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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Proline-mediated responses of lettuce to alternating fresh and saline water irrigation: impacts on soil salinity, evapotranspiration and yield

Marulun dönüşümlü tatlı ve tuzlu su sulamasına prolin uygulamalarının tepkileri: toprak tuzluluğu, evapotranspirasyon ve verim üzerindeki etkileri

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ABSTRACT

This study investigated the effects of alternating applications of freshwater and saline irrigation waters combined with exogenous proline treatments on soil salinity dynamics, evapotranspiration (ET), water use efficiency (WUE), and yield performance of lettuce (*Lactuca sativa* L.). The experiment was conducted under greenhouse conditions in Hatay, Türkiye. Lettuce plants were initially irrigated with freshwater until the 8-10 leaf stage and subsequently irrigated with saline water at salinity levels of 2, 4, and 6 dS m^{-1} until harvest, while foliar proline was applied at doses of 0, 5, and 10 mM. The results indicated that increasing irrigation water salinity progressively limited soil salinity accumulation (ECe) and plant water consumption, with more pronounced effects under higher salinity levels. Proline applications, particularly at the 5 mM dose, reduced soil salt accumulation and contributed to the maintenance of ET under high salinity conditions. The optimal seasonal ET range for lettuce was determined to be approximately 9.1-9.5 L, whereas water consumption below or above this range negatively affected commercial head weight and marketability. Low to moderate salinity treatments (EC2-EC4) did not completely suppress yield and quality, whereas at 6 dS m^{-1}, losses in commercial quality became dominant. Overall, the findings suggest that appropriate irrigation management combined with careful selection of proline dose can effectively mitigate the adverse effects of salinity stress in lettuce cultivation.

ÖZET

Bu çalışma, tatlı ve tuzlu sulama sularının dönüşümlü uygulanmasının ve eksojen prolin uygulamalarının marulda (*Lactuca sativa* L.) toprak tuzluluğu dinamikleri, evapotranspirasyon (ET), su kullanım etkinliği (WUE) ve verim performansı üzerine etkilerini incelemiştir. Deneme, Hatay koşullarında sera ortamında yürütülmüş; marul bitkileri 8-10 yapraklı döneme kadar tatlı suyla, ardından hasada kadar 2, 4 ve 6 dS m^{-1} tuzluluk düzeylerindeki sulama suları ile sulanmış ve 0, 5 ve 10 mM prolin dozları yapraktan uygulanmıştır. Bulgular, artan sulama suyu tuzluluğunun toprak tuzluluğunu (ECe) ve bitki su tüketimini sınırladığını, bu etkinin özellikle yüksek tuzluluk düzeylerinde belirginleştiğini göstermiştir. Prolin uygulamaları, özellikle 5 mM dozunda, toprakta tuz birikimini sınırlandırmış ve yüksek tuzluluk koşullarında ET'nin korunmasına katkı sağlamıştır. Marulda optimum ET aralığının yaklaşık 9.1-9.5 L olduğu, bu aralığın dışındaki su tüketimlerinin ticari baş ağırlığı ve pazarlanabilirliği olumsuz etkilediği belirlenmiştir. Düşük ve orta düzey tuzluluk uygulamaları (EC2-EC4) verim ve kaliteyi tamamen baskılamazken, 6 dS m^{-1} düzeyinde ticari kalite kayıpları ön plana çıkmıştır. Sonuçlar, marul yetiştiriciliğinde uygun sulama yönetimi ve dikkatli prolin dozu seçiminin, tuzluluk stresinin olumsuz etkilerini azaltmada etkili olabileceğini göstermektedir.

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INTRODUCTION

Soil salinity is one of the most critical abiotic stresses constraining agricultural production, particularly in arid and semi-arid regions where irrigation is indispensable. The intensive use of low-quality or saline irrigation water accelerates salt accumulation in the soil profile and leads to significant yield reductions (Qadir et al., 2007). The progressive depletion of freshwater resources -especially in the Mediterranean Basin and the Middle East, including Türkiye- has forced farmers to increasingly rely on saline or moderately saline water for irrigation in recent years (FAO, 2023). Consequently, the development of sustainable irrigation strategies that minimize salt accumulation in the root zone has become a major priority. Among the proposed approaches, the alternate application of freshwater and saline water -often referred to as cyclic or rotational irrigation strategies- has attracted considerable attention. These strategies may mitigate rapid salt buildup in soils while maintaining crop productivity. However, their effectiveness strongly depends on crop salt tolerance, growth stage, and the salinity level of the irrigation water. In addition to irrigation management, the application of osmoprotective compounds such as proline has been widely suggested as a means to alleviate the adverse effects of salinity stress. Proline is a multifunctional amino acid that accumulates in plant tissues under stress conditions, stabilizes cellular structures, scavenges reactive oxygen species, and enhances overall stress tolerance (Ashraf & Foolad, 2007). Exogenous proline application has been shown to improve growth, plant water relations, and photosynthetic efficiency in salt-stressed plants (Misra & Gupta, 2005; Szabados & Savouré, 2010), mainly through its role in osmotic adjustment, chlorophyll preservation, and activation of antioxidant defense systems (El Moukhtari et al., 2020). Furthermore, proline contributes to osmotic balance (Szabados & Savouré, 2010), protein and membrane stabilization (Verbruggen & Hermans, 2008), antioxidant protection (Hoque et al., 2007; Sharma & Dietz, 2006), and serves as an energy and nitrogen reserve, thereby reinforcing plant stress resilience (Misra & Gupta, 2005). Recent studies have demonstrated that foliar application of proline reduces growth losses in a wide range of crops exposed to salinity stress (e.g., rice, wheat, tomato, and lettuce), regulates ionic homeostasis-particularly the Na^+/K^+ balance-and enhances photosynthetic efficiency (Khedr et al., 2003; Heuer, 2010).

Lettuce (*Lactuca sativa* L.), a widely cultivated leafy vegetable, is considered salt-sensitive, with yield reductions occurring even under moderate salinity levels (Shannon & Grieve, 1999). The threshold soil salinity (ECe) for lettuce is generally reported to range between 1.3 and 2.0 dS m^{-1}, beyond which yield losses become evident. Therefore, lettuce represents an appropriate model crop for evaluating the combined effects of saline irrigation and stress mitigation strategies (Adhikari et al., 2021). Although studies on proline application in lettuce are limited, research on various crops -including rice, wheat, tomato, and lettuce- indicates that foliar-applied proline can reduce growth inhibition, regulate ion homeostasis (particularly Na^+/K^+ balance), and enhance photosynthetic performance under salinity stress (Khedr et al., 2003; Heuer, 2010). Nevertheless, plant responses to proline are often heterogeneous and depend on both proline dosage and salinity intensity, necessitating crop-specific evaluations.

Despite growing interest, information remains limited regarding the combined effects of alternate freshwater–saline irrigation and exogenous proline application on soil salinity dynamics, evapotranspiration, and yield performance in lettuce. Therefore, the present study aimed to assess the effects of alternating freshwater with saline water at different salinity levels (2, 4, and 6 dS m^{-1}), in combination with varying foliar proline doses (0, 5, and 10 mM), on evapotranspiration, water use efficiency, soil salinity dynamics, and yield-related traits of lettuce. The findings are expected to contribute to the development of integrated management strategies for improving lettuce production under saline conditions.

MATERIALS and METHODS

Soil, climate, and crop characteristics

The experiment was conducted between 8 February and 20 April 2022 in a plastic greenhouse belonging to the Department of Biosystems Engineering, Faculty of Agriculture, Hatay Mustafa Kemal University, Türkiye. The greenhouse is located at an altitude of approximately 134 m above sea level (36°19'34.57" N, 36°11'39.29" E). Clay-textured soil collected from the Amik Plain was used as the growth medium. Initial soil analyses indicated a pH of 8.11, electrical conductivity of the saturation extract (ECe) of 0.498 dS m^{-1}, total nitrogen content of 0.11%, organic matter content of 2.25%, field capacity of 34.25% (w/w), and permanent wilting point of 24.84% (w/w). As the soil was suitable for vegetable cultivation, no sand or organic amendments were added. The soil was sieved to ensure homogeneity and filled into plastic pots at 11 kg per pot. Pots (25 cm diameter, 25 cm height, 12.5 L volume) with perforated bottoms were arranged in the greenhouse according to the experimental design.

The Amik Plain exhibits a typical Mediterranean climate, which enhances its agricultural potential. During the experimental period (February-April), greenhouse air temperature and relative humidity were recorded hourly using a HOBO data logger positioned approximately 150 cm above plant canopy level. Mean air temperatures during February, March, and April were 19.2, 13.8, and 21.9 °C, respectively, while corresponding relative humidity values were 60.6%, 56.2%, and 49.4%. The lettuce cultivar 'Davidole' (*Lactuca sativa* L.) was used as plant material. This cultivar is characterized by curly, wavy green leaves with a crisp texture and is suitable for both greenhouse and open-field production, with good tolerance to winter conditions.

Experimental treatments

Lettuce plants were irrigated with two types of irrigation water during the growth period. Initially, all plants were irrigated with tap water until reaching the 8-10 leaf stage, after which saline irrigation treatments were applied until harvest. Salinity levels were prepared using NaCl to obtain irrigation waters with electrical conductivities of 2, 4, and 6 dS m^{-1}. Stock saline solutions were thoroughly mixed before each irrigation to prevent salt precipitation and ensure homogeneity.

The experiment was arranged in a factorial design consisting of four irrigation water salinity levels and three proline doses, with three replications and two pots per replication, resulting in a total of 72 pots. A leaching fraction (LF) of 20% was applied to all treatments except the control. The control treatment received irrigation water with an ECw of 548 µmhos cm^{-1} (C_2S_1 class). Saline treatments received irrigation water with EC levels of 2 (EC2), 4 (EC4), and 6 dS m^{-1} (EC6). Foliar proline applications (0, 5, and 10 mM) were initiated after the onset of saline irrigation and applied multiple times during the experimental period.

Irrigation scheduling

Prior to each irrigation event, pots were weighed to determine the required irrigation water amount, which was calculated using Eq. (1) (Ayers & Westcot, 1985):

$$I = \frac{\mathrm{Wfc}-\mathrm{W})/\rho\mathrm{w}}{1-\mathrm{LF}} \qquad \text{Eq. (1)}$$

where I is the applied irrigation water (L), Wfc and W are pot weights at field capacity and immediately before irrigation (kg), respectively, ρw is the density of water (1 kg L^{-1}), and LF is the leaching fraction. Irrigations were performed approximately every seven days during both freshwater and saline irrigation periods.

Evapotranspiration

Crop evapotranspiration (ET) between two consecutive irrigations was calculated using Eq. (2):

$$ET = \frac{\mathrm{Wn}-\mathrm{Wn+1}}{\rho \mathrm{w}} + I - Dp \quad \text{Eq. (2)}$$

where ET is plant water consumption (L), Wn and Wn+1 are pot weights before the n th and (n+1) th irrigations (kg), I is the applied irrigation water (L), and Dp is the drainage water collected one day after irrigation (L)

Water use efficiency (WUE)

Water use efficiency was calculated based on yield and evapotranspiration values using Eq. (3) (Howell et al., 1984):

$$WUE = \frac{\mathrm{Y}}{\mathrm{ET}} \quad \text{Eq. (3)}$$

where WUE is water use efficiency (g L^{-1}), Y is yield (g), and ET is evapotranspiration (L).

Soil salinity

To assess temporal changes in soil salinity (ECe, dS m^{-1}), disturbed soil samples were collected at depths of 0-15 and 15-25 cm on the 20th day after the initiation of saline irrigation and at harvest (45th day). Soil samples were analyzed for total salinity using a 1:2.5 soil-water extract (Ayers & Westcot, 1985). Electrical conductivity and pH were measured using an EC meter (Consort C533).

Yield and yield components

Plants were harvested with roots intact. Aboveground biomass was separated and weighed to determine head fresh weight (g $plant^{-1}$). Commercial head weight was determined after removing non-marketable leaves. In addition, dry biomass (g), root length (cm), root width (cm), commercial head height (cm), commercial head diameter (cm), total leaf number, commercial leaf number, leaf width (cm), and leaf length (cm) were measured to evaluate morphological responses.

Statistical analysis

Yield and yield-related parameters were subjected to analysis of variance (ANOVA) using SPSS software (version 18.0). Mean comparisons were performed using Duncan's multiple range test (Bek & Efe, 1988). Relationships between lettuce yield, irrigation water salinity, and proline levels were evaluated using regression analysis in Microsoft Excel.

RESULTS and DISCUSSIONS

Soil salinity dynamics

During the experiment, lettuce plants received a total of 14 irrigations. The first seven irrigations were applied using freshwater with an electrical conductivity (ECw) of 548 µmhos cm^{-1} while the remaining seven irrigations consisted of saline waters with ECw levels of 2, 4, and 6 dS m^{-1}. Until the initiation of saline irrigation on 4 March, soil salinity remained relatively stable at approximately 0.7 dS m^{-1}. Following the onset of saline irrigation, soil salinity (ECe) increased progressively. At the first sampling date (29 March 2022), ECe values ranged from 0.767 to 3.189 dS m^{-1}, whereas at harvest (26 April 2022) they increased markedly, ranging from 1.193 to 10.757 dS m^{-1} (Figure 1).

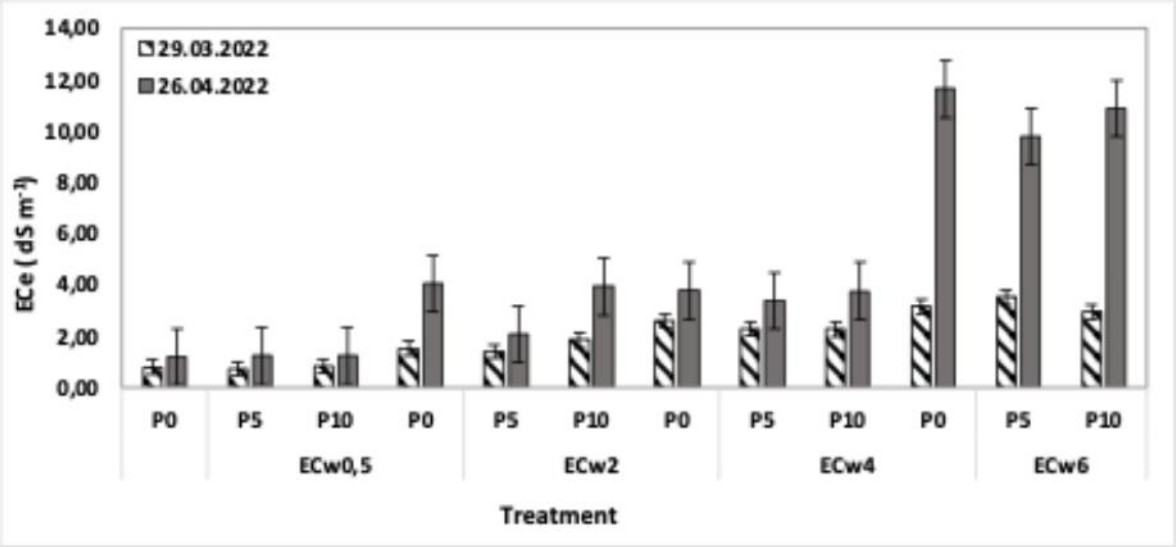

Figure 1. Temporal variation of soil salinity as affected by proline doses
Şekil 1. Toprak tuzluluğunun zamana ve prolin dozlarına bağlı değişimi

In the control treatment (ECw = 548 µmhos cm^{-1}), ECe at harvest was 1.193 dS m^{-1}, while the highest soil salinity was recorded in the ECw6 treatment (mean 10.757 dS m^{-1}) (Table 1). The progressive increase in soil salinity was particularly pronounced under EC4 and EC6 treatments. As irrigation water salinity increased, the effectiveness of the applied leaching fraction in limiting soil salinity decreased, resulting in accelerated salt accumulation in the root zone and increased ionic concentrations. Similar trends have been reported by Grattan and Grieve (1999) and Rengasamy (2010).

While irrigation water salinity significantly increased soil ECe, proline applications tended to reduce soil salinity levels. In particular, the 5 mM proline treatment resulted in lower ECe values compared to the corresponding control treatments. This effect is likely associated with the role of proline in regulating osmotic balance at the soil-plant interface. As an osmoprotectant, proline helps maintain cellular water potential, reduce ion toxicity, and regulate ion uptake, thereby contributing to a more balanced soil-plant system (Ashraf & Foolad, 2007; Szabados & Savouré, 2010). This mechanism may explain the lower ECe values observed under moderate salinity conditions (ECw2 and ECw4) combined with 5 mM proline application.

Table 1. Effects of treatments on evapotranspiration (ET), water use efficiency (WUE), and soil salinity (ECe)
Çizelge 1. Evapotranspiration (ET), su kullanım etkinliği (WUE) ve toprak tuzluluğunun (ECe) konulara bağlı değişimi

Teatment	ECe 29 March	ECe Harvest	Mean	ET (L)	WUE (g/L)
ECw0P0	0.783	1.168	0.975	9.98	7.03
ECw0P5	0.716	1.206	0.961	10.05	5.02
ECw0P10	0.804	1.207	1.005	9.81	6.78
ECw2P0	1.512	4.043	2.777	9.40	9.07
ECw2P5	1.378	2.071	1.725	9.55	9.55
ECw2P10	1.829	3.914	2.872	9.50	8.51
ECw4P0	2.579	3.759	3.169	9.40	8.19
ECw4P5	2.265	3.367	2.816	9.23	8.47
ECw4P10	2.248	3.753	3.000	8.79	10.40

Table 1 (continue). Effects of treatments on evapotranspiration (ET), water use efficiency (WUE), and soil salinity (ECe)

Çizelge 1 (devamı). Evapotranspiration (ET), su kullanım etkinliği (WUE) ve toprak tuzluluğunun (ECe) konulara bağlı değişimi

ECw6P0	3.135	11.628	7.381	8.17	9.52
ECw6P5	3.509	9.790	6.649	8.85	8.05
ECw6P10	2.923	10.854	6.889	8.71	10.91
ECw0	0.767	1.193	0.980	9.94	6.27
ECw2	1.573	3.343	2.458	9.48	9.04
ECw4	2.364	3.626	2.995	9.14	9.00
ECw6	3.189	10.757	6.973	8.58	9.49
P0	2.002	5.149	3.576	9.24	8.40
P5	1.967	4.108	3.038	9.42	7.72
P10	1.951	4.932	3.441	9.20	9.07

Under the applied leaching fraction of approximately 15-20%, the relationship between irrigation water salinity (ECw) and root-zone soil salinity (ECe) was evaluated in relation to the commonly used approximation ECe ≈ 1.5 x ECw (Ayers & Westcot, 1985; Corwin & Rhoades, 1982). At the first sampling date, the calculated coefficients ranged between 0.49 and 0.91, indicating that soil salinity had not yet reached equilibrium. At harvest, the coefficient increased to approximately 1.46 (ECe ≈ 1.46 x ECw), closely approaching the theoretical value. Ayers and Westcot (1985) suggested that the 1.5 coefficient is a practical approximation under long-term equilibrium conditions and conventional irrigation management with LF ≈ 0.15-0.20. However, Letey et al. (2011) emphasized that this coefficient may overestimate leaching requirements under heterogeneous soil, plant, and management conditions. Overall, soil salinity in the lettuce-growing soil increased over time in a linear relationship with irrigation water salinity, while appropriately dosed proline applications contributed to limiting salt accumulation. These findings indicate that proline may exert a mitigating effect on soil salinity dynamics under saline irrigation.

Evapotranspiration and soil water dynamics

Increasing irrigation water salinity generally resulted in reduced evapotranspiration (ET), increased irrigation water requirements, and fluctuating deep percolation (Dp) amounts. During the initial growth phase, when all treatments received freshwater irrigation, ET values were similar across treatments, ranging between 2.42 and 2.70 L. Following the application of saline irrigation, ET declined in response to increasing soil salinity, particularly under ECw6 conditions. Seasonal ET values were recorded as 9.94, 9.48, 9.14, and 8.58 L for EC0, EC2, EC4, and EC6 treatments, respectively.

Differences in ET among proline treatments were relatively small; however, certain proline doses mitigated the adverse effects of salinity on plant water consumption. ET increased from 9.20 L in the control (P0) to 9.42 L under 5 mM proline application. In the salinity x proline interaction, the highest ET (10.05 L) was observed in EC0P5, while the lowest ET (8.17 L) occurred in EC6P0. These results indicate that increasing salinity restricts plant water uptake, likely due to enhanced osmotic stress reducing root water potential and limiting water absorption (Munns & Tester, 2008). Proline application, particularly under EC6 conditions, increased ET compared to the control, which may be attributed to its role in osmotic adjustment and membrane stabilization. Similar findings were reported by Ashraf & Foolad (2007), who noted that proline improves water use efficiency and maintains transpiration and photosynthetic activity under saline stress.

Irrigation water amounts showed a slight increase with rising salinity levels, ranging from 7.74 L in EC0 to 9.09–9.14 L in ECw4 and ECw6 treatments. This increase can be explained by the reduction in soil solution osmotic potential under saline conditions, which decreases plant-available water and necessitates higher irrigation volumes (Shannon et al., 1994). Proline application did not significantly alter irrigation water requirements, suggesting that its primary effects occurred at the plant physiological level rather than through changes in soil water balance.

Deep percolation values ranged from 0.15 to 0.94 mm, with the highest value recorded in ECw0P5 and the lowest in ECw2P5. Higher Dp values were generally observed under low salinity conditions, whereas increased salinity reduced deep percolation. This pattern suggests that salinity alters soil water retention and infiltration characteristics, leading to greater water retention in the upper soil layers and reduced percolation under high salinity conditions (Chartzoulakis, 2005).

Overall, increasing salinity constrained ET in lettuce, while proline application partially alleviated this effect, particularly under high salinity. These results are consistent with studies highlighting the role of proline in maintaining plant water balance and improving water use efficiency under salt stress (Szabados & Savouré, 2010; Hayat et al., 2012).

Evapotranspiration–yield and water use efficiency

Even relatively small variations in ET (8.58-9.94 L) resulted in noticeable differences in lettuce yield and quality parameters (Table 1 and 3). Yield increased with increasing ET up to a certain threshold, beyond which further increases in ET led to yield reductions. Commercial head weight was more sensitive to ET variation than total head weight, with optimal performance observed at ET levels of approximately 9.1-9.5 L. Higher ET levels negatively affected head compactness and marketability, indicating that ET influences yield primarily through quality rather than quantity.

Table 2. Analysis of variance results for yield and yield-related parameters as affected by treatments

Çizelge 2. Verim ve verim parametrelerinin uygulamalara bağlı varyans analiz sonuçları

Source	df	Head weight			Comm. head weight			Biomass			Root lenght		
		SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
ECe	3	3003.97	1001.32	38.39**	3163.31	1054.43	64.17**	17.84	5.94	12.36**	69.889	23.29	5.29*
P	2	685.80	342.90	13.14**	488.01	244.00	14.85**	5.56	2.78	5.78**	4.264	2.13	0.48ns
ECe* P	6	1420.37	236.72	9.07**	734.07	122.34	7.44**	20.35	3.39	7.05**	36.236	6.03	1.37ns
Err	22	573.71	26.08		361.45	16.43		10.585	0.48		96,736	4,39	
		Root width			**Comm. head height**			**Comm head diameter**			**Number of leaves**		
Source	**df**	**SS**	**MS**	**F**	**SS**	**MS**	**F**	**SS**	**MS**	**F**	**SS**	**MS**	**F**
ECe	3	71.66	23.88	16.45**	0,38	0,13	0,24ns	29,18	9,72	5,40*	8,75	2,91	0.46ns
P	2	12.38	6.19	4.26*	1.51	0.75	1.41ns	1.43	0.71	0.39ns	26.00	13.00	2.05ns
ECe* P	6	38.50	6,41	4.41**	0.65	0.10	0.20ns	30.62	5.10	2.83*	47.33	7.88	1.24ns
Err	22	31.94	1.45		11.73	0.53		39.56	1.799		139.16	6.32	
		Comm. number of leaves			**Leaf width (cm)**			**Leaf lenght (cm)**					
Source	**df**	**SS**	**MS**	**F**	**SS**	**MS**	**F**	**SS**	**MS**	**F**			
ECe	3	104.30	34.76	7.32**	1.57	0.52	2.04ns	1.81	0.606	2.63ns			
P	2	4.66	2.33	0.49ns	0.51	0.25	0.99ns	2.30	1.154	5.01*			
ECe* P	6	29.11	4.85	1.02ns	9.42	1.57	6.13**	1.27	0.212	0.92ns			
Err	22	104.50	4.75		5.63	0.25		5.06	0.230				

ECe: Electrical conductivity of soil paste in harvest (dSm^{-1}), P: Proline, SS: Sum of Squares, MS: Mean Square, * and ** is significant in $p<0.05$ and $p<0.01$ level, ns is not significant

Total biomass exhibited lower sensitivity to ET variations and fluctuated within a narrow range. However, partial reductions in biomass were observed under low ET conditions. Root development reached a balanced level as ET increased, whereas low ET limited root width and functionality. Under high ET conditions, despite improved water availability, root growth did not show a distinct advantage, suggesting that optimal root response occurs only under balanced water regimes.

Leaf morphological traits responded differently to ET levels. Leaf number remained relatively stable, while leaf width and length showed slight sensitivity to ET. Morphological uniformity was highest at moderate ET levels, whereas excessive ET caused irregularities in leaf quality. The reduction in commercial leaf number at high ET levels suggests that excessive water may induce leaf deformation and reduce marketable quality.

A strong negative relationship was observed between ET and water use efficiency (WUE). WUE decreased significantly as ET increased, with the highest WUE values obtained under low to moderate ET conditions (approximately 8.7-9.3 L). However, despite higher WUE at very low ET levels, commercial yield declined, indicating that WUE alone is not a sufficient indicator of optimal production. Sustainable lettuce production under saline conditions therefore requires a combined evaluation of yield and WUE.

Table 3. Changes in yield and yield components as affected by different treatments

Çizelge 3. Verim ve verim parametrelerinin uygulamalara bağlı değişimi

Treatment	Head weight (g)	Comm. head weight (g)	Biomass (g)	Root lenght (cm)	Root width (cm)	Comm. head height (cm)	Comm head diameter (cm)	Number of leaves	Comm. number of leaves	Leaf width (cm)	Leaf lenght (cm)
ECw0P0	72.14ef	70.10de	16.10bc	9.67	13.00 bc	11.33	15.67bc	28.00	19.33	9.50e	9.50
ECw0P5	66.17fg	50.39g	16.53bc	11.33	13.33b	11.00	17.33ab	26.67	21.00	9.90cd	9.50
ECw0P10	84.59bc	66.54fg	16.76ab	12.67	10.33de	11.33	16.33bc	30.33	20.00	10.10bc	10.21
ECw2P0	85.29bc	76.65bc	16.22bc	15.67	13.33b	11.50	18.67a	28.67	23.33	11.60a	9.40
ECw2P5	91.14ab	82.30ab	15.38cd	15.33	12.67bc	11.33	16.00bc	30.00	24.33	9.83cd	9.50
ECw2P10	80.77cd	75.24bc	**18.13a**	13.00	13.00bc	11.67	15.33cd	30.33	25.33	9.70de	9.30
ECw4P0	77.01de	71.69cd	15.55cd	15.33	11.00cd	11.67	17.00ab	30.00	24.67	9.77de	9.67
ECw4P5	78.18de	70.48de	17.14ab	14.17	15.00a	11.00	17.00ab	29.00	21.67	10.16bc	9.87
ECw4P10	91.41ab	83.43ab	16.68ab	14.17	11.00cd	11.67	18.50a	28.00	22.67	10.83ab	10.50
ECw6P0	77.79de	59.19fg	16.12bc	12.00	9.00e	11.17	14.67cd	27.67	23.67	9.67de	9.50
ECw6P5	71.27ef	46.30g	13.62e	14.67	9.00e	11.17	16.00bc	26.67	23.33	10.00cd	9.67
ECw6P10	94.95a	57.83fg	14.89de	12.67	10.00de	11.83	14.33d	31.67	25.67	10.43bc	10.40
					Mean						
ECw0	74.30 b	62.34b	16.46a	11.22b	12.22a	11.22	16.44a	28.33	20.11b	9.83	9.73
ECw2	85.73a	78.06a	16.58a	14.66a	13.00a	11.50	16.66a	29.66	24.33a	10.37	9.40
ECw4	82.20a	75.20a	16.45a	14.55a	12.33a	11.38	17.50a	29.00	23.00a	10.25	10.01
ECw6	81.34a	54.44c	14.88b	13.11ab	9.33b	11.44	15.00b	28.66	24.22a	10.03	9.85
					Mean						
P0	77.54de	69.92b	16.00b	13.16	11.58ab	11.41	16.50	28.58	22.75	10.13	9.51b
P5	72.74c	66.31c	15.66b	13.87	12.50a	11.12	16.58	28.08	22.58	9.97	9.63b
P10	83.42a	75.27a	16.61a	13.12	11.08b	11.62	16.12	30.08	23.41	10.26	10.10a

Soil salinity–yield

Because the first half of the irrigation period involved freshwater irrigation followed by saline irrigation, yields under higher salinity treatments were higher than expected. This response may be attributed to early root system development under low salinity, which enhanced osmotic adjustment capacity and allowed plants to better tolerate subsequent salt stress. Additionally, foliar proline application may have contributed to stress priming rather than abrupt salt stress. The application of a leaching fraction (LF = 0.20) at each irrigation and relatively frequent irrigation intervals further mitigated the negative effects of salinity. The highest head weight and commercial head weight were observed under EC2 and EC4 treatments, while the lowest values occurred under EC6. Low to moderate salinity levels did not completely suppress lettuce growth and, in some cases, slightly stimulated development. However, high salinity (EC6) caused a substantial reduction in commercial head weight, indicating that salinity primarily affects marketable yield rather than total biomass. Under EC6, total head weight declined by approximately 5%, whereas commercial head weight decreased by more than 30%, highlighting the pronounced impact of salinity on quality attributes such as head compactness and uniformity.

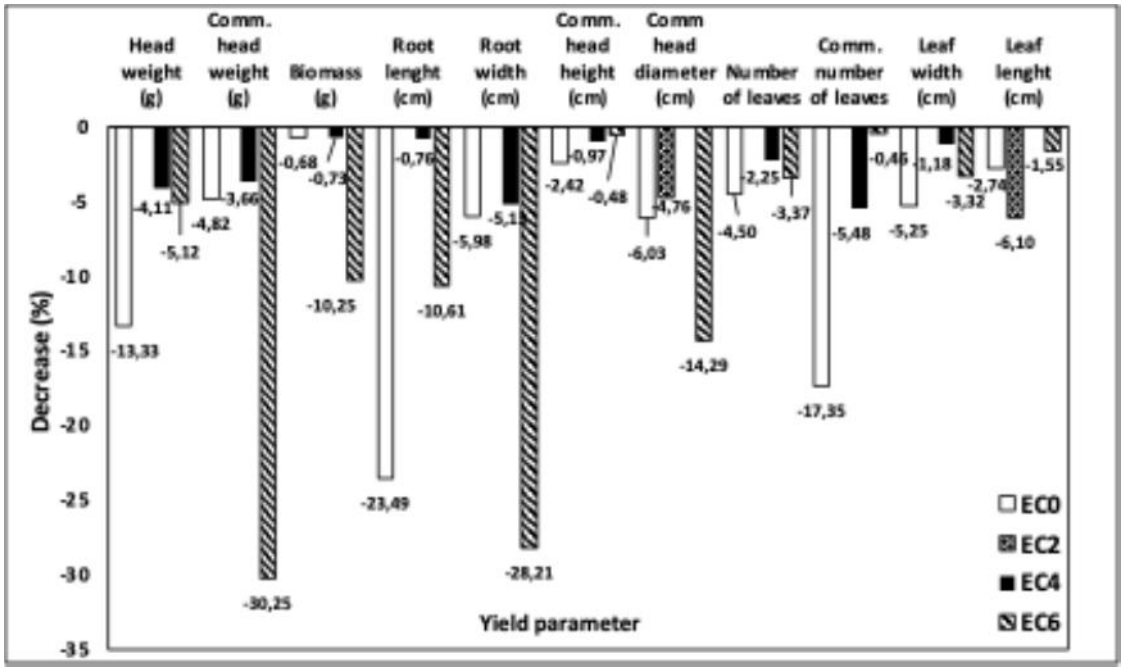

Figure 2. Salinity-induced reduction ratios in yield and yield-related parameters (Reduction ratios were calculated relative to EC4 for commercial head diameter and leaf length, and relative to EC2 for the remaining parameters, where the maximum values were observed.)

Şekil 2. Verim ve verim parametrelerinde tuzluluğa bağlı azalma oranları (Ticari baş çapı ve Yaprak uzunluğu en yüksek EC4 de diğer parametrelerde en yüksek ECw2' de olduğundan azalma oranları ilgili parametrelerde anılan tuzluluk konularına göre hesaplanmıştır)

These findings are consistent with previous studies reporting moderate salt tolerance in lettuce up to soil salinity levels of 3-4 dS m^{-1} (Shannon & Grieve, 1999), while higher salinity levels significantly restrict root and shoot growth (Ünlükara et al., 2008). Kurunc (2021) similarly reported that lettuce yield and water consumption increase up to a threshold soil salinity level, beyond which yield declines. Collectively, the results indicate that irrigation water salinity is a critical factor in lettuce production, with ECw levels of 2-4 dS m^{-1} being partially tolerable, whereas 6 dS m^{-1} severely limits commercial yield.

Proline–yield

Proline application exerted positive effects on yield-related parameters of lettuce. Both head weight and commercial head weight were responsive to proline dose (Figure 3). Compared with the control treatment (P0), both parameters increased under the P10 dose, with head weight rising from 77.55 g (P0) to 83.42 g (P10) and commercial head weight increasing from 69.92 g (P0) to 75.28 g (P10).

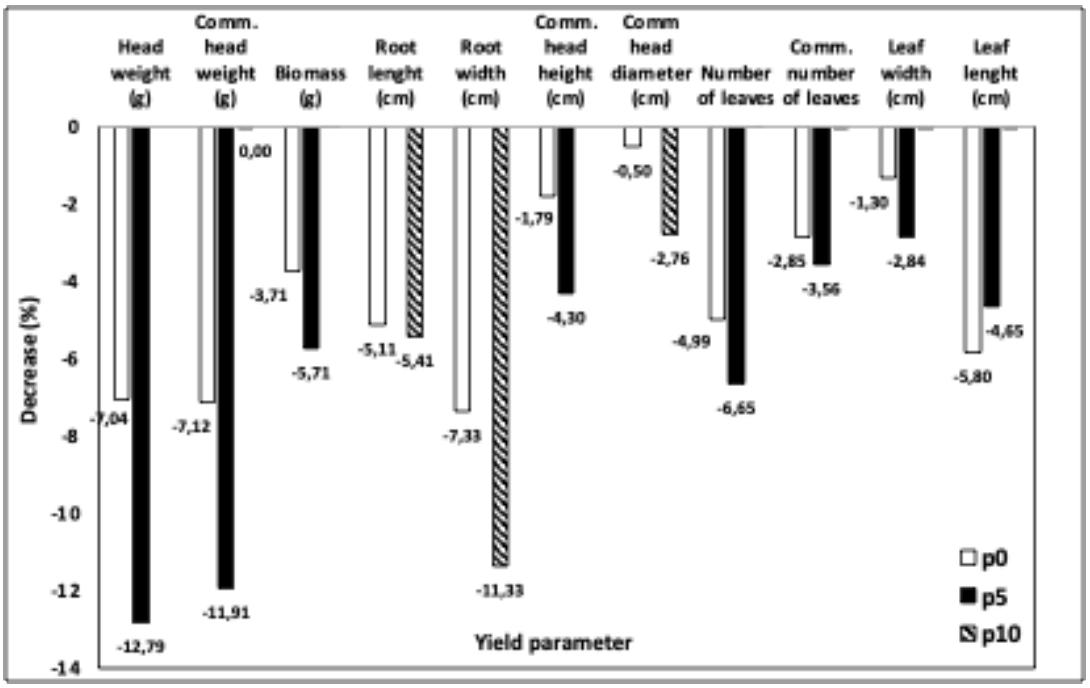

Figure 3. Proline dose-dependent reduction ratios in yield and yield-related parameters (Reduction ratios were calculated relative to P5 for root length, root width, and commercial head diameter, and relative to P10 for the remaining parameters, where the maximum values were observed.)

Şekil 3. Verim ve verim parametrelerinde prolin dozlarına bağlı azalma oranları (Kök uzunluğu, Kök genişliği ve Ticari baş çapı en yüksek P5 konusunda diğer parametrelerde en yüksek P10 dozunda ölçüldüğünden ilgili parametrelerde anılan prolin dozlarına göre hesaplanmıştır)

Leaf number was approximately 28 leaves per plant under P0 and P5 treatments, whereas it increased to 30.08 leaves under P10. Similarly, commercial leaf number, leaf width, and leaf length reached their highest values at the P10 dose. In contrast, dry biomass was highest at P10 (16.61 g) and lowest at P5 (15.66 g). Root length exhibited a different trend, with the highest value recorded at P5 (13.87 cm) and the lowest at P10 (13.12 cm).

Overall, proline did not induce a linear, dose-dependent improvement in growth parameters. Instead, the transition from P0 to P5 resulted in a pronounced decline in most traits, except for leaf length, whereas at the P10 dose, partial recovery of several shoot-related parameters was observed, accompanied by a marked suppression of root morphology (Table 3). These findings suggest that exogenous proline imposes a metabolic burden at low to moderate doses, while at higher doses it confers partial tolerance primarily in the shoot system. For instance, parameters critical for commercial quality, such as head weight, biomass, and leaf width, were suppressed at P5 but rebalanced at P10. Conversely, root architecture was substantially affected at the P10 dose, with a pronounced reduction in root width indicating that exogenous proline preferentially benefits aboveground growth rather than root development. Although increasing proline doses helped maintain shoot yield, the concomitant weakening of the root system may restrict long-term water and nutrient uptake, indicating that proline is not protective at all doses.

These results demonstrate that a 5 mM proline dose did not produce a clear improvement in yield parameters and, in some cases, resulted in values lower than those of the control, whereas the 10 mM dose positively affected lettuce yield components and may act as a potential protective agent under high salinity conditions. The beneficial effects of 10 mM proline are likely associated with its osmoregulatory role, which reduces water loss and stabilizes metabolic processes. Proline accumulation and osmotic adjustment mechanisms allow plants to sustain growth under moderate stress conditions; however, these mechanisms become insufficient under excessive salinity stress (Munns & Tester, 2008). Proline has been reported to maintain cell membrane stability, reduce ionic toxicity, and enhance photosynthetic efficiency under stress conditions (Ashraf & Foolad, 2007), acting both as an osmoprotectant and an antioxidant, thereby improving plant growth parameters (Szabados & Savouré, 2010). Consistent with these reports, the findings of the present study indicate that proline, particularly at the 10 mM dose, positively influences lettuce yield parameters and may serve as an effective mitigation strategy under high salinity stress.

CONCLUSIONS

Applying 14 irrigations during the lettuce growth period, with the first seven irrigations using low-salinity water and the remaining seven using saline irrigation waters of varying salinity levels (ECw 2-6 dS m^{-1}), combined with a leaching fraction of 20% (LF = 0.20) at each irrigation, reduced the adverse effects of salinity on yield and yield components. Following the initiation of saline irrigation, soil salinity (ECe) increased progressively over time, with a more pronounced accumulation observed under EC4 and EC6 treatments. At harvest, the relationship between irrigation water salinity and soil salinity approximated ECe ≈ 1.46 x ECw, which is consistent with salinity relationships reported for soils exposed to prolonged saline irrigation.

Proline application, particularly at the 5 mM dose, exhibited a limiting effect on soil salt accumulation, suggesting that proline contributes to maintaining osmotic balance within the soil–plant system through its osmoprotective role. This effect was more evident under moderate salinity conditions (ECw2 and ECw4). Increasing salinity constrained evapotranspiration (ET); however, proline applications partially alleviated this negative effect. Under high ECe conditions, 5 mM proline maintained higher plant water consumption compared with the untreated control, likely due to its stabilizing effects on cell membranes and osmotic regulation.

Under the experimental conditions, the optimal seasonal ET range for lettuce was approximately 9.1-9.5 L. Both lower and higher ET levels negatively affected commercial head weight and marketability, indicating that ET is more closely associated with commercial quality than with total yield. A strong negative relationship was observed between ET and water use efficiency (WUE), with the highest WUE values obtained under low to moderate ET levels; however, these conditions did not always correspond to maximum commercial yield.

Low to moderate salinity levels (ECw2–ECw4) did not fully suppress lettuce growth and, in some cases, resulted in higher yield and quality parameters than the control. In contrast, at EC6, although total head weight declined only slightly, commercial head weight decreased by more than 30%, indicating that high salinity primarily impairs marketable quality rather than total biomass.

Exogenous proline application did not show a linear dose-dependent response. While 5 mM proline resulted in yield parameters comparable to or lower than the control, 10 mM proline improved head weight, commercial head weight, and leaf morphology. However, the marked suppression of root morphology at the 10 mM dose suggests that proline may enhance shoot tolerance at the expense of root development, highlighting the critical importance of dose selection.

The results indicate that irrigation waters with salinity levels up to 2–4 dS m^{-1} can be used in lettuce cultivation when appropriate irrigation management and sufficient leaching (LF ≈ 0.20) are applied. In contrast, continuous use of irrigation water with salinity levels of 6 dS m^{-1} or higher should be avoided due to substantial losses in

commercial quality. Proline application should not be considered a single-dose protective strategy; instead, the appropriate dose should be selected based on salinity level and production objectives. Under short-term and high-salinity conditions, 10 mM proline may be an effective strategy for maintaining aboveground yield and commercial quality, provided that its potential inhibitory effects on root development are carefully considered.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

AUTHOR'S CONTRIBUTIONS

All author contributed equally for analyses, writing and interpretation of the article. The authors read and approved the final version of the manuscript.

STATEMENT OF ETHICS CONSENT

This article does not require ethical approval as there are no experiments with human or animal subjects.

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