelated with the other variables. In contrast, pH did not exhibit correlations with any measured parameter.

At the flowering stage (Figure 2b), the correlation patterns became more defined and structured. NO_3^- -N showed strong negative correlations with P, K^+ , Ca^{2+} , Na^+ , Cl^- , and $^{\circ}\text{Brix}$ ($r = -0.66$ to -0.95). Conversely, a strong positive cluster was observed among P, Na^+ , Cl^- , K^+ , and $^{\circ}\text{Brix}$. In particular, Na^+ and Cl^- were positively correlated ($r = 0.96$), while Na^+ and Cl^- were also positively correlated with $^{\circ}\text{Brix}$ ($r = 0.83$ and 0.84 , respectively). Calcium also displayed moderate positive associations within this group, while pH remained weakly related to the rest of the variables.

During fruit development (Figure 2c), Na^+ , Cl^- , P, and Ca^{2+} were positively correlated, with the highest correlation observed between Na^+ and Cl^- ($r = 0.93$). In contrast, these variables showed strong negative correlations with NO_3^- -N and K^+ ($r = -0.55$ to -0.86). Additionally, $^{\circ}\text{Brix}$ presented weaker and less consistent correlations compared to previous developmental stages.

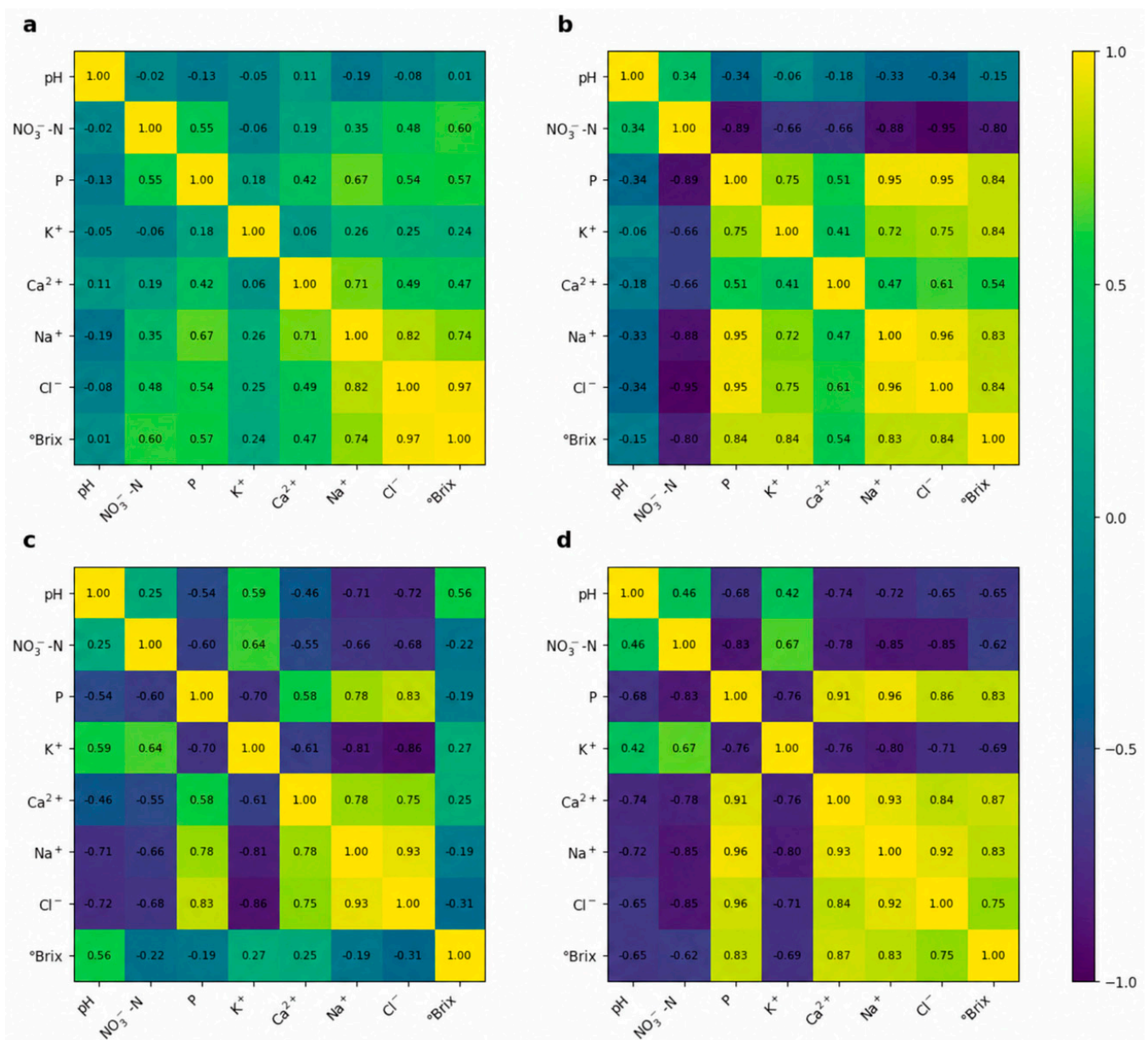


Figure 2. Pearson correlation heatmaps illustrate the relationships among petiole sap parameters at different phenological stages: (a) vegetative, (b) flowering, (c) fruit development, and (d) harvest. Color intensity represents the strength and direction of correlations (−1 to 1). Abbreviations: NO₃⁻-N (nitrate-nitrogen), P (phosphorus), K⁺ (potassium), Ca²⁺ (calcium), Na⁺ (sodium), Cl⁻ (chloride), and °Brix (total soluble solids).

At harvest (Figure 2d), positive correlations were observed among Na⁺, Cl⁻, Ca²⁺, P, and °Brix. The highest correlations were recorded between P and Na⁺ ($r = 0.96$), Ca²⁺ and Na⁺ ($r = 0.93$), and Na⁺ and Cl⁻ ($r = 0.92$). In contrast, NO₃⁻-N and K⁺ showed negative correlations with this group. Furthermore, °Brix was positively correlated with P, Ca²⁺, Na⁺, and Cl⁻ ($r = 0.75$ to 0.87). The values of pH showed negative correlations with P, Ca²⁺, Na⁺, Cl⁻, and °Brix ($r = -0.65$ to -0.74).

