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EVALUATION OF SALT STRESS TOLERANCE IN LETTUCE (*Lactuca sativa* L.) CULTIVARS UNDER HYDROPONIC CONDITIONS

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Abstract:
Salt stress is one of the most important abiotic stress factors that significantly negatively affect plant growth and yield. This study evaluated the morphological and physiological responses of eight lettuce (*Lactuca sativa* L.) varieties to salt stress under hydroponic conditions. The lettuce varieties were grown in an aerated hydroponic system under control (1.8 dS/m) and salt stress (6.0 dS/m) conditions. The results showed that salt stress caused significant reductions in shoot biomass, particularly compared to root biomass characteristics. The varieties with the greatest reductions in biomass parameters under salt stress conditions were Chicarita, Triplex, and Levistro, while Baeza and Cherokee produced higher biomass. Morphological characteristics such as leaf number, stem diameter, plant height, and leaf area were also significantly affected by salt stress, with leaf area—the edible part of the lettuce—being the most affected by salt stress. As with other parameters, the Baeza, Kireve, and Cherokee varieties were more stable in these parameters. The Chicarita variety again showed the lowest performance in these parameters. Leaf color parameters such as L, a*, b*, chroma, and hue angle were significantly affected by salt stress. Under salt stress conditions, the Expedition and Kireve varieties had more vibrant colors than other varieties. Chlorophyll-a, chlorophyll-b, and total chlorophyll content increased in some varieties due to salt stress, while they decreased in others. Salt stress generally increased leaf sap EC and SSC values. The effect of salt stress on fruit juice pH was not statistically significant. In conclusion, salt stress negatively affected shoot parameters in lettuce varieties, while in some varieties it caused an increase in root morphology. These differences in response to salt stress are related to stress adaptation ability, so the parameters used in this study can be used as selection criteria for lettuce or other plants. Additionally, the varieties identified as salt-tolerant in this study can be used in breeding programs aimed at growing plants under saline conditions.

Keywords: Lettuce, Salt stress, Chlorophyll, Leaf color

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1. Introduction

Salt stress, one of the most harmful abiotic stress factors, causes physiological disorders in plants, which ultimately results in plant death and leads to a decrease in crop yield and quality. Salt stress is increasing from arid to semi-arid regions, thereby progressively limiting agricultural production (Hussain et al., 2019; Sakadevan and Nguyen, 2010; Rouphael et al., 2018). Due to excessive salt concentrations, approximately 2,000 hectares of arable land are lost every day worldwide (Shrivastava and Kumar, 2015). Salt concentration in irrigation water accumulates in the root zone of plants, causing stunted plant growth. In plants grown under saline conditions, sodium and chloride ion accumulation disrupts physiological balance by accumulating in plant cell compartments (Muhammad et al., 2022; Sakadevan and Nguyen, 2010). In addition, all soluble salts that contribute to soil salinity cause physiological stress in plants in arid and semi-arid regions (Chinnusamy et al., 2005; Hussain et al., 2019). To mitigate the adverse effects of salt stress on plants, it is necessary to adopt strategies for the more appropriate use of land and water resources (Yamaguchi and Blumwald, 2005). These strategies may enable vegetable production under saline conditions (Moncada et al., 2018, 2021). The use of hydroponic cultivation systems helps overcome salt stress and other abiotic stresses and ensures more efficient and higher quality yields in some vegetable species (Moncada et al., 2018, 2021; Settanni et al., 2013). In hydroponic systems, plants are supplied with a mineral nutrient solution, and their roots are supported by mineral or organic substrates or floating panels (floating systems) that hold the plants above the nutrient solution. The water used to prepare nutrient solutions should have a particularly low salt concentration, as the addition of soluble mineral fertilizers can affect the electrical conductivity (EC) of the nutrient solution (Moncada et al., 2020). Hydroponics is one of the most promising systems in protected agriculture due to its simplicity, ease of operation, higher yield, economic feasibility and nutritionally superior products. Compared to traditional cultivation, hydroponics provides more efficient, early, high-quality and healthy products. In addition, water and fertilizer are used more efficiently than in traditional cultivation. Due to the widespread use of intensive agricultural practices in Mediterranean regions, excessive use of irrigation water has caused significant seawater leakage, which in turn has led to increased salinity in groundwater. The use of this water, which has a high EC value, in hydroponic systems can lead to nutrient solutions that exceed the tolerance thresholds of many vegetable species (Mariani and Ferrante, 2017). For these reasons, researchers have focused on understanding the adaptation mechanisms of plants to salt stress to increase the salt tolerance of vegetables (Rao et al., 2006). Lettuce (*Lactuca sativa* L.), which is rich in iron, which is essential for human health, is an important vegetable in terms of nutrition. It is mostly consumed as part of the daily diet in salads (Moncada et al., 2020). However, lettuce cultivation is quite sensitive to the adverse effects of salinity stress. Additionally, excessive accumulation of sodium and nitrate in vegetables is a particularly important issue for humans (Shi et al., 2022). Adverse soil conditions, challenging environmental factors, and irrigation with low-quality water severely limit lettuce production, significantly reducing its yield potential. Salt stress reduced seed germination, leaf water content, and chlorophyll content in lettuce plants, and reduced root and shoot growth in terms of biomass, while increasing sodium and chloride ion concentrations and lipid peroxidation in leaf tissues (Barassi et al., 2006; Eraslan et al., 2007; Kaya et al., 2002; Mohammadi and Khoshgoftarmanesh, 2014; Pérez-López et al., 2013). Cultivated lettuce varieties are more sensitive to salt stress than wild species due to differences in root structure and varying uptake and accumulation of sodium. Lettuce production in soils and water with high salt concentrations can be increased by selecting salt-tolerant varieties through intraspecific genetic diversity (Wei et al., 2014).

