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Agro-morphological and enzymatic responses of onion to salinity stress under deficit irrigation conditions

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Abstract

In this study, the responses of onion to drought and salinity stress were investigated. In the research, a total of 15 treatments were designed including water stress levels (I_1: non-stress, I_2: %25 water stress, I_3: %50 water stress) and different NaCl concentrations (S_1: 0.3 dS/m-control, S2: 2.5 dS/m, S_3: 5.0 dS/m, S_4: 7.5 dS/m, S_5: 10.0 dS/m). During the experiment, 9.25, 7.66 and 6.07 L/pot irrigation water was applied to I_1, I_2 and I_3 treatments, respectively. As a result of the study, both drought and salinity stress showed destructive effects on the agro-morphological parameters of onion. Especially salinity levels above 7.5 dS/m triggered significant decreases in yield and yield components in onion. Onion yield decreased by 17.3% in S_2, 30.3% in S_3, 37.5% in S_4 and 56.2% in S_5 compared to the control group (S_1) according to salinity levels. When the effects of water stress and salinity stress were evaluated separately, the highest membrane damage (58.4%) was determined in severe water stress treatment (I_3), and the highest H_2O_2 content was determined in severe salinity stress treatment S_5. On the other hand, antioxidant enzymes such as catalase (CAT), peroxidase (POD) and superoxide dismutase undertook more significant tasks under salinity stress compared to water stress and these enzymes reached maximum values especially under severe salinity stress. Increasing salinity stress decreased the water productivity (WP) of onion, whereas increasing water stress provided significant increases in WP values. According to the obtained data; the use of water with electrical conductivity above 2.5 dS/m in irrigation shows that there will be significant yield decreases in onion. On the other hand, it was clearly understood from the results of this study that 25% to 50% water saving can be achieved by deficit irrigation in onion cultivation in water stressed environments.

Introduction

Water is an indispensable natural resource for agricultural production, and on a global scale, population growth and drought are increasing the demand for clean water resources year by year. Drought is generally defined as a decrease in precipitation and creates serious pressure on water-dependent sectors such as agriculture (Türkeş, 2014; Erişmiş, 2023). Especially in arid and semi-arid regions, increasing temperatures and irregular precipitation with climate change are increasing the frequency and severity of drought events, and all of these threaten food security (Partigöç and Soganci, 2019; Özüpekçe, 2021). Drought adverse effects the development and production of plants in approximately 45% of the world's agricultural areas (Asraf and Foolad, 2007; Yavuz et al., 2021).

On the other hand, salinity as much as drought, is an important environmental stress factor that negatively affects the physiological functions and general development of crops. In particular, the use of saline irrigation water reduces the water uptake of plants from the soil, which has negative effects on photosynthesis and general plant health (Kal et al., 2023; Borromeo et al., 2023; Machado and Serralheiro, 2017). Salt stress can prevent water uptake by creating high osmotic pressure in the root system of plants, which can lead to damage to cellular components (Kahraman, 2024; Yetişsin and Karakaya, 2022). In arid and semi-arid areas, both irrigation water and irrigated areas should be regularly checked in terms of salinity (Smedema and Shiati, 2022; Fernández-Cirelli et al., 2009). For this purpose, it is necessary to know the quality of water resources and determine the salt tolerance threshold values of plants irrigated with these waters (Castellanos et al., 2016). Investigating the salt resistance of plants is important in order to select and grow plants that can produce economically, especially in areas where soil salinity cannot be reduced below a certain level (Kotuby et al., 1997; Zhang and Shi, 2013). In this context, studies are carried out to determine the effects of salinity stress on plants and their morpho-physiological and biochemical response criteria are evaluated (Zhang et al., 2014; Mukherjee et al., 2014; Kostopoulou et al., 2015; Wang et al., 2016; Arora and Bhatla, 2017; Chen et al., 2017; Li et al., 2017; Yavuz et al., 2022).