3.4. Leaf Physiological Traits

Table 4 shows the values of physiological parameters including SPAD index, N content, LRH, and LTemp, evaluated at different phenological stages during the experimental period. Under vegetative conditions, SPAD values, N content, LRH, and temperature remained

statistically unchanged across the treatments, with all the treatments grouped within the same significance level.

Table 4. Effects of salinity treatments on physiological parameters including SPAD index, nitrogen content (N mg g^{−1}), leaf relative humidity (LRH %), and leaf temperature (LTemp °C) at different phenological stages.

Growth Stage		SPAD	N	LRH	LTemp
T0	Vegetative growth	43.43 ± 1.22 a	16.82 ± 0.43 a	64.32 ± 2.02 a	26.36 ± 0.85 a
T1		42.76 ± 1.58 a	15.31 ± 0.42 a	62.76 ± 1.63 a	26.92 ± 0.56 a
T2		42.62 ± 1.93 a	15.66 ± 0.84 a	63.14 ± 2.09 a	26.86 ± 0.42 a
T3		43.20 ± 1.74 a	16.76 ± 0.49 a	64.94 ± 2.79 a	27.72 ± 0.32 a
T0	Flowering	47.37 ± 1.43 a	17.49 ± 0.74 a	69.71 ± 2.75 a	25.42 ± 0.13 b
T1		38.57 ± 1.30 b	15.28 ± 1.15 b	65.16 ± 3.19 a	27.04 ± 0.16 a
T2		39.41 ± 2.17 b	15.17 ± 1.18 b	60.02 ± 5.66 b	27.45 ± 0.42 a
T3		36.26 ± 1.95 b	14.44 ± 0.45 b	56.42 ± 1.94 b	27.35 ± 0.25 a
T0	Fruit development	44.71 ± 1.79 a	16.80 ± 1.22 a	67.20 ± 2.76 a	25.80 ± 0.92 b
T1		44.93 ± 1.18 a	15.13 ± 0.90 b	65.38 ± 2.51 a	28.18 ± 0.27 a
T2		41.91 ± 1.14 b	15.35 ± 1.16 b	61.58 ± 2.19 b	28.39 ± 0.34 a
T3		41.01 ± 1.36 b	15.83 ± 1.29 b	62.45 ± 4.90 b	27.61 ± 0.27 a
T0	Harvest	45.92 ± 4.26 a	16.11 ± 1.33 a	75.70 ± 2.53 a	30.71 ± 0.81 b
T1		35.24 ± 5.37 b	16.23 ± 2.82 a	69.03 ± 2.08 b	33.90 ± 0.04 a
T2		30.51 ± 1.10 c	11.94 ± 0.53 b	51.31 ± 2.65 c	33.43 ± 0.46 a
T3		29.53 ± 0.92 d	11.73 ± 0.51 b	50.16 ± 2.64 c	32.92 ± 0.53 a

* Values are means ± standard error (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$), while “ns” denotes non-significant differences.