The aim of this study is to determine the tolerance levels of eight different lettuce (*Lactuca sativa* L.) varieties to salt stress and to evaluate their physiological and morphological responses to salinity under static water culture conditions.

2. Materials and Methods

The experiment was conducted in a fully automated Venlo-type glass greenhouse at Kırşehir Ahi Evran University (38°08'02"N, 34°07'08"E) between January and April 2025. The seeds of the lettuce varieties (Table 1) were supplied by RIJK ZWAAN (Rijk Zwaan Seed Production and Seed Trade B.V.).

Table 1. provides information on lettuce varieties

Variety	Type
Levistro	Lollo type
Cencibel	Lollo type
Baeza	Mini Romaine
Kireve	Oak leaf lettuce
Expedition	Incised leaf sweet crisp frisée
Triplex	incised leaf sweet crisp frisée
Chicarita	Crunchy
Cherokee	Batavia

2.1. Sowing Seeds and Planting Seedlings

Seeds were sown on January 20, 2025, and seedlings were transplanted on February 17, 2025. Seed sowing was carried out in 128-cell pots filled with a 3:1 peat:perlite mixture. Transplanting was performed when the seedlings reached the 3-4 true leaf stage. Greenhouse climate control operations were carried out using an automation system.

2.2. Establishment of the Hydroponic System and Salt Stress Testing

Eight lettuce varieties were planted in a hydroponic system (130 L containers covered with aerated and perforated composite panels) according to a randomized plot design containing three replicates and three plants per replicate (9 control, 9 salt). The nutrient solution used in the hydroponic system consisted of 1500 μM $Ca(NO_3)_2$, 750 μM K_2SO_4, 650 μM $MgSO_4$, 500 μM KH_2PO_4, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 0.4 μM Na_2MoO_4, and 80 μM Fe EDDHA, with a pH adjusted to 6.5. Salt application began two days after planting and gradually increased until reaching 6.00 dS/m on the sixth day. The salt level was adjusted by adding NaCl to the nutrient solution. The same genotypes were also grown under control conditions (1.8 dS/m) in addition to the saline water application. After reaching full salt concentration (6.00 dS/m), the plants were grown in the hydroponic system for 30 days, after which the experiment was terminated. Greenhouse cooling (fan-pad cooling) and humidification (high-pressure misting) were automated. When the temperature exceeded 27 °C, the cooling system was activated, and when the relative humidity dropped below 50%, misting continued until the humidity reached 65%. When the temperature dropped below 14°C, the geothermal heating system was activated.

2.3. Parameters Measured in Plants under Salt Stress Conditions

2.3.1. Determination of biomass parameters

The stem diameters of the plants were measured in millimeters (mm) using a digital caliper just below the first leaves. The main stem length and canopy diameter were measured in centimeters (cm) using a tape measure. The number of leaves (number of leaves per plant) was recorded by counting all the leaves on the plant at the end of the experiment. The fresh weights of leaves and roots were determined by separating the plants into root and above-ground parts and weighing them during harvest (g). The dry weights of leaves and roots were obtained by drying fresh samples in an oven at 65°C for 48 hours (g). To determine root length (cm), diameter (mm), and volume (cm^3), 5 g fresh root material subsamples were collected from each treatment group. These root subsamples were scanned and analyzed using a root imaging system (WinRhizoRegular LA2400, Regent Instruments). The values obtained for 5 g of roots were then calculated proportionally to the total fresh root weight to determine the root length and root volume for each plant.