Salinity and drought stress trigger the defense mechanisms of plants and significantly affect antioxidant enzyme activities. Under stress conditions, the activities of enzymes such as SOD, CAT and POD increase (Jebara et al., 2005; Gondim et al., 2012) and increase the defense of plants against oxidative damage by detoxifying reactive oxygen species (ROS) formed under stress conditions (Corpas et al., 2015; Yasar et al., 2020, Sachdev et al., 2021). These enzymes, whose activities change depending on the severity of stress (Ashraf and Harris 2004), catalyze the splitting of high concentrations of H_2O_2 into O_2 and H_2O, thus stabilizing the physiological functions of the plant. Each enzyme manages different steps of this process; However, CAT is unique in that it does not require any cellular reducing equivalent (Scandalios et al. 1997). Although the experienced stress is first perceived through the roots, its reflections on the leaves play a more important role in understanding the negative effects of stress on plant health (Kirecci, 2018; Yakar and Tuna, 2023). Evaluation of biochemical expressions such as chlorophyll values, carotenoids content and membrane damage are criteria that will increase success in managing abiotic stresses such as drought and salt stress (Al-Sammarraie et al., 2020; Giordano et al., 2021).

The Konya Plain, located in Central Anatolia of Türkiye and having a semi-arid climate, has limited water resources and therefore the increase in yield and quality in plant production depends on irrigation (Yavuz et al., 2022). Onion (*Allium cepa* L.), which has been widely cultivated throughout the world for thousands of years and is an important agricultural product, has a production of 124 631 tons in 6 775 hectares of land in Türkiye (FAO, 2022). Like many crops, onion is an agricultural product that requires irrigation throughout the vegetation period, especially in arid areas. Adequate irrigation is critical for the growth and productivity of onion (Geries et al., 2021). Onion is sensitive to drought because it is a shallow-rooted plant (Ghodke et al., 2020), and Chaudhry et al. (2024) evaluated the effects of different salinity levels in a period without irrigation and reported that photosynthesis decreased significantly, which was reflected in the yield loss. Different researchers also stated that onion is sensitive to drought and salinity stresses (Sönmez et al., 2005; Chaudhry et al, 2024), however, studies examining the responses of onion to conditions where these abiotic stresses are applied together are limited (Ghodke et al., 2020; Khandagale et al., 2024). In this study, the use of low-quality saline waters in onion cultivation in Konya, where drought and salinity problems are experienced, was investigated. In this context, the responses of onion to salinity stress under deficit irrigation conditions were determined agro-morphologically, physiologically and biochemically.

Materials and Methods

Study location and greenhouse climate

The research was carried out in pots in a glass greenhouse at Selcuk University Faculty of Agriculture in April and May 2022 (Latitude: 38°02 N, Longitude: 32° 30′ E; Altitude: 1105m). During the research, climate characteristics inside the greenhouse were measured and recorded regularly by means of a meteorological station (Davis Vantage Pro2). During the 43-day vegetation period, the average temperature of the greenhouse varied between 14.3 and 27.5 °C (aver. 20.5 °C) while relative humidity values ranged from 16.4% and 64.9% (average 38.1%) (Figure 1).

Soil properties and trial subjects used in the research

In the experiment, clay-loam textured soil with an organic matter content of 2.72%, pH 7.52, electrical conductivity (EC): 0.91 dS/m and lime content 10.7% was used. The field capacity and permanent wilting point moisture content of the soil were determined as 30.1% and 13.6%, respectively, on a weight percentage