At the flowering stage, treatment effects became evident. SPAD values were significantly reduced under the saline treatments compared to T0 (23.45% in T3 compared to T0), whereas temperature showed an inverse pattern (7.59% increase in T3 compared to T0). A comparable response was observed for N concentration, which also showed significant reductions at the highest salinity treatments (17.44% in T3 compared to T0). Leaf relative humidity followed the same pattern, with significantly lower values recorded in the T2 and T3 treatments (19.06% reduction in T3 compared to T0).

During the fruit developmental stage, SPAD values were significantly reduced under the highest saline treatments (8.28% in T3 compared to T0). Similarly, N concentration and relative humidity were significantly affected (5.77% in T3 compared to T0), while relative humidity showed significantly lower values in the T2 and T3 treatments (7.07% reduction in T3 compared to T0). Temperature showed the lowest value in plants fertigated without NaCl, with an increase of 7.02% in T3 compared with T0.

At the harvest stage, the effects of the saline treatments were more pronounced. SPAD values showed a significant decline with increasing salinity, with T3 presenting the lowest values (35.69% reduction compared to T0). Nitrogen concentration followed a similar trend, with significant reductions observed in the T2 and T3 treatments (27.19% in T3 compared to T0). The LRH also differed significantly among the treatments, decreasing under higher salinity levels (33.74% in T3 compared to T0). In contrast, LTemp displayed limited variability and no consistent significant differences, with the highest value in control plants (7.20% increase in T3 compared to T0).

3.5. Leachate Parameters

The volume of leachate, water uptake, and the chemical composition of the leachate throughout the experimental period are presented in Figure 3. Leachate volume (Figure 3a) and EC (Figure 3c) increased progressively over time in all the treatments, with the highest

values in plants fertiligated at the highest salinity level. In contrast, water uptake (Figure 3b) showed an opposite trend, decreasing as salinity increased and reaching the lowest values recorded under the highest salt concentration. Differences among the treatments became more evident from the middle of the experimental period onward.

The pH of the leachate (Figure 3d) remained stable throughout the experiment without significant differences detected among the treatments at any sampling time.

Nutrient concentrations in the leachate showed variable temporal patterns. The concentration of NO_3^- (Figure 3e) gradually increased over time in all the treatments but without significant differences among the treatments during most of the experimental period. Phosphorus concentration (Figure 3f) showed an initial decrease followed by a progressive increase, with significant differences among the treatments emerging from the mid-experimental period onward. In contrast, K^+ concentration (Figure 3h) remained relatively stable over time without significant differences observed among the treatments.

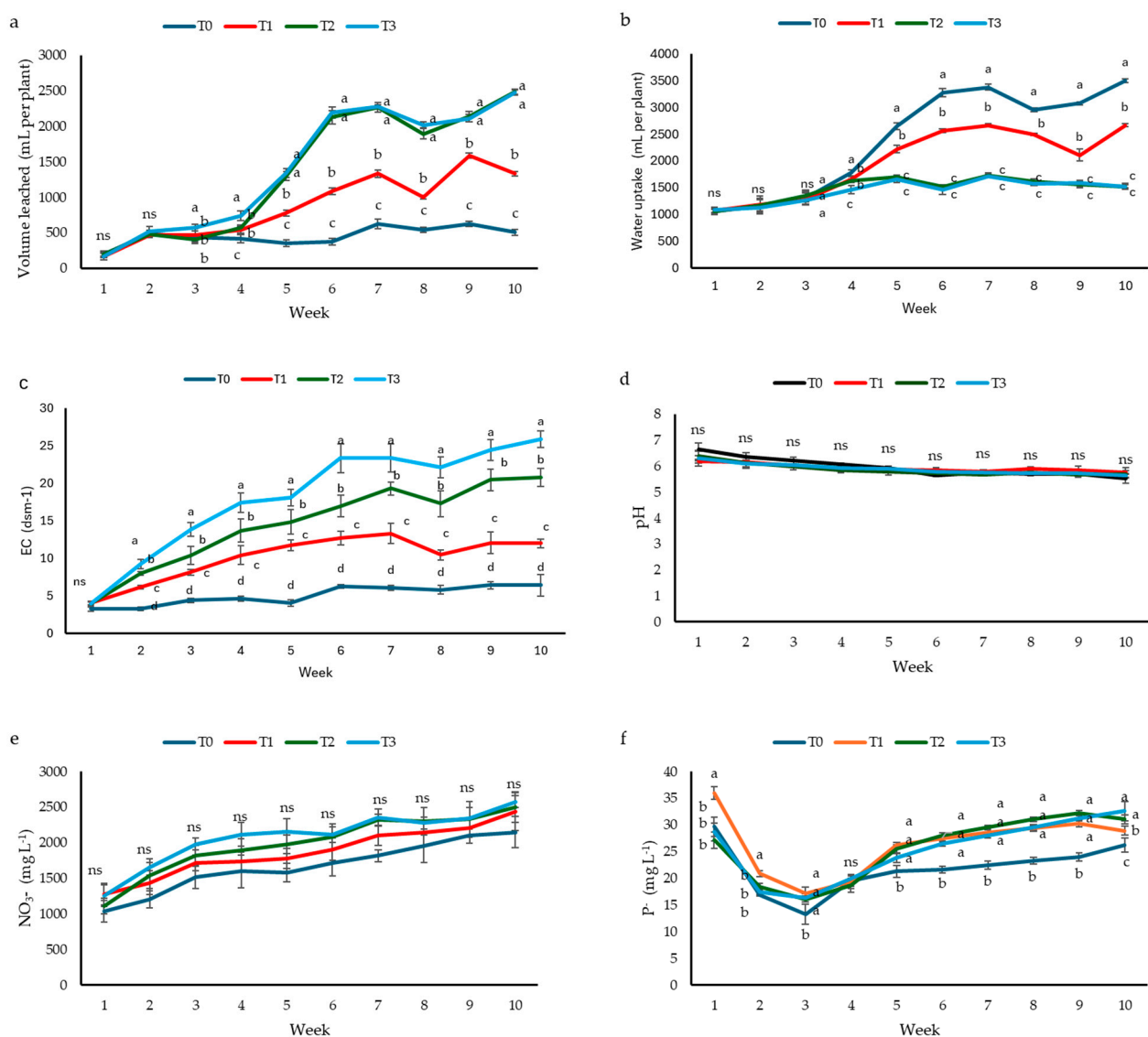


Figure 3. Cont.