2.3.2. Determination of leaf sap electrical conductivity (EC), pH, and soluble solids content (SSC)

Leaf sap EC and pH were measured using an Extech EC meter and pH meter, respectively. Soluble solid content (SSC) was measured using a Hanna HI96801 digital refractometer.

2.3.3. Determination of hue angle value

Color parameters such as CIE L, a*, b*, chroma (C), and hue angle (h°) were measured using a Minolta CR-400 colorimeter (Konica Minolta, Japan). Measurements were taken at three different points for each lettuce leaf sample. The device was calibrated using a standard calibration plate prior to measurement (Özdemir, 2001). The hue angle was calculated using the following formula (equation 1):

$$\text{Hue angle } (h^\circ) = \left(\frac{180}{\pi}\right) \times \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

The hue angle value indicates the color shade: 0° = red, 60° = yellow, 120° = green, 180° = cyan, 240° = blue, 300° = magenta, 360° or 0° = red.

2.3.4. Chlorophyll a, b, total chlorophyll, and carotenoid analysis

Total chlorophyll and carotenoid, chlorophyll a, and chlorophyll b contents were determined according to the Arnon, (1949) method. Measurements were performed using a spectrophotometer (Shimadzu Model 1208, Tokyo, Japan) for each process.

2.4. Statistical Analysis

Data obtained from the hydroponic experiment were analyzed using one-way analysis of variance (ANOVA) at a 5% significance level with the SPSS 18.0 statistical software package (IBM, Chicago, IL, USA). Differences between means were determined using Duncan's multiple range tests.

3. Results

Under control and salt stress conditions, the leaf and root weights (both fresh and dry) of lettuce plants were evaluated in Table 2. Salt stress caused a decrease in fresh leaf weight in all varieties, with the greatest decreases observed in the Chicarita (−58%), Levistro (−49%), and Triplex (−47%) varieties, respectively. The smallest decreases were observed in the Baeza (−7%) and Cherokee (−19%) varieties. Under control and salt stress conditions, the highest fresh leaf weight was determined in the Expedition variety. Leaf dry weight results showed a similar trend to fresh leaf weight results. In some varieties, an increase in root fresh weight was observed under salt stress conditions. Significant increases were observed in the Levistro (+12%), Baeza (+3%), Cencibel (+59%), Cherokee (+45%), Expedition (+24%), and Triplex (+17%) varieties. In the other two varieties, Chicarita (−29%) and Kireve (−13%), decreases were observed. As shown in Figure 1, despite the differences between varieties, the Kireve variety had the highest root fresh weight under both control and salt stress conditions.

Table 2. Effect of salt stress on leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, and their percentage changes in lettuce varieties

Variety	Leaf Fresh Weight (g)			Leaf Dry Weight (g)			Root Fresh Weight (g)			Root Dry Weight (g)		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	126.07cd	64.23de	-49	18.44cd	9.61de	-48	11.40bc	12.73abc	12	1.59bc	1.84abc	16
Cencibel	82.17e	56.80e	-31	12.12e	8.50e	-30	7.20d	11.47abc	59	1.15cd	1.52abc	32
Baeza	99.27de	91.90cd	-7	14.61de	13.51cd	-8	9.23cd	9.53bc	3	1.24cd	1.36bc	10
Kireve	205.47b	139.43ab	-32	29.78b	20.35ab	-32	21.83a	18.97a	-13	2.90a	2.62a	-9
Expedition	275.90a	169.60a	-39	39.79a	24.66a	-38	14.20b	17.63ab	24	2.03b	2.29ab	13
Triplex	136.57c	72.33de	-47	19.94c	10.71de	-46	8.73cd	10.20abc	17	1.18cd	1.44bc	23
Chicarita	106.30c-e	44.33e	-58	15.57c-e	6.76e	-57	6.77d	4.83c	-29	1.01d	0.77c	-24
Cherokee	140.27c	113.63bc	-19	20.47c	16.61bc	-19	9.80cd	14.2ab	45	1.39cd	2.03ab	46
p value	0.000	0.000		0.000	0.000		0.000	0.001		0.000	0.001	

%C= percentage change compared to the control. N.S= not significant. Means that do not share a letter are significantly different (P<0.05).

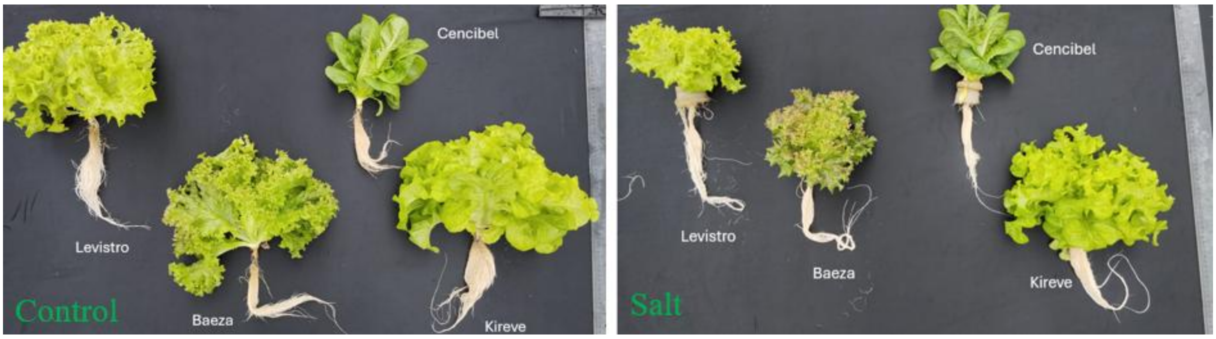

Figure 1. Effect of salt stress on growth and morphology of levistro, cencibel, baeza and kireve lettuce varieties.

Under control conditions, the highest number of leaves was observed in the Kireve variety, while the lowest number of leaves was observed in the Cencibel variety. Similarly, under salt stress conditions, the highest number of leaves was observed in the Kireve variety, as was the case under control conditions. Under salt stress, the lowest number of leaves was observed in the Cencibel and Levistro varieties. Salt stress had a statistically significant effect on stem diameter in all varieties ($p < 0.001$). Under both control and salt stress conditions, the tallest plants were measured in the Kireve variety, while the shortest plants were measured in the Triplex variety. Salt stress caused a reduction in plant height ranging from 11% to 31% depending on the variety. Leaf areas also decreased significantly in all varieties under salt stress. The greatest reduction in the leaf area was observed in the Chicarita variety (58%), while the lowest reduction was observed in the Baeza variety (7%) (Table 3). Despite the general decrease under salt stress conditions, the Expedition variety had the highest leaf area in both control and salt stress treatments (Figure 2).

Table 3. Effect of salt stress on number of leaves, main stem diameter, plant height, leaf area, and their percentage changes in lettuce varieties

Variety	Number of Leaves			Main Stem Diameter (mm)			Plant Height (cm)			Leaf Area (cm^2)		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	13.67de	12.00d	-12	12.94a	7.51bc	-42	23.50a-c	18.67cd	-21	4724.00cd	2407.00de	-49
Cencibel	11.33e	11.33d	0	8.59c	7.29bc	-15	23.67a-c	17.00d	-28	3079.00e	2129.00e	-31
Baeza	20.33bc	22.67ab	11	11.76ab	13.61a	16	20.83bc	17.33d	-17	3719.90de	3443.80cd	-7
Kireve	26.67a	27.33a	2	10.48bc	9.99b	-5	26.83a	22.67a	-16	7700.00b	5225.10ab	-32
Expedition	21.33b	19.67bc	-8	9.49bc	9.45b	0	25.00ab	22.33ab	-11	10338.00a	6357.00a	-39
Triplex	17.00b-d	14.33cd	-16	8.37c	5.95c	-29	19.67c	16.67d	-15	5117.70c	2711.00de	-47
Chicarita	19.33bc	14.33cd	-26	10.10bc	8.11bc	-20	25.67ab	17.67d	-31	3983.50c-e	1661.30e	-58
Cherokee	15.67c-e	16.00cd	2	11.29ab	10.38ab	-8	25.00ab	20.33bc	-19	5256.00c	4258.3bc	-19
p value	0.000	0.000		0.000	0.000		0.003	0.000		0.000	0.000	

%C= percentage change compared to the control. N.S= not significant. Means that do not share a letter are significantly different (P<0.05).

Figure 2. Effect of salt stress on growth and morphology of expedition, triplex, chicarita and cherokee lettuce varieties.

A decrease in canopy diameter was observed in all lettuce varieties grown under salt stress. The highest canopy diameter under salt stress was measured in the Kireve variety. In terms of root length, the Kireve variety had the longest roots under both control and salt stress conditions, while the Chicarita variety had the shortest roots under salt stress. Under salt stress conditions, the highest root volume was observed in the Expedition variety (11.64 cm^3), followed by the Kireve (10.75 cm^3) and Levistro (10.02 cm^3) varieties. The lowest root volumes were observed in the Chicarita (3.83 cm^3) and Triplex (4.77 cm^3) varieties. Under salt stress, root diameter decreased in the Levistro, Cencibel, and Kireve varieties, while in other varieties, root diameter increased by 2% to 27% under salt conditions. Canopy diameter, root length, root volume, and root diameter were significantly influenced by genotype at the $p < 0.001$ level (Table 4).

Table 4. Effect of salt stress on canopy diameter, root length, root volume, root diameter, and their percentage changes in lettuce varieties

Variety	Canopy Diameter (cm)			Root Length (cm)			Root Volume (cm^3)			Root Diameter (mm)		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	29.00bc	23.33bc	-20	3861.53b	4869.87b	26	8.26b	10.02a	21	0.52 ab	0.52a	-1
Cencibel	24.33cd	23.00c	-5	3062.31c	3557.58c	16	6.81b	6.16b	-10	0.53 ab	0.47b	-12
Baeza	18.00d	17.33cd	-3	3328.71c	3164.86c	-5	6.96b	7.08b	2	0.52 ab	0.53a	3
Kireve	37.00a	30.67a	-17	4255.52ab	4201.27b	-1	13.24a	10.75a	-19	0.63a	0.57a	-9
Expedition	32.67ab	29.67ab	-9	4545.95a	5514.79a	21	7.84b	11.64a	49	0.47 ab	0.52a	10
Triplex	24.00cd	19.00cd	-21	3998.28b	2318.25d	-42	4.50c	4.77c	6	0.40b	0.51a	27
Chicarita	19.67d	16.00d	-19	2980.23d	2293.28d	-23	4.77c	3.83c	-20	0.45 ab	0.46b	2
Cherokee	29.00bc	23.67bc	-18	3241.67c	4197.39b	29	6.70b	9.93a	48	0.51ab	0.55a	7
p value	0.000	0.000		0.000	0.000		0.000	0.000		0.000	0.000	

%C= percentage change compared to the control. N.S= not significant. Means that do not share a letter are significantly different (P<0.05).

In lettuce varieties, all leaf color parameters, including lightness (L), redness/greenness (a*), yellowness (b*), color (C), and color tone angle (°), were significantly affected by salt stress. ($p < 0.05$ or $p < 0.001$ depending on the parameter) (Table 5). The lightness value (L) decreased in most varieties under salt stress, resulting in a duller appearance of the leaves. The most pronounced decrease in L was observed in the Levistro (-13%) and Cencibel (-12%) varieties, while a significant increase (+17%) was detected in the Expedition variety. This variety exhibited lighter-colored leaves under salt stress conditions. The a* value, representing the red-green axis, decreased in most varieties, with the largest decrease observed in the Cencibel (-44%) variety, followed by the Triplex (-13%) variety. Interestingly, under salt stress conditions, the Expedition (+13%) and Kireve (+5%) varieties exhibited a redder color tone. The b* value decreased in the Triplex (-14%), Chicarita (-14%), and Cencibel (-13%) varieties due to salt stress, while the b* value increased in the Levistro (+8%), Expedition (+10%), and Kireve (+4%) varieties, which were more affected by salt stress and exhibited increased yellow pigmentation in their leaves. Under salt stress, the chroma values decreased in most varieties, with the greatest decrease observed in the Cencibel (-19%) and Triplex (-14%) varieties. However, the chroma values increased in the Expedition (+11%), Levistro (+6%), and Kireve (+4%) varieties, showing stronger color saturation under stress conditions. Regarding the color tone angle, which reflects the overall perceived color tone, salt stress had little effect on most varieties. The most significant decrease occurred in the Cencibel (-8%) variety. In the Baeza (+1%) and Chicarita (+2%) varieties, slight increases were observed. Under salt stress, the Kireve, Expedition, Triplex, and Cherokee varieties maintained relatively stable color tone angles.

Table 5. Effect of salt stress on leaf color parameters (l, a*, b*, chroma, hue angle) and their percentage changes in lettuce varieties

Variety	L			a*			b*			Chroma (C)			Hue Angle (°)		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	56.69a	49.33a-d	-13	-17.81bc	-17.33cd	-3	30.52b	33.00b	8	35.27a-c	37.28b	6	120.08bc	117.70b	-2
Cencibel	46.00b	40.67d	-12	-15.00ab	-8.33a	-44	26.33b-d	23.00c	-13	30.33b-d	24.48cd	-19	119.57c	109.99c	-8
Baeza	45.67b	45.00b-d	-1	-15.67ab	-15.00bc	-4	23.00cd	21.00cd	-9	27.84cd	25.82cd	-7	124.29a	125.62a	1
Kireve	56.33a	57.33a	2	-21.33c	-22.33e	5	38.00a	39.67a	4	43.58a	45.53a	4	119.30c	119.37b	0
Expedition	46.00b	54.00ab	17	-18.33bc	-20.67de	13	29.67bc	32.67b	10	34.88bc	38.68b	11	121.75a-c	122.25ab	0
Triplex	45.67b	41.67d	-9	-16.00ab	-14.00bc	-13	28.67bc	24.67c	-14	32.83bc	28.38c	-14	119.17c	119.50b	0
Chicarita	43.33b	44.00cd	2	-13.00a	-12.00ab	-8	19.33d	16.67d	-14	23.30d	20.54d	-12	123.86ab	125.81a	2
Cherokee	56.00a	51.00a-c	-9	-19.00bc	-17.33cd	-9	33.00ab	31.00b	-6	38.08ab	35.53b	-7	119.90c	119.19b	-1
p value	0.000	0.000		0.001	0.000		0.000	0.000		0.000	0.000		0.001	0.000	

%C= percentage change compared to the control. N.S= not significant. Means that do not share a letter are significantly different (P<0.05).

The chlorophyll-a, chlorophyll-b, total chlorophyll, and total carotenoid contents and % changes of lettuce varieties under control and salt stress conditions are presented in Table 6. Salt stress causes significant differences in pigment contents among varieties. While all chlorophyll contents were significantly affected by salt stress ($p < 0.05$), the change in total carotenoid content was not statistically significant ($P>0.05$). Salt stress caused an increase in chlorophyll-a content in some lettuce varieties, while it caused a decrease in others. The most significant increase was observed in the Levistro variety (64%), followed by Baeza (37%) and Chicarita (14%). In contrast, chlorophyll-a content decreased significantly in the Triplex (-28%), Expedition (-25%), and Cherokee (-10%) varieties. When evaluating these results in response to salt stress, it can be concluded that Levistro and Baeza can increase chlorophyll-a accumulation, while other varieties are negatively affected by salt stress. Similar trends were observed in chlorophyll-b content as in chlorophyll-a content. Increases were observed in the Levistro (+50%), Baeza (+36%), and Chicarita (+21%) varieties under salt stress, while decreases were observed in the Triplex (-43%), Expedition (-26%), and Cencibel and Cherokee (-19%) varieties. Similar results to those observed in chlorophyll-a and b were obtained for total chlorophyll content. The Levistro variety showed the highest increase (+60%), while the Triplex variety showed the greatest decrease (-33%). Changes in total carotenoid content among varieties under control and salt stress conditions were not statistically significant.

Table 6. Effect of salt stress on chlorophyll-a, chlorophyll-b, total chlorophyll, total carotenoid contents, and their percentage changes in lettuce varieties (mg L^{-1})

Variety	Chlorophyll-a (mg l^{-1})			Chlorophyll-b (mg l^{-1})			Total Chlorophyll (mg l^{-1})			Total Carotenoid (mg l^{-1})		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	0.28b	0.47ab	64	0.12b	0.17b	50	0.40c	0.64c	60	0.06a	0.09a	63
Cencibel	0.5ab	0.48ab	-4	0.24ab	0.20b	-19	0.74a-c	0.67c	-9	0.11a	0.09a	-11
Baeza	0.64ab	0.88a	37	0.45a	0.61a	36	1.10ab	1.50a	37	0.11a	0.08a	-11
Kireve	0.33b	0.33b	1	0.14b	0.14b	-1	0.47bc	0.48c	0	0.07a	0.08a	18
Expedition	0.64ab	0.48ab	-25	0.34ab	0.26b	-26	0.99a-c	0.74bc	-25	0.12a	0.07a	-36
Triplex	0.47ab	0.34b	-28	0.26ab	0.15b	-43	0.73a-c	0.49c	-33	0.08a	0.08a	-1
Chicarita	0.77a	0.87a	14	0.48a	0.58a	21	1.25a	1.45ab	16	0.11a	0.11a	-2
Cherokee	0.57ab	0.51ab	-10	0.24ab	0.20b	-19	0.82a-c	0.71bc	-13	0.11a	0.11a	-1
p value	0.008	0.004		0.004	0.000		0.006	0.001		0.075	0.141	

%C= percentage change compared to the control. N.S= not significant. Means that do not share a letter are significantly different (P<0.05).

The effects of salt stress on leaf sap electrical conductivity (EC), pH, and soluble solids content (SSC) in lettuce varieties are presented in Table 7. It shows that significant variety differences were observed in EC and SSC values ($p < 0.05$) and that pH was not statistically affected. Salt stress significantly increased EC in all varieties, representing increased ion accumulation in leaf tissue. The highest increase in EC was observed in the

Chicarita variety (+221%), followed by Triplex (+109%) and Cherokee (+106%). The lowest increase in EC was observed in the Kireve (+34%) and Baez (+49%) varieties. Leaf sap pH under control and salt stress conditions was not significantly different among varieties (P>0.05). The soluble solid content (SSC) in leaf sap was significantly affected by salt stress ($p < 0.01$). Salt stress increased SSC content in the Expedition (+78%), Baeza, Chicarita (+41%), and Cherokee (+30%) varieties. The lowest increase was observed in the Levistro (+7%) variety.

Table 7. Effect of salt stress on leaf sap electrical conductivity (ec), ph, soluble solids content (ssc), and their percentage changes in lettuce varieties

Variety	Leaf Sap EC (dS/m)			Leaf Sap pH			Leaf Sap Soluble Solids Content (%)		
	Control	Salt	%C	Control	Salt	%C	Control	Salt	%C
Levistro	4.57	8.06ab	76	6.59	6.52	-1	2.70a	2.90b	7
Cencibel	4.87	9.44a	94	6.81	6.39	-6	2.60a	3.20a	23
Baeza	4.24	6.33b	49	6.57	6.47	-2	2.20b	3.10ab	41
Kireve	5.15	6.89b	34	6.55	6.36	-3	2.40ab	2.90b	21
Expedition	4.17	7.47b	79	6.42	6.39	0	1.80c	3.20a	78
Triplex	4.08	8.51ab	109	6.54	6.58	1	1.90c	2.40c	26
Chicarita	2.07	6.64b	221	6.42	6.50	1	2.20b	3.10ab	41
Cherokee	4.55	9.39a	106	6.48	6.65	3	2.30b	3.00ab	30
p value	N.S	0.004		N.S	N.S		0.002	0.003	

4. Discussion

Salt stress inhibits plant growth and biomass accumulation by reducing water uptake, altering ion homeostasis, and disrupting metabolic activities (Aydın, 2024; Aydın and Yetişir, 2022; Munns and Tester, 2008). In the present study, salt stress significantly affected both shoot and root biomass in lettuce varieties, particularly causing notable decreases in leaf fresh and dry weights (Table 2). These findings are consistent with previous studies showing that salt stress inhibits shoot growth through osmotic stress and reduced cell expansion (Ashraf and Akram, 2009; Parida and Das, 2005). Leaf fresh weight decreased the most in the Baeza (−7%) and Cherokee (−19%) varieties, indicating that these varieties produced a more stable shoot biomass under salt stress. This relative tolerance mechanism may be associated with better osmotic adjustment or antioxidant capacity, which help maintain cellular functions under stress (Adhikari et al., 2021). The responses of varieties to root fresh weight were found to be different. Under salt stress, increases in root fresh weight were observed in the Cencibel (+59%), Cherokee (+45%), and Expedition (+24%) varieties. These varieties may have developed a tolerance mechanism by increasing root volume to enhance water uptake. Under salt stress, Chicarita (-29%) and Kireve (-13%) varieties showed decreases in root fresh weight. The low root development in these varieties may be due to reduced access to water and nutrients under salt stress. Root dry weight results are like root fresh weight results. These findings reflect the variability in genotype responses, as the Cherokee, Cencibel, and Expedition varieties showed superior root biomass performance under salt stress. Increased root development under saline conditions is a positive adaptive trait as it facilitates better water uptake and nutrient acquisition (Munns and Gilliham, 2015; Zhu, 2002). Overall, the findings indicate that the shoot biomass of 8 different lettuce varieties is more sensitive to salt stress than root biomass. While all varieties experienced reductions in shoot growth, some varieties were able to maintain or increase root growth under stress. Varieties such as Cherokee, Cencibel, and Expedition demonstrated greater adaptability to salt stress conditions due to their improved root performance, while the Chicarita variety was highly sensitive to salt stress, with significant reductions in both shoot and root growth. These responses of varieties to salt stress could serve as a useful selection criterion for breeding programs aimed at selecting salt-tolerant lettuce genotypes.

Salt stress caused significant changes in the parameters of leaf number, main stem diameter, plant height, and leaf area in lettuce varieties (Table 3). While most varieties showed a decrease in leaf number, some varieties showed an increase in leaf number. The most significant decreases in leaf number were observed in the Chicarita (-26%) and Triplex (-16%) varieties. These decreases are consistent with findings (Aydin, 2024) indicating that salt stress restricts leaf initiation and expansion due to osmotic and ionic stress. On the other hand, some varieties such as Baeza (+11%), Kireve (+2%), and Cherokee (+2%) maintained or increased their leaf count under salt stress. The variability in leaf count decreases and increases may be due to a potential genotype tolerance mechanism related to sustainable meristem activity. In plants under salt stress, stem diameter decreased significantly, particularly in the Levistro (-42%) and Triplex (-29%) varieties. This decrease may be due to salt stress affecting the development of the vascular bundle and stem thickening through changes in cell turgor or lignin accumulation (Zhu, 2002). The Baeza variety, on the other hand, increased its stem diameter by 16% under salt stress conditions. While the plant heights of all lettuce varieties grown under salt stress decreased significantly, the highest decrease was observed in the Chicarita (-31%) variety. Similar findings regarding reduced plant height have been reported in other studies on lettuce and leafy vegetables (Adhikari et al., 2021; Ahmed et al., 2019). Among all the parameters examined, the leaf area was the trait most affected by salt stress. The greatest reductions were observed in the Chicarita (-58%), Triplex (-47%), and Levistro (-49%) varieties, respectively. This sharp reduction in leaf area may be attributed to the inhibitory effects of salt stress on cell growth and stomatal closure, which limit the photosynthetic surface area (Parida and Das, 2005). The Baeza (-7%) and Cherokee (-19%) varieties showed more stable decreases in leaf area compared to other varieties. These varieties may have the ability to tolerate efficient osmotic regulation and better tissue hydration. When all biomass parameters were considered, the morphological responses of the varieties to salt stress showed significant differences. Under salt stress conditions, lettuce varieties showed different responses in canopy diameter and root morphology parameters. The Expedition and Cherokee varieties increased root volume and diameter under stress. These findings suggest that salt-tolerant genotypes may have maintained or increased root development to improve water and nutrient uptake under saline conditions (Munns, 2011; Munns and Gilliham, 2015; Munns and Tester, 2008; Zhu, 2002). Under salt stress conditions, some varieties attempted to maintain their biomass characteristics, which aligns with previous studies emphasizing the importance of morphological stability under stress conditions (Ashraf and Harris, 2013). It is thought that genetic diversity may affect the plant's response to salinity. Başak et al. 2025 reported that genotypes have different hormone levels in root and leaf tissues in saline conditions.

Overall, salt stress caused a decrease in L values (openness) in most varieties. The most significant decreases in L were observed in the Levistro (-13%) and Cencibel (-12%) varieties, while increases were observed in Expedition (+17%) and Kireve (+2%). Under salt stress, the a* value decreased significantly in Cencibel (-44%), Triplex (-13%), and Chicarita (-8%). This indicates a shift toward a greener leaf color under salt stress (Ashraf and Harris, 2013). Salt stress caused decreases in b* values in Cencibel (-13%) and Chicarita (-14%) varieties. This may be due to the loss of yellow pigments such as carotenoids (Parida and Das, 2005). Chroma, which represents color vibrancy, decreased in most varieties due to the effect of salt stress. The greatest decreases were observed in the Cencibel (-19%) and Triplex (-14%) varieties. However, Expedition (+11%) and Kireve (+4%) exhibited stronger colour saturation. Changes in colour tone were relatively minor among the varieties. These findings are consistent with previous studies (Ashraf and Harris, 2013; Zhao et al., 2017). Salt stress significantly affected the chlorophyll a, b, and total chlorophyll content in lettuce varieties ($p < 0.01$). The highest increases were observed in the Levistro, Baeza, and Chicarita varieties. These varieties may have activated pigment synthesis or retention mechanisms under saline conditions (Munns and Gilliham, 2015). In contrast, decreases in pigment levels were observed, particularly in the Triplex and Expedition varieties. In the study, total carotenoid content did not change statistically between control and salt stress conditions. Salt stress significantly increased leaf sap electrical conductivity (EC) and soluble solid content (SSC) in most lettuce varieties, particularly Chicarita, Triplex, and Cherokee, which may be related to enhanced ion accumulation and osmotic adjustment mechanisms under salt stress (Aydin, 2024).

5. Conclusion

In this study, the effects of salt stress on 8 different lettuce varieties on their morphological, physiological and biochemical properties were investigated. Salt stress had negative effects on all lettuce varieties. In the study, it was generally determined that the shoot mass decreased more than the decrease in root biomass. In addition, some varieties, especially those determined tolerant under salt stress conditions, either maintained or increased their root growth. In addition, in some varieties, leaf color parameters and pigment contents (chlorophyll-a, b and total chlorophyll) increased under the effect of salt stress, while in others they decreased. Salt stress caused a general increase in parameters such as leaf sap electrical conductivity (EC) and soluble solid content (SSC). In general, lettuce varieties gave different responses to salt stress. Cherokee, Cencibel, Baeza and Expedition varieties used in the study were determined to be more tolerant to salt than other varieties. These varieties can be starting materials for cultivation in saline environments and breeding studies to increase salt tolerance in lettuce.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	A.A.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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