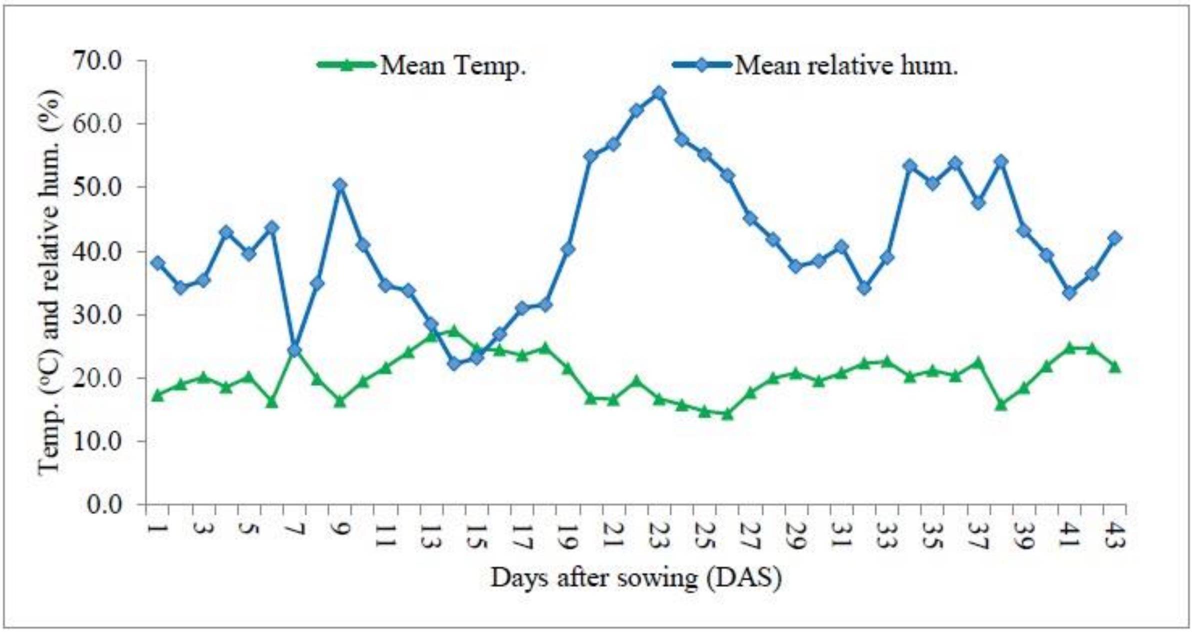


Figure 1. Temperatures and relative humidity values inside the greenhouse during the trial

basis. The soil used in the experiment does not pose a problem for onions in terms of physical and chemical properties. In the research, 12 kg of soil was weighed and placed in each of the plastic pots with a volume of approximately 12 L.

The quality of the mains water used as control is T_2S_1. Irrigation waters with different salt concentrations were created by adding NaCl salt to the tap water (EC: 0.3 dS/m). In the experiment, 15 experimental subjects including 5 different irrigation water salinities (0.3 (control), 2.5, 5.0, 7.5 and 10.0 dS/m) and 3 water levels (I_1: non-stress, I_2: 25% stress, and I_3: 50% stress) were carried out in randomized plots the factorial experimental design with 3 replications. The experimental subjects are shown in Figure 2.

Planting, harvesting and irrigation

In the study, 10 onion seeds were planted in each pot. After sowing, water with different NaCl concentrations was applied to each pot as 1400 mL. Approximately 2 days after seed planting, 1500 mL of irrigation water was applied to all pots to increase soil moisture to field capacity. Then, programmed irrigations were started and moisture losses of control pots (no water deficit and salt stress applied, S_1-I_1) were monitored daily by weighing method. In the experiment, the same amount of irrigation water as the control subject was applied to salinity subjects S_2-I_1, S_3-I_1, S_4-I_1 and S_5-I_1. After programmed irrigations started, 75% and 50% of the water applied to I_1 was given to subjects I_2 and I_3, respectively. In the study, when approximately half of the total usable water of the S_1-I_1 subject decreased, irrigation water with different NaCl values prepared according to the trial subjects was measured and applied to all pots. The onions reached maturity 43 days after seed planting and were harvested on May 24, 2022.

Determination of actual evapotranspiration (ETa)

In the study, ETa for each trial subject was calculated using Equation 1.

$$ET = I - D_P \pm \Delta S \qquad \text{(Eq.1)}$$

Eta = Actual evapotranspiration (L),
I = Volume of irrigation water (L),
D_p = Losses through drainage (L),
ΔS = Soil moisture content change (L) dir.

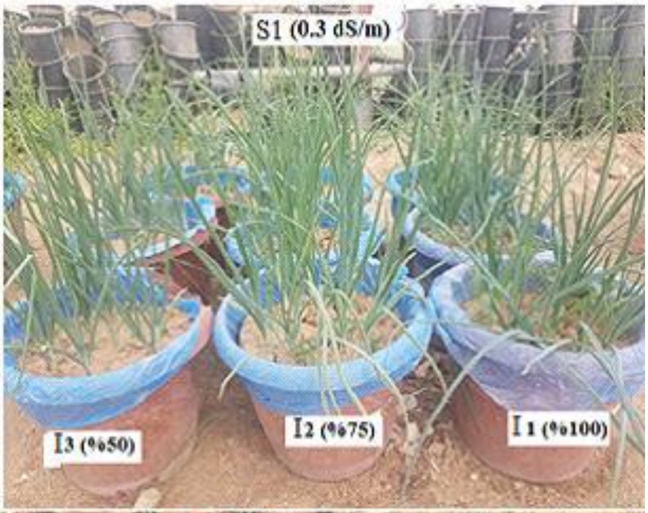
ΔS was calculated from the difference between the available soil moisture at the time of seed sowing and the available soil moisture at the time of harvest.

Water use efficiency (WP) was calculated using yield per plant (g) and ETa (L) per plant values.

Determination of agro-morphