Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 29 (2), 552-568
Mustafa Kemal University Journal of Agricultural Sciences, 29 (2), 552-568
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of the physiological response of lettuce to different irrigation water salinities (NaCl) and leaching fractions

Marulun farklı sulama suyu tuzluluklarına ve yıkama fraksiyonlarına karşı fizyolojik tepkisinin belirlenmesi

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ARTICLE INFO

Article history:
Recieved / Geliş: 07.04.2024
Accepted / Kabul: 24.05.2024

Keywords:
Chlorophyll concentrations
Chlorophyll content
Greenhouse
Lactuca sativa L.
Soil salinity
Stomatal conductance
Washing rate

Anahtar Kelimeler:
Klorofil konsantrasyonu
Klorofil içeriği
Lactuca sativa L.
Sera
Toprak tuzluluğu
Stoma iletkenliği
Yıkama oranı

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ABSTRACT

The Amik Plain, where the experiment was conducted, is increasingly salinised owing to improper irrigation methods, excessive irrigation, drainage and groundwater use. This situation indicates that soil salinity will increase even more in the future. The study was conducted using a factorial experimental design in potted conditions inside the greenhouse to investigate the impacts of three distinct levels of irrigation water salinity (EC_i) (EC_{i-0}= 0.5 dS m^{-1} (control), EC_{i-2}= 2 dS $m^{-1}$and EC_{i-4}= 4 dS m^{-1}) and four different leaching fraction (LF) (LF_0=0%, LF_{10}=10%, LF_{20}=20%, LF_{30}=30%) on stomatal conductance (g_s), leaf surface temperature (LSt), chlorophyll content (SPAD), chlorophyll concentrations (Chl-*a*, Chl-*b*, and Chl-*tot*) and yield parameters in 'Cospirina' lettuce plants. As a result of the study, soil salinity (EC_e) increased from 0.82 dS m^{-1} to 2.09 dS m^{-1} with increasing EC_i. As EC_i increased, plant water consumption (PWC) decreased from 8.92 to 5.71 L pot^{-1}, yield decreased from 276 g pot^{-1} to 198 g pot^{-1}, g_s decreased from 266 mmol m^{-2} s^{-1} to 215 mmol m^{-2} s^{-1}. LSt increased by 2.17% in EC_{i-2} and 6.4% in EC_{i-4} compared to the control. As EC_e increased, yield decreased by 10% in EC_{i-2} and 28% in EC_{i-4} compared to the control treatment. Chl-*tot* and Chl-*a* were significantly affected by the increase in EC_e (r^2=0.96*, r^2=0.99**, respectively), while Chl-*b* was not affected. In contrast to soil salinity, leaching fraction had a positive effect on plant physiology.

ÖZET

Denemenin yürütüldüğü Amik Ovası yanlış sulama yöntemleri, aşırı sulama, drenaj ve yeraltı su kullanımı nedeniyle hızla tuzlanmaktadır. Bu durum, toprak tuzluluğunun gelecekte daha da artacağını göstermektedir. Araştırma, 'Cospirina' çeşidi marul bitkisinde 3 farklı sulama suyu tuzluluğu (EC_i) (EC_{i-0}= 0.5 dS m^{-1} (kontrol), EC_{i-2}= 2 dS $m^{-1}$and EC_{i-4}= 4 dS m^{-1}) ve 4 farklı yıkama oranlarının (LF) (LF_0=0%, LF_{10}=10%, LF_{20}=20%, LF_{30}=30%) stoma iletkenliği (g_s), yaprak yüzey sıcaklığı (LSt), klorofil içeriği (SPAD) ve klorofil konsantrasyonları (Chl-*a*, Chl-*b* ve Chl-*tot*) ve verim parametrelerine etkilerini belirlemek amacıyla seradaki saksı koşullarında faktöriyel deneme deseninde yürütülmüştür. Araştırma sonucunda, EC_i arttıkça, toprak tuzluluğu (EC_e) 0.82 dS m^{-1} den, 2.09 dS m^{-1}'ye artmıştır. EC_i'nin artmasıyla bitki su tüketimi (PWC) 8.92'den 5.71 L pot^{-1}'ye, verim 276 g pot^{-1}'dan 198 g pot^{-1}'a, g_s, 266 mmol m^{-2} s^{-1}'dan 215 mmol m^{-2} s^{-1}'a azalmıştır. LSt, EC_{i-0}'a göre EC_{i-2}'de %2.17, EC_{i-4}'de %6.4 artmıştır. EC_e arttığında verim tanık konuya göre, EC_{i-2}'de %10 ve EC_{i-4}'de %28 azalmıştır. Chl-*tot* ve Chl-*a*, EC_e'deki artıştan önemli ölçüde etkilenirken (sırasıyla r^2=0.96*, r^2=0.99**), Chl-*b* etkilenmemiştir. Toprak tuzluluğunun aksine yıkama oranları bitki fizyolojisini olumlu etkilemiştir.

Cite/Atıf Ödemiş, B., Kazgöz Candemir, D., & Karaca, C. (2024). Determination of the physiological response of lettuce to different irrigation water salinities (NaCl) and leaching fractions. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 29* (2), 552-568. https://doi.org/10.37908/mkutbd.1466659

INTRODUCTION

Türkiye is among the Mediterranean countries that will be most affected by climate change. Hatay province, located in the Eastern Mediterranean region, has been experiencing drought and decreased precipitation due to climate change in recent years. Farmers in this region have to use salty groundwater or drainage water for irrigation, resulting in yield and quality losses, particularly in field crops and vegetables. The use of irrigation water with a high electrical conductivity of 7-8 dS m^{-1} in certain areas of the Amik and Samandağ plains in Hatay province has resulted in soil salinization (Ödemiş et al., 2019) Salinity and alkalinity issues are prevalent in about 1.92% (1.5 million ha) of Türkiye due to the widespread use of saline water, and approximately 32.5% of irrigated lands are affected by salinity problems (Kanber et al., 2005). Research indicates that over 20% of the world's arable land is negatively affected by salinity stress and 7% of the world's 930 million ha of land has problems due to soil salinity (Arora, 2019; Szabolcs, 1994). Khamidov et al. (2022) reported that climate change will significantly affect soil salinization in the future, and according to the results of model studies, slightly saline soils in Uzbekistan will decrease between 2050 and 2100, while medium (from 31.2% to 32.5%) and high saline soils (from 13.4% in 2050 to 15.1% in 2100) will increase in the same period.

Lettuce is one of the most significant leafy vegetables consumed raw and can be cultivated year-round using breeding varieties in both open fields and under protective cover (Aydinsakir et al., 2019; Ibrahim et al., 2024; Islam et al., 2021). Lettuce, an annual and cool-climate vegetable, can be grown in a short period of 2-3 months. In Türkiye, 562 000 tons were produced in 2022 (TUİK, 2024), and the highest production was in the Mediterranean region (Gün, 2019). Lettuce (*Lactuca sativa L.*), which is rich in iron, contains 94-95% water in its leaves and is widely preferred for daily nutrition. However, high salinity is one of the most significant problems in its production (Shi et al., 2022). Lettuce yields are reduced due to poor soil quality, adverse environmental conditions, and inadequate water quality for irrigation. Although it is possible to increase salt tolerance and yield by applying necessary nutrients, soil compaction due to over-fertilization is also an important cause of yield reduction (Sardar et al., 2023). To mitigate the negative effects of salt stress on yield and growth, it is recommended to irrigate lettuce frequently with good quality water (Yurtseven & Bozkurt, 1997). However, in areas where the use of saline water is unavoidable, it is important to understand the effects of salt stress. It should be noted that the response to salinity varies depending on plant varieties and production conditions, resulting in different salt-yield relationships for lettuce, as shown in previous studies. Qin et al. (2013) reported that lettuce is sensitive or moderately sensitive to salinity. De Pascale and Barbieri (1995) found that lettuce is moderately salt tolerant, but growth and yield decrease at soil salinity levels (EC_e) higher than 2-2.6 dS m^{-1}. Ünlükara et al. (2008) reported that yield decreased by 9.3% per 1 dS m^{-1} after EC_e of 1.1 dS m^{-1}. Cahn and Ajwa (2004) found that growth ended at EC_e greater than 2 dS m^{-1}, and Barassi et al. (2006) reported that plant growth, seed germination, and leaf moisture content decreased at EC_e greater than 2 dS m^{-1}. Other researchers have obtained similar results (Al-Maskri et al., 2010; Mekki & Orabi, 2007; Miceli et al., 2003).

Salt-tolerant plant breeding, soil reclamation, and soil leaching are methods commonly used to reduce the negative effects of soil salinity and increase plant productivity. Leaching applications are particularly effective in areas with drainage systems (Ödemiş, 2001). Leaching is necessary to prevent the accumulation of soluble salts in the root zone and to promote sustainable irrigated agriculture. Several studies have shown that leaching practices using saline water have a significant effect on increasing plant yield, dry matter content, fruit quality, and decreasing soil salinity (Erdem & Kale Çelik, 2018; Isayenkov, 2012; Xu & Mou, 2015).

Plant adaptation studies, carried out at physiological, molecular and biochemical levels to better understand the plant response to salinity, continue in both model plants and cultivated plants (Acosta-Motos et al., 2017; Gupta & Huang, 2014). Salt stress occurs physiologically in a manner similar to drought stress. Insufficient water uptake in the root zone causes stomata to close, leaf area to shrink, transpiration, photosynthesis, and chlorophyll concentration to decrease because of stress. Measuring these responses of plants (time and severity of stress) and taking measures in a short time increases the amount of product. Various tools have been developed to show the stress levels of plants to recognize early that the plant is stressed and to take precautions. These tools can be used to easily measure the physiological responses of plants under field conditions. The reduction in gas transfer by the closure of the plant's stomata can be measured using a porometer under water stress conditions, and the increased leaf temperature due to reduced transpiration as a result of stomatal closure can be measured with an infrared thermometer. The decrease in leaf chlorophyll content as a result of stress was measured using the SPAD. SPAD can also provide insights into the nitrogen content and health of plants. It is important to know which parameter is the first physiological response to stress, whether caused by salinity or drought, and its effect on yield. Thus, stress can be predicted early, and measures can be taken. Ödemiş and Çalışkan (2014) reported that among the parameters of photosynthesis, transpiration (T_r), and stomatal conductance (g_s) in potato plants, gs was the most affected by salinity and photosynthesis rate (P_n) was the least affected. Vos and Groenwold (1989) reported that stomatal conductance responded to water deficiency in soil earlier than photosynthesis.

This study investigated the effects of different irrigation water salinity levels and leaching fractions on yield, vegetative characteristics, soil salinity, Plant water consumption, and physiological parameters (stomatal conductance, chlorophyll content, leaf surface temperature, chlorophyll a, chlorophyll b, and total chlorophyll) in lettuce plants grown in potted media under greenhouse conditions. Regression models were used to determine the effect of leaching on salinity-induced stress, the physiological parameters that are most sensitive to stress, and their effects on yield and yield components.

MATERIALS and METHODS

Soil, climate, and crop characteristics

The research was carried out in an unheated plastic greenhouse in Hatay Province between February and April 2021 in medium-textured soil. Soil analyses showed pH 8.06, soil salinity (EC_e) 0.492 dS m^{-1}, $CaCO_3$ 2.05 (%), nitrogen 0.05 %, organic matter 1.04 (%), field capacity 24.5 (% P_w), wilting point 11.5 (% P_w). The region where the study area is located reflects the typical climatic characteristics of the Mediterranean region: hot and dry summers and mild and rainy winters. Temperature and relative humidity values inside the greenhouse were measured hourly using the climate sensor (HOBO, MX1101, Onset, BlvdBourne, USA) instrument at a height of 1.5 meters from the ground and were determined as 16.8°C - 17.6°C and 58.8% - 63.5%, respectively. The study was conducted using potted plants with a width of 30 cm and a height of 40 cm (Ödemiş & Çalışkan, 2014). Each pot was filled (23.9 kg) equally with after sieving the soils. Beneath the pots, bottom plates were placed to collect the drainage water. *Lactuca sativa L.* var. Longifolia Lam. cv. Cospirina was used as plant material. This variety is suitable for greenhouse and open field production, summer, and early autumn cultivation, and has strong leaves and a heavy core structure. It also has a high tolerance to leaf tip burns (Syngenta, 2020).

Experimental treatments

Lettuce was irrigated with tap water until it had 8-10 leaves. The experiment was conducted with irrigation waters of three different irrigation water salinity levels (EC_{i-0} = 0.5 dS m^{-1}(control), EC_{i-2} = 2 dS m^{-1}, EC_{i-4} = 4 dS m^{-1}) and four different leaching fractions ((LF_0=0%, LF_{10}=10%, LF_{20}=20%, LF_{30}=30%). It was conducted in a factorial trial design with 5 replications. Leaching fractions were obtained by adding 10%, 20% and 30% irrigation water to the irrigation water required for the field capacity of the control pot before each irrigation. The salinity levels of the irrigation water were adjusted using NaCl. The electrical conductivity of the applied irrigation water (EC_i) was controlled using a portable Consort C533 EC meter (Consort bvba, Yunhout, Belgium) before each irrigation.

Measurements

Soil salinity (EC_e, dS m^{-1}): Soil samples were taken on March 13, 19, and 30 from the pots exposed to the same treatments outside the experiment after the saline water treatments were started and the plants reached the growth and development stage. Saturated soil pastes were prepared from each soil sample, and soil water was extracted after 24 hours. The electrical conductivities of the extracted soil water (EC_e, dS m^{-1}) for each treatment were measured using a Consort C533 EC meter (Consort bvba, Yunhout, Belgium).

Yield and vegetative characteristics: At harvest, the aboveground parts of the plants were cut and first the head mass (yield g $plant^{-1}$) was weighed. Then, the commercial head mass (g $plant^{-1}$) weight was determined by discarding the poor-quality leaves in the head mass. Root length (cm), root width (cm), commercial head length (cm), commercial head diameter (cm), number of leaves (number), number of commercial leaves (pieces), leaf width (cm), and leaf length (cm) were measured to determine the effects of the treatments on the vegetative characteristics of lettuce.

Physiological measurements: stomatal conductivity (g_s) (Kim et al., 2004), leaf surface temperature (LSt), chlorophyll content (SPAD) Gianquinto et al. (2004) and chlorophyll concentrations (Chl-*a*, Chl-*b* and Chl-*tot*) were measured on two leaves between 11:00-14:00 h, one day before irrigation in each replicate after the leaves reached a measurable size during the experiment, and Chl-*a*, Chl-*b*, and Chl-*tot* concentration (mg g^{-1}) were measured only at harvest. The stomatal conductivity was measured using an SC-1 leaf porometer (Decagon Devices Inc., Pullman, WA, USA). The instrument was calibrated using standard calibration paper before each measurement. SPAD values related to chlorophyll content in the leaves were measured with a hand-held SPAD-502 (Konica–Minolta, Inc., Osaka, Japan). The leaf surface temperature was measured 2 times from four sides of each crop using an infrared thermometer (IR Temp Meter, Spectrum Tech. Inc., Aurora, USA).

Chlorophyll concentration was determined by the weight of the leaves taken from each treatment at harvest, they were homogenized by adding 1-2 ml of 80% acetone in a porcelain mortar and then filtered through coarse filter paper into 10 ml glass tubes and then added 10 ml of 80% acetone completed with acetone (Arnon, 1949). The obtained solutions were read on a spectrophotometer at wavelengths of 645, 663 and 652 nm, and chlorophyll a, chlorophyll b and total chlorophyll concentrations (mg g^{-1}) were determined (Lichtenthaler & Wellburn, 1983).

Plant water consumption (PWC): The pots were weighed and the required irrigation water for the field capacity was determined and applied to the plants before each irrigation. The pots were weighed again just before the next irrigation cycle (after a period of 7 ± 1 days), and the difference was evaluated as weekly PWC. Electronic weighing scale was used for pot weighing. This process was repeated throughout the experiment, and the seasonal PWC was determined (Eq. 1).

Irrigation scheduling and Plant water consumption (PWC): To determine the irrigation water to be applied in each irrigation, the pots were weighed just before irrigation and calculated using Equation 1 (Ayers & Westcot, 1985).

$$I = \frac{(W_{fc} - W)/\rho_w}{1 - LF} \qquad \textbf{Eq. (1)}$$

where I is the amount of applied irrigation water (L); W_{fc} and W are the weight of lysimeter at field capacity and just before irrigation (kg); ρ_w is bulk density of water (1 kg L^{-1}) and LF is the leaching fraction.

During the growing season, the pots were weighed every 5-6 days, and 40-50% of the available water was considered as the threshold for irrigation. In the growing season irrigation was performed every 7 ± 1 days. Irrigation water was applied using graduated containers, with an accuracy of 0.1 liters. Plant water consumption between two consecutive irrigations was determined by using Equation 2 (Ödemiş et al., 2019).

$$PWC = \frac{W_n - W_{n+1}}{\rho_w} + I - D_p \qquad \textbf{Eq. (2)}$$

where PWC is the plant water consumption between two consecutive irrigations (L); W_n and W_{n+1} are the weight of pot before n^{th} and n + 1^{th} irrigation application (kg); ρ_w is bulk density of water (1 kg L^{-1}); I is the amount of applied irrigation water (L) and D_p is the amount of drainage water accumulated in the pots 1 day after irrigation (L).
This process was repeated throughout the experiment. The actual PWC was calculated by subtracting the weight of the lettuce from the weight of the observation pots at the end of the experiment.

Statistical analyses

The relationships between soil salinity and g_s, LSt, SPAD, Chl-*a*, Chl-*b*, Chl-*tot* and yield parameters were determined using the linear regression model given in Equation 3.

$$y = ax + b \qquad \textbf{Eq. (3)}$$

where y is the soil salinity; x is the measured value of the selected variable; a and b are the slope and intercept of the fitted line, respectively.

The determination coefficients (r^2) for the linear relationships between each variable and soil salinity were calculated using Equation 4.

$$r^2 = \frac{\left[\sum_{i=1}^{n}\left(X_i - \overline{X_i}\right)\left(Y_i - \overline{Y_i}\right)\right]^2}{\sum_{i=1}^{n}\left(X_i - \overline{X_i}\right)^2 \sum_{i=1}^{n}\left(Y_i - \overline{Y_i}\right)^2} \qquad \textbf{Eq. (4)}$$

where: r^2 the determination coefficient; x_i and y_i are individual data points; and $\bar{X}_I$ and $\bar{Y}_i$ are the means of the variables. r^2 equal to 1 indicates the strongest possible linear relationship between the variables in the regression model. All statistical analyses were performed using OriginPro v2023b (OriginLab Corporation, Northampton, MA, USA).

RESULTS and DISCUSSIONS

Effect of climatic conditions in greenhouse on soil salinity

Greenhouse temperature ranged between 2.7°C and 43.7°C and relative humidity between 17.1% and 88.7% from planting to harvest. The temperature increased by 0.66°C and the relative humidity increased by 0.03% per day on average. The soil temperature increased because of the increase in greenhouse temperature and decrease in soil moisture. Differences of up to 5°C in the soil temperature were measured before and after irrigation. The effect of greenhouse temperature on soil temperature increased as soil salinity increased, and soil moisture decreased. The daily increase in soil temperature was 1°C at EC_{i-2} and 1.3°C at EC_{i-4}. Doubling irrigation water salinity (from 2 dS m^{-1} to 4 dS m^{-1}) increased soil temperature by 30%. This situation shows that soil warming will be affected by soil salinity as well as air temperature in the process of climate change.
Increased soil salinity can be exacerbated by human activities, such as increasing temperatures, rising sea water levels, seawater intrusion, erosion of minerals, over-fertilization, and over-irrigation (Shrivastava & Kumar, 2015). The saline drainage water used in areas where water resources are insufficient on a global scale can increase the severity of soil salinity and temperature. Soil temperature caused by irrigation water salinity during plant growth and development may cause yield losses in plants with shallow root structures. In our study, the soil temperature

decreased with an increase in leaching rates applied to reduce the effect of irrigation water salinity on salt accumulation in the soil. The soil temperature decreased by 1°C at 10% leaching fraction and by 2°C at 20% and 30% after irrigation. Considering that soil temperature has a high correlation with salinity, it is thought that the main factor in the decrease in temperature with increasing leaching fraction is due to a less saline and more saturated layer with leaching. Although leaching is considered as an option for preventing soil salinity, it is not possible to be a definite solution due to the unfavorable quality and quantity of water resources in the future.

Soil salinity (EC_e)

The soil salinity varied depending on the applied irrigation water salinity, leaching rate, soil depth, and time. Salt accumulation in the soil increased as the irrigation water salinity increased. Soil salinity increased from 0.569 dS m^{-1} to 1.159 dS m^{-1} at EC_{i-0} with no leaching ($EC_{i-0}xLF_0$) at the beginning of the trial. Leaching prevented reaching the equilibrium of soil salinity, and drainage water and soil salinity reached balance only after the 4th irrigation. Similarly, Ayers and Westcot (1985) stated that soil and drainage water salinity can reach equilibrium only after 4-6 irrigations and that differences in climate, soil conditions, and management practices change the equilibrium period.

Leaching has been the most important practice in the reduction of soil salinity. It was observed that the efficiency of leaching was higher, and it could leach more salt, particularly at high soil salinities. 30% leaching rate reduced soil salinity by 23% in EC_{i-2} (from 1.416 dS m^{-1} to 1.145 dS m^{-1}) and 40% in EC_{i-4} (from 2413 µmhos cm^{-1} to 1719 µmhos cm^{-1}) (Table 1). Saline water (up to 11 dS m^{-1}) has been used successfully in combination with commercial irrigation to irrigate a number of crops globally (Karlberg, 2005) and Israel has successfully used saline groundwater by 3.0 dS m^{-1} in 25-30% leaching rate (Miyamoto et al., 1984).

The difference in salinity between the upper and lower soil layers increased over time, as water transported upwards by capillary action evaporated. However, the time-dependent rate of salt accumulation in the upper soil layers increased at the beginning of the experiment due to capillarity and the rate of increase decreased as the harvest time approached. In the first sampling, EC_e in the 0-10 cm layer increased by 186% in EC_{i-0} and 287% in EC_{i-4} compared to the 30-40 cm. The rate of salt transport decreased towards harvest, despite the difference between the 0-10 cm and 30-40 cm layers was 2-3 fold during the irrigation period. These results are in agreement with those of previous studies. Studies have shown that the highest salt accumulation between two irrigations is observed in the upper layers and under the root zone under normal conditions (under homogeneous soil profile and same soil structure conditions), and that the soil salinity in these layers can be up to 2 to 3 folds higher than that in the other layers (Shalhevet, 1984).

Table 1. Effects of different levels of irrigation water salinity and leaching fractions on ECe, yield, PWC and some growth parameters

Çizelge 1. Farklı sulama suyu tuzluluk seviyeleri ve yıkama fraksiyonlarının ECe, verim, PWC ve bazı gelişim parametreleri üzerindeki etkileri

Treatments		EC_e ($dS\ m^{-1}$)	Yield ($g\ pot^{-1}$)	PWC ($L\ pot^{-1}$)	g_s ($mmol\ m^{-2}\ s^{-1}$)	LSt (°C)	SPAD	Chl a (*mg g⁻¹*)	Chl b (*mg g⁻¹*)	Chl tot (*mg g⁻¹*)
$EC_{i-0.5}$		0.83 b	276 a	8.92 a	266 a	21.27 c	39.60	0.381 a	0.118	0.547 a
EC_{i-2}		1.31 ab	248 ab	7.71 a	255 a	22.15 b	39.45	0.366 ab	0.116	0.532 b
EC_{i-4}		2.09 a	198 b	5.71 b	215 b	22.78 a	38.44	0.327 b	0.102	0.472 c
Sig. Level of EC_i		*	*	**	***	*	ns	*	ns	*
LF_0		1.58 a	222.67 b	6.44 b	231.4 b	23.08 a	37.62	0.353 ab	0.112	0.512 b
LF_{10}		1.44 ab	232.00 b	7.07 ab	257.2 b	22.37 b	38.47	0.353 ab	0.112	0.512 b
LF_{20}		1.38 ab	253.22 a	7.81 ab	275.4 a	21.84 b	40.04	0.398 a	0.120	0.571 a
LF_{30}		1.24 b	254.33 a	8.47 a	217.4 c	20.97 c	40.51	0.327 b	0.103	0.472 c
Sig. Level of LF		*	***	**	**	**	ns	*	ns	*
$EC_{i-0.5}$	LF_0	0.91	267	7.32	220	21.98	37.88	0.355	0.116	0.549
	LF_{10}	0.80	275	8.25	254	21.52	38.75	0.377	0.118	0.589
	LF_{20}	0.74	282	9.64	284	21.38	40.79	0.410	0.128	0.544
	LF_{30}	0.85	280	10.50	305	20.21	40.99	0.383	0.110	0.505
EC_{i-2}	LF_0	1.41	219	7.00	229	23.42	38.58	0.334	0.117	0.507
	LF_{10}	1.36	233	7.52	235	22.71	39.42	0.426	0.125	0.523
	LF_{20}	1.31	268	7.84	272	21.46	39.61	0.357	0.116	0.610
	LF_{30}	1.14	271	8.46	284	21.00	40.17	0.345	0.105	0.486
EC_{i-4}	LF_0	2.41	182	5.00	203	23.85	36.40	0.292	0.104	0.479
	LF_{10}	2.14	188	5.44	205	22.87	37.24	0.392	0.093	0.426
	LF_{20}	2.10	210	5.94	216	22.69	39.73	0.292	0.116	0.559
	LF_{30}	1.72	212	6.46	238	21.69	40.38	0.332	0.095	0.425
EC_i x LF		ns	ns	ns	ns	ns	ns	ns	ns	ns

Where ECe is the electrical conductivity of the extracted soil water, and ECi-0.5, ECi-2, and ECi-4 indicate the irrigation water salinity levels of 0.5 $dS\ m^{-1}$, 2 $dS\ m^{-1}$ and 4 $dS\ m^{-1}$, respectively. LF0, LF10, LF20, and LF30 represent the 0, 10%, 20%, and 30% leaching fraction rates, respectively. The means indicated with the same small letter or without any letter in the same column are not significantly different ($p < 0.05$). *, **, ***, and ns, significant at the $p < 0.05$, $p < 0.01$ level, $p < 0.001$ and not significant, respectively.

Plant water consumption (PWC)

PWC is a complex process involving the interaction of crops (cultivar and genetics), soil (soil moisture and nutrient content), and climatic conditions (vapor pressure deficit, air temperature, wind and sunshine period and intensity). Plants grown under cool conditions are more tolerant to salinity than those grown under hot conditions (Niu et al., 2019). In our study, PWC was measured at a low level because lettuce was grown during a cool period, even though it was grown under greenhouse conditions. In addition, PWC decreased as the salinity increased. Similar results have been reported in many studies and in other crops (Heidarpour et al., 2009; Jiang et al., 2012; Çebi et al., 2018; Yavuz et al., 2023). PWC varied between 7.32-10.5 L (mean 8.92 L) in EC_{i-0}, 7.0-8.46 L (mean 7.71 L) in EC_{i-2}, and 5.0-6.46 (mean 5.71 L) in EC_{i-4}. Compared with the control treatment, PWC decreased by 14% in EC_{i-2} and 36% in EC_{i-4}. The PWC increased as the leaching rates increased (Table 1). The average PWC was 5.0 L in LF_0, 5.44 L in LF_{10}, 5.94 L in LF_{20} and 6.46 L in LF_{30}. Compared to LF_0, the PWC increased proportionally by 8% in LF_{10}, 19% in LF_{20}, and 29% in LF_{30}. On average, PWC decreased by 2.5 L for a 1 $dS\ m^{-1}$ increase in EC_e (r^2=0.99**). A decrease in the leaching fraction significantly reduces PWC by increasing irrigation water salinity (Heidarpour et al., 2009). Since the amount of leaching caused different amounts of EC_e at each salinity treatment, the relationship between PWC due to leaching rates was different; PWC decreased by 5.08 L at EC_{i-2} and 2.12 L at EC_{i-4} for 1 dS m^{-1} EC_e increase. Ünlükara et al. (2008) reported that PWC decreased with the proportional increase in EC_e and PWC decreased by 0.24 g pot^{-1} per unit increase in EC_e and the lowest PWC was measured at the highest salinity.

The salt concentration of the layer close to the soil surface and the time the plant is exposed to salinity are some of the most important factors affecting plant yield. Although it is very important that the soil salt concentration exceeds the threshold value of the plant in terms of yield reduction, many studies have shown that the time the root zone is exposed to salinity is the most important factor (Maas et al., 1986; Maas & Poss, 1989). The lettuce yield ranged from 182 g $plant^{-1}$ ($EC_{i-4}xLF_{10}$) to 282 g $plant^{-1}$ ($EC_{i-0}xLF_{20}$) (Table 1), and the yield decreased with increasing soil salinity (EC_e) and increased with increasing leaching rate. After EC_e of 0.825 dS m^{-1} (threshold value), the yield decreased by 22% for every 1 dS m^{-1}. When EC_e increased from 0.825 dS m^{-1} (EC_{i-0}) to 1.31 dS m^{-1} (EC_{i-2}) and 2.09 dS m^{-1} (EC_{i-4}), yield decreased by 10% and 28%, respectively. Ünlükara et al. (2008) reported that lettuce yield decreased by 25.3%, 31.4%, 48.5%, 56.8% and 69.7% for EC_i 1.5, 2.5, 3.5, 5 and 7 dS m^{-1}, respectively. Heidarpour et al. (2009) reported that an increase in irrigation water salinity (EC_i) and a decrease in leaching fraction caused a significant decrease in yield, and increased EC_i increased Cl^- and Na^+ accumulation in the plant, but leaching application decreased this accumulation. Exogenous nitric oxide (NO) application partially reduced the effects of salt stress in lettuce, whereas salt stress (compared to the control) caused a significant decrease in growth, yield, carotenoids, and photosynthetic pigments and significantly affected oxidative compounds. In addition, salt stress decreased nitrogen (N), phosphorus (P), and potassium (K^+) ions and increased Na^+ ions in lettuce leaves (Sardar et al., 2023).

Stomatal conductivity

Stomata are responsible for gas exchange between the intercellular space of the leaf and the atmosphere and for the control of water vapor outflow (Kerepesi & Galiba, 2000). Owing to their immediate response to changes in soil moisture, stomatal conductivity decreases when water decreases or osmotic pressure increases in the root zone. In our experiment, irrigation water salinity ($p<0.01$) and leaching fractions ($p<0.05$) were effective on stomatal conductance. Increasing soil salinity decreased the water uptake of the roots and caused a decrease in stomatal conductance (Figure 1). The average EC_e and g_s values were 0.83 dS m^{-1} - 266 mmol m^{-2} s^{-1} at $EC_{i-0.5}$, 1.31 dS m^{-1} - 255 mmol m^{-2} s^{-1} at EC_{i-2} and 2.09 dS m^{-1} - 215 mmol m^{-2} s^{-1} at EC_{i-4}. The highest g_s was 305 mmol m^{-2} s^{-1} at $EC_{i-0.5}xLF_{30}$ (0.85 dS m^{-1}), and the lowest g_s was 203 mmol m^{-2} s^{-1} at $EC_{i-4}xLF_0$ (2.41 dS m^{-1}). g_s decreased as the soil salinity increased and the leaching fraction decreased (Figure 2).

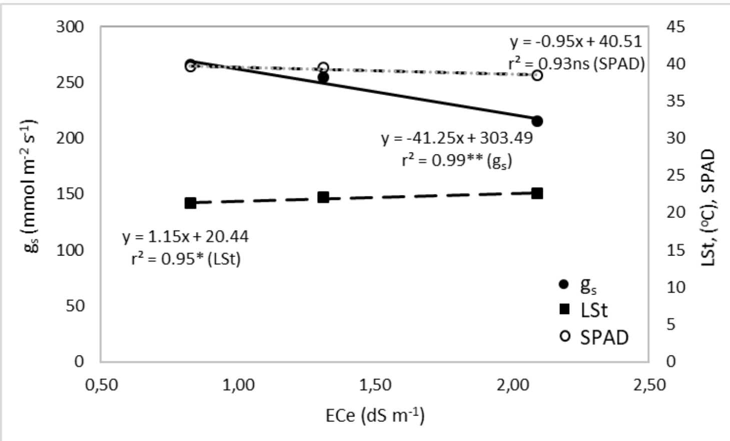

Figure 1. Relationship between seasonal average g_s, LSt and SPAD values and soil salinity at different irrigation water salinity levels

Şekil 1. Farklı sulama suyu tuzluluk seviyelerinde sezonluk ortalama g_s, LSt ve SPAD değerleri ile toprak tuzluluğu arasındaki ilişki

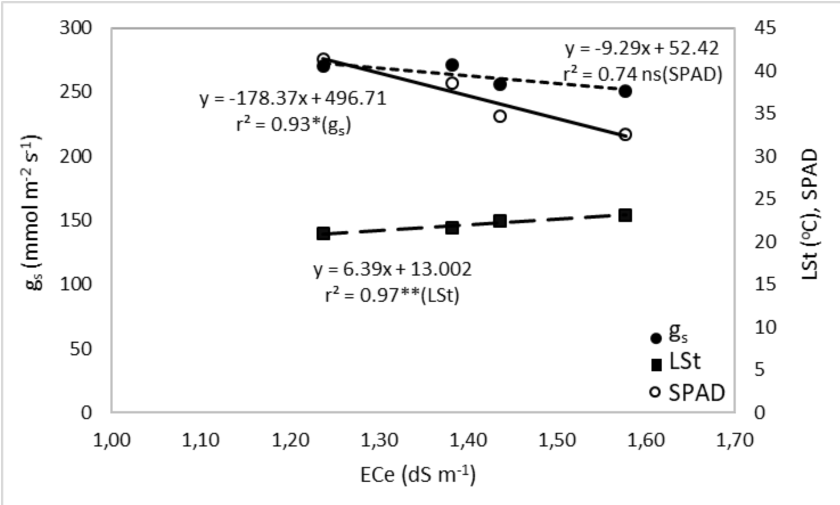

Figure 2. Relationship between seasonal average g_s, LSt and SPAD values and soil salinity in different leaching fractions

Şekil 2. Farklı yıkama fraksiyonlarında sezonluk ortalama g_s, LSt ve SPAD değerleri ile toprak tuzluluğu arasındaki ilişki

The most stable effect of leaching fraction on g_s was observed at EC_{i-4}. g_s was more stable at low soil salinity; the positive contribution of leaching fraction to soil salinity did not affect g_s in EC_{i-0} and EC_{i-2}. Yield increased linearly in all treatments as g_s increased. However, the yield was linear and insignificant (r^2=0.86ns) in EC_{i-0}, linear and significant (r^2 = 0.96*) in EC_{i-2}, and polynomial and significant (r^2=0.99**) in EC_{i-4} owing to the increase in g_s.
The stomatal conductivity increased and then decreased in all treatments on the measurement dates. While the average g_s was 257 mmol m^{-2} s^{-1} in the first measurements, it was 382 and 209 mmol m^{-2} s^{-1} at the beginning and end of the maximum development period, respectively, and 143 mmol m^{-2} s^{-1} at harvest. In addition to the normal senescence cycle of lettuce, salt stress accelerates plant senescence and weakens stomatal conductivity, and the stomata react later, even if irrigation is applied. The change in stomatal conductivity between the two irrigation cycles was similar to the change in stomatal conductivity from sowing to harvest. It was also observed that g_s did not increase immediately after irrigation but decreased after reaching a maximum value following a recovery phase of 1 or 2 days. These results are in agreement with those of other studies investigating the effects of salt stress in potatoes (Ödemiş & Çalışkan, 2014) and drought stress in cotton (Kazgöz Candemir & Ödemiş, 2021) on stomatal conductivity.

Leaf surface temperature (LSt)

After a healthy plant is irrigated, g_s increases and cooling occurs on the leaf surface. However, when the plant loses water and g_s begins to decrease, LSt approaches the air temperature (T_a). If the stress continues after this stage, LSt becomes slightly warmer than Ta. An increase in the difference between LSt and T_a indicated an increase in the stress level of the plant. Plants respond similarly to drought and salinity stress (Oosterhuis, 2001). In our experiment, irrigation water salinity ($p<0.05$) and leaching fractions ($p<0.01$) were effective on leaf surface temperature. The LSt increased as salinity increased (Figure 1). LSt increased by 2.07% at EC_{i-2} and 7.09% at EC_{i-4} compared with the control. The max. and min LSt was measured in $EC_{i-4}xLF_0$ (23.85°C) and $EC_{i-0}xLF3$ (20.21°C) interactions. As the leaching fractions increased in EC_{i-2} and EC_{i-4}, LSt also decreased as EC_e decreased (Figure 2). The regression coefficient (r^2) between EC_e and LSt varied according to salinity, and the highest relation was observed in EC_{i-4} (3.07°C increase in 1 dS m^{-1}). The difference between LSt-T_a was affected by EC_e as well as LF ($p<0.01$). The max. LSt-Ta difference was observed in LF_{30} (5.66 °C) and the minimum in LF_0 (4.00 °C). The increase in T_a caused a significant difference between LSt-T_a and the plants were exposed to more stress during these periods. Previous studies examining the change in LSt under water stress conditions showed that the LSt-T_a difference (leaf surface temperature-air temperature difference) increased as the irrigation time was getting closer and decreased after irrigation because of the increase in g_s (Ödemiş & Kazgöz Candemir, 2023). Hancı and Tuncer, (2020) reported that LSt made no difference at 0 and 100 mM salt concentrations but increased slightly at 200 mM.

SPAD

Chlorophyll molecules are the basic biological components of the photosynthetic process, and any reduction in leaf chlorophyll content can disrupt photosynthetic mechanisms and reduce yields (Shin et al., 2020). Chlorophyll is the primary pigment involved in the capture of light for photosynthesis and other photochemical and non-photochemical reactions; thus, the amount of light absorbed by a leaf is related to its chlorophyll content. Leaf chlorophyll content, besides its importance in light capture, can also be used as an indicator of the light environment during plant growth. Traditional methods for determining leaf chlorophyll content have been found to cause damage to the leaves and result in time-consuming processes to achieve accurate and clear results, prompting the development of a portable chlorophyll meter (SPAD-502) by the Minolta Corporation in the 1990s. The SPAD-502 was used as a tool to assess leaf nitrogen content in plants. Since there is a close relationship between leaf nitrogen and leaf chlorophyll content subsequent research has demonstrated that the SPAD device could be used to determine plant chlorophyll content under field conditions (Marenco et al., 2009). The increase in soil salinity did not cause a significant change in the SPAD (Figure 1). The mean SPAD value was measured at 39.60 at $EC_{i-0.5}$, 39.45 at EC_{i-2} and 38.44 at EC_{i-4}. Adhikari et al. (2019) reported that salinity stress decreased the chlorophyll index by 5-14%. SPAD values were not affected by EC_e measured at leaching fractions (Figure 2). SPAD was measured as 38.47 at 10% leaching, 40.04 at 20% leaching, and 40.51 at 30% leaching. Similar to stomatal conductance, SPAD showed a decreasing trend towards harvest. SPAD was 44.29 at the first measurement approximately 15 days after lettuce planting and decreased to 35.77 at harvest. The decreasing trend varied among treatments; there was a linear and significant decrease in EC_{i-2} and EC_{i-4}, whereas a polynomial and insignificant decrease was determined in $EC_{i-0.5}$. The lowest SPAD values at harvest were measured at 33.12 and 35.02 in EC_{i-2} and EC_{i-4} treatments, respectively. The relationship between SPAD and yield was influenced by the EC_e and leaching levels. A unit increase in the SPAD value resulted in a 47 g increase in yield ($y = 47.123x - 1612.7$ $r^2 = 0.99^{**}$). However, the effect of leaching fractions on SPAD-yield was significant only for EC_{i-4} ($y = 7.88x - 105.11$ $r^2 = 0.99^{**}$).

Chlorophyll concentration

Apart from the stomata, which regulate gas exchange during photosynthesis, chloroplasts containing chlorophyll significantly influence photosynthesis. Initially, although photosynthesis is reduced by stomatal factors, continued stress or increased severity leads to depression in chloroplast and enzyme activity, resulting in a decrease in chlorophyll concentration (Miller et al., 2010). In our study, as soil salinity increased, both Chl-*tot* and Chl-*a* concentrations decreased ($r^2=0.96^*$, $r^2=0.99^{**}$), whereas Chl-*b* remained unaffected (Figure 3). The highest Chl concentration was measured in EC_{i-0}. Although lower concentrations were measured in EC_{i-2} and EC_{i-4} than in EC_{i-0}, the concentrations in EC_{i-0} and EC_{i-2} were approximately the same. Chl-*b* was the lowest in EC_{i-0} (0.102 mg g^{-1}), whereas it was measured at approximately the same level in EC_{i-0} and EC_{i-2} (0.116 mg g^{-1}-0.118 mg g^{-1}). Chl-*a*, Chl-*b*, and Chl-*tot* chlorophyll concentrations were measured at the same level in the LF_0 and LF_{10} treatments (0.353 mg g^{-1} in Chl-*a*, 0.112 mg g^{-1} in Chl-*b*, and 0.512 mg g^{-1} in Chl-*tot*). The effects of the leaching fractions on the SPAD and chlorophyll concentrations were similar.

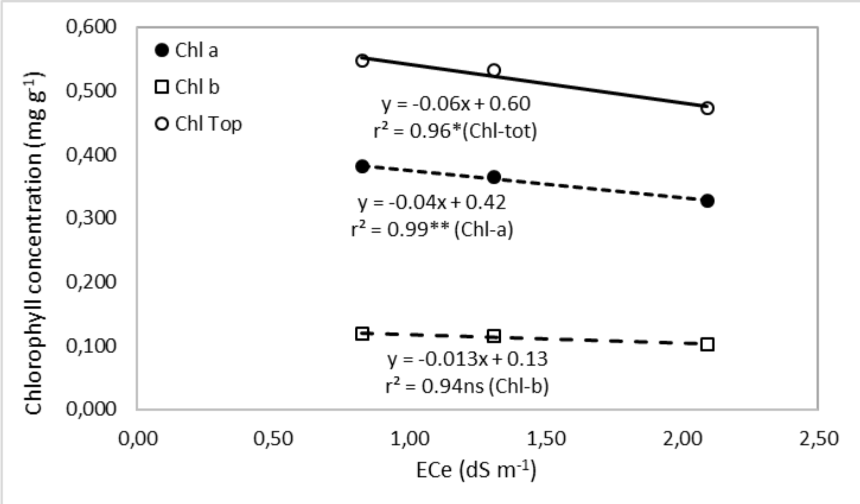

Figure 3. The relationship between chlorophyll concentrations and soil salinity at different irrigation water salinity levels

Şekil 3. Farklı sulama suyu tuzluluk seviyelerinde klorofil konsantrasyonları ile toprak tuzluluğu arasındaki ilişki

Our findings indicate that the salinity levels we studied caused a significant decrease in chlorophyll a and total chlorophyll contents in lettuce leaves. The level of soil salinity that causes stress affects chlorophyll concentration by leading to higher salt accumulation in the leaves and oxidizing chlorophyll and chloroplasts, resulting in decreased concentrations of pigment proteins (Aftab et al., 2011). These results are consistent with previous research showing a significant decrease in chlorophyll concentration with increasing salt stress (Shin et al., 2020; Babaousmail et al., 2022; Sardar et al., 2023). Linear and significant relationships were found between chlorophyll concentrations and SPAD values ($p<0.01$). However, Ödemiş et al. (2019) did not find a consistent relationship between chlorophyll concentration and SPAD in cotton plants exposed to drought stress. There were insignificant relationships between Chl-*a*, Chl-*b* and Chl-*tot* and SPAD in the first year and significant relationships only with Chl-*a* in the second year (r^2=0.65). The differences between studies are thought to stem from differences in soil, plant, and climate conditions, as well as the severity of stress and cultural practices.