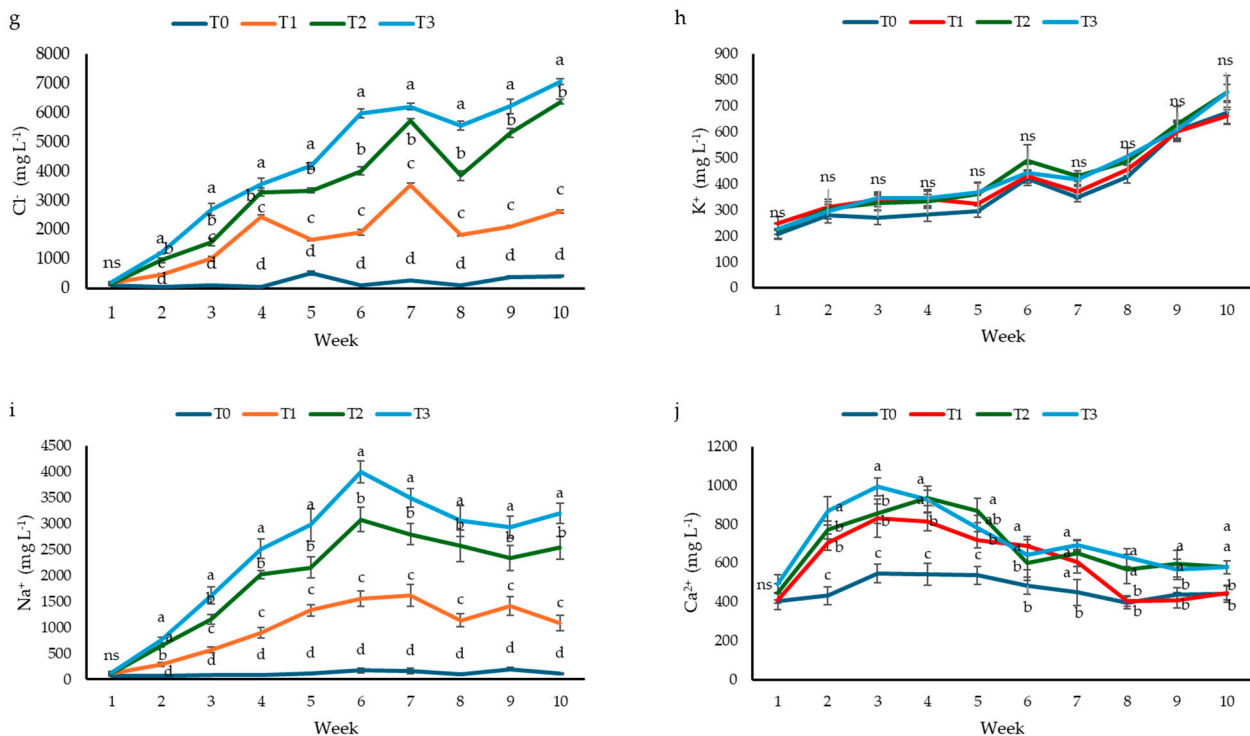


Figure 3. Temporal variation in leachate volume, water uptake, and leachate chemical composition under different salinity treatments. Panels represent: (a) leachate volume, (b) water uptake, (c) electrical conductivity (EC), (d) pH, (e) NO₃⁻-N, (f) P, (g) Cl⁻, (h) K⁺, (i) Na⁺, and (j) Ca²⁺. Values are means ± standard error (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$), while “ns” denotes non-significant differences.

Salinity-related ions exhibited the most pronounced responses. The concentrations of Cl⁻ and Na⁺ (Figure 3g,i) increased markedly throughout the experimental period, with clear and significant differences among the treatments, reaching the highest values under the highest salinity level. Similarly, Ca²⁺ concentration (Figure 3j) followed a comparable trend, although greater variability was observed and differences among the treatments were less consistent during the early stages.

4. Discussion

The present study demonstrated that increasing salinity levels exerted pronounced effects on growth, biomass accumulation, physiological performance, petiole sap composition, and leachate chemistry in tomato plants cultivated under protected conditions in a tropical climate.

The progressive decline in the plant biomass parameters reported with increasing EC confirms the sensitivity of tomato to saline conditions even when this cultivar has a higher salt tolerance than other cultivars [5,38]. These findings indicate that salt stress has led to a clear restriction in plant growth primarily linked to the osmotic and ionic effects of stress associated with saline conditions, as has been widely reported in other experiments growing tomato under salinity conditions [39–41]. Interestingly, among the evaluated organs, fruits showed the greatest reduction in both fresh and dry weights, indicating that reproductive structures were more sensitive to salinity than vegetative tissues, as reported by Mitchell et al. [42]. This fact can be explained by the alteration of assimilate partitioning under salt conditions, aiming to favor the maintenance of vegetative organs at the expense of reproductive development [43]. The increase in root and leaf weight ratios under higher salinity levels recorded in our study further supports this

interpretation. A higher biomass allocation to roots is indicative of an adaptive strategy to enhance water and nutrient acquisition under osmotic stress conditions [44], while increased biomass allocation to leaves represents a typical feature in salt-stressed plants to maintain photosynthetic capacity [45], even when there is a clear reduction in leaf area index as occurs in our experiment.

The spectral vegetation responses recorded in our experiment confirmed the physiological effect of saline conditions on leaf tissues in tomato plants. For instance, the decline in the red component and NRI values under salt stress conditions may be linked to modifications in pigment concentration and leaf optical properties [46]. Conversely, the increase in NGI values under higher salinity levels could be associated with structural and biochemical adjustments in leaf tissues caused by salt stress [47]. The absence of significant differences in NBI indicates that some spectral indicators may be less sensitive to different abiotic stresses [48]. Overall, our results showed that some parameters of the spectral analysis can serve as useful non-destructive tools for detecting plant salinity-induced changes.

Petiole sap composition was greatly influenced by salt stress, particularly during the reproductive stage. These results indicate that ion accumulation and nutrient imbalance are more accentuated as plant development progressed. The progressive rise in Na^+ and Cl^- concentrations in sap represents a typical pattern widely reported in plants under salt stress based on the accumulation of these ions in leaf tissues, aiming to enhance cell osmotic adjustment [49]. Besides the accumulation of these ions, the increase in °Brix values under saline conditions further supports the importance of higher soluble solid concentrations to maintain cellular turgor and water retention under osmotic stress [50]. In contrast, NO_3^- -N concentrations showed a progressive decline with increasing salinity conditions, especially during flowering, fruit development, and harvest, which are critical phases in the final yield. This reduction in NO_3^- -N uptake can be associated with the antagonism between Cl^- and NO_3^- -N that occurs under salinity conditions, since both ions have similar transport pathways in plants [51,52]. Potassium also tended to decline at advanced developmental stages (fruit development and harvest) under higher salinity levels, indicating another classic antagonism that occurs between K^+ and Na^+ under salt stress [53,54]. Interestingly, Ca^{2+} concentrations increased markedly under higher saline levels, particularly during fruit development and harvest. This pattern may be ascribed to the passive transport of Ca^{2+} and its accumulation in reproductive organs to enhance membrane stabilization, thereby reducing the damage caused by Na^+ toxicity [55]. Phosphorus concentration in sap tended to increase in the advanced developmental stages, which can be ascribed to the energy (ATP) required to transport the excess ions into the vacuoles under salt stress conditions [35].

The correlation analyses among the different variables tested in this study reported that the relationship between ions became more pronounced under higher salinity conditions. During the vegetative stage, ion interactions were weak, suggesting that in this phenological stage the plants were able to maintain ionic homeostasis under moderate stress. Nevertheless, as the experimental period progressed, particularly in flowering, there were clear antagonistic relationships between NO_3^- -N and salinity-related ions, while Na^+ , Cl^- , Ca^{2+} , P, and °Brix showed positive relationships. In fruit development, the trend was not very accentuated but in the harvest stage, the results obtained reported the same trend obtained during flowering, with K^+ included in the antagonistic relationships. These coordinated responses indicate that osmotic adjustment became increasingly dependent on ion accumulation during reproductive stages [43].

Water relations and leachate chemistry dynamics recorded in our experiment provided additional evidence of the physiological constraints suffered by plants cultivated under saline conditions. The reduction in water uptake under increasing saline conditions reflects

the osmotic stress caused by increasing NaCl concentrations in the nutrient solution, thereby reducing the capacity of plants to take up water [56]. Consequently, there was an increase in leachate volume and EC among saline treatments over the experimental period, indicating a lower capacity for accumulation of Na^+ and Cl^- within the substrate solution [57]. No variations in leachate pH over the experimental period indicate that salinity treatments only affected ionic concentration but not the acid-base balance in the substrate solution [58]. The results obtained in the chemical composition of the leachate mirrored the ion dynamics reported in petiole sap. The progressive increase in Na^+ and Cl^- concentrations in the leachate confirms the continuous accumulation and movement of salts within the substrate–plant system under saline conditions, and the respective higher EC values. The increasing trend of accumulation of NO_3^- and K^+ in the leachate also confirms the antagonisms in the uptake between NO_3^- and Cl^- and K^+ and Na^+ , respectively, observed in petiole sap. The absence of clear treatment differences in NO_3^- and K^+ concentration during the experiment may indicate that NO_3^- and K^+ availability in the nutrient solution was higher than the nutritional plant requirements [59]. The increasing trend in phosphorus concentration in the leachate from the mid-experimental period onward can be ascribed to higher P supplies in the nutrient solution than plant uptake requirements [60].

5. Conclusions

The results of this study demonstrate that increasing salinity in the nutrient solution significantly reduced plant growth with modifications in the nutrient balance throughout the tomato cycle. Fresh and dry biomass declined progressively in all the organs, with fruits showing the highest sensitivity to salinity stress. Fruit fresh weight decreased by 64.00% at an EC of 8 dS m^{-1} compared to the control. These reductions were associated with reduced leaf area index (56.92%), water uptake (55.75%), SPAD values (33.58%), N concentration (25.88%), and leaf relative humidity (33.73%), suggesting clear limitations on plant growth and physiological activity under salt stress. Salinity also modified biomass partitioning, favoring roots and leaves over fruits, and caused a progressive ionic imbalance characterized by Na^+ and Cl^- accumulation, especially during the reproductive stage.

SPAD values and RGB-derived indices, particularly NRI and NGI, showed potential as rapid and non-destructive indicators of salt stress. Overall, petiole sap analysis, leachate monitoring, SPAD measurements, and RGB imaging provided complementary tools for monitoring the nutritional and physiological status of tomato plants under saline irrigation conditions.

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