Effect of soil salinity and leaching rates on vegetative characteristics

The root length, root width, leaf number, number of commercial leaves, and leaf length decreased as salinity increased ($p<0.01$). Commercial fruit length, diameter, and biomass were not affected by the increase in salinity. Root length 1.16 cm, root width 0.35 cm, leaf number 2.90, number of commercial leaves 1.75, leaf width 0.92 cm and leaf length 0.88 cm decreased with one unit increase in EC_e.

The vegetative parameters affected by the leaching rates and the level of the effect varied depending on the increase in EC_e. Leaching fractions affected only leaf number in $EC_{i-0.5}$, root length, root width, commercial fruit diameter, number of leaves, number of commercial leaves, and biomass in EC_{i-2}, and root width, commercial fruit diameter, number of leaves, number of commercial leaves, leaf width, leaf length, and biomass in EC_{i-4}. When both the EC_e and leaching rates were considered, it was observed that increases in both variables did not form a consistent model for vegetative characteristics. Al-Maskri et al. (2010) stated that some vegetative traits decreased with salinity at 0, 50 mM, and 100 mM, while others were not affected by salinity increase, showing inconsistent changes. The findings of Bar-Yosef et al. (2005) indicated that lettuce vegetative characteristics are not equally affected by salinity at the same level. For example, while leaf number decreased with salinity in Ünlükara et al. (2008) and our study, it was not affected by salinity in Andriolo et al. (2005). Additionally, although biomass was not affected by the salinity increase in our study, it decreased in Al-Maskri et al. (2010).

Table 2. Significance levels of regression analysis of physiological parameters and yield parameters under irrigation water salinity level and leaching fractions (y=ax+b, r^2, n=4)

Çizelge 2. Sulama suyu tuzluluk seviyesi ve yıkama fraksiyonları altında fizyolojik parametreler ve verim parametrelerinin regresyon analizinin anlamlılık düzeyleri (y=ax+b, r^2, n=4)

Treat.	Physiological parameters	Yield	Root Length	Root Width	Com. Fruit Length	Com. Fruit Diam.	Leaf Num.	Num. Comm Leaves	Leaf Width	Leaf Length	Biomass
EC$_i$	g_s	**	**	**	ns	ns	ns	**	**	**	ns
	LSt	**	**	**	ns	ns	ns	**	**	**	**
	SPAD	**	**	**	ns	ns	ns	**	**	**	**
	Chl-*tot*	*	**	ns	ns	ns	ns	**	*	**	ns
	Chl-*a*	**	**	*	ns	ns	ns	**	**	**	ns
	Chl-*b*	*	*	ns	ns	ns	ns	**	*	*	ns
LF	g_s	**	**	**	**	ns	ns	ns	ns	ns	ns
	LSt	**	**	**	**	ns	**	ns	ns	ns	ns
	SPAD	**	**	**	ns	ns	**	ns	ns	ns	ns
	Chl-*tot*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Chl-*a*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Chl-*b*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns

where EC$_i$ is the irrigation water salinity level; LF is the leaching fractions; **, * and ns indicate the significance level of p<0.01, p<0.05 and non -significant, respectively.

Effect of physiological parameters on yield and vegetative characteristics

The average soil salinity had approximately the same level of impact on g_s, LSt, and SPAD values, affecting both yield and vegetative characteristics of lettuce (Table 2). Only the relationship between g_s and Biomass was not significant. The effects of Chl-*tot*, Chl-*a*, and Chl-*b* on the yield and vegetative characteristics were mostly insignificant. This is because the chlorophyll concentrations in $EC_{i-0.5}$ and EC_{i-2} were measured close to each other.

The physiological parameters measured at the leaching fractions affected fewer vegetative traits. g_s, LSt, and SPAD values were effective on yield, root length, root width, and leaf number, whereas only g_s and LSt were effective on commercial fruit lengths, respectively (Table 2). The chlorophyll concentration values, depending on leaching fraction rates, did not significantly affect any of the yield or vegetative traits.

In this study, the responses of Chl-a, Chl-b, Chl-tot, g_s and LSt to soil salinity were significant (r^2=0.99** and r^2=0.95*). However, salinity changes caused by leaching prevented a clear response from g_s and LSt. The fact that both g_s and LSt responded to stress in a short time and showed a high regression relationship with yield shows that they have a high potential for use in predicting crop yield. SPAD measurements, which are recommended because chlorophyll analyses are damaging to the plant and time consuming, were found to have a high relationship with yield but a low relationship with salinity. This suggests that the relationship between yield and salinity is more closely related to the duration of exposure to salinity than to instantaneous soil salinity. In our experiment, with an irrigation interval of approximately one week, g_s and LSt responded shortly after being relieved from stress, while the same response was not observed in SPAD measurements. Therefore, it was concluded that SPAD would respond significantly only under prolonged conditions of salinity or water stress. g_s, LSt and SPAD values decreased towards harvest. The particularly high decreasing trend in EC_{i-4} indicates that lettuce enters a rapid aging process under stress. Soil salinity significantly decreased lettuce yield and the development of a significant part of the vegetative parts, whereas leaching application had a positive effect on yield and vegetative characteristics. Although the increase in leaching rate decreased soil temperature, doubling the irrigation water EC_i (from 2 dS m^{-1} to 4 dS m^{-1}) increased soil temperature by an average of 30%. An increase in soil temperature with increasing salinity will increase the importance of soil management planning and irrigation scheduling during climate change. Despite the positive effects of leaching on soil salinity and plant water consumption have been observed, continuous leaching practices have not been considered as a sustainable option in water-limited areas. Instead, it may be recommended to use saline water in rotation (freshwater-drainage water-freshwater) or saline water during growth periods when salinity tolerance is high. In our study, the salt threshold value of lettuce was determined to be 0.825 dS m^{-1}, and the yield decreased by 22% at 1 dS m^{-1}. This value provided by continuous irrigation reflects an average response. If we had used rotational irrigation or different developmental stages of lettuce, the response to salinity would probably have been different. In addition, towards the end of the growth period, the plant's salt tolerance would increase, and the possibility of using saline water would increase. For this reason, in salt-yield relationships, studies that reveal the response of developmental periods to salinity should be emphasized instead of seasonal salt-yield relationships.

ACKNOWLEDGEMENTS

We would like to thank Hatay Mustafa Kemal University for the financial support of the project numbered 22YL022.

STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR’S CONTRIBUTIONS

The authors declare that they have contributed equally to the article.

STATEMENT OF ETHICS CONSENT

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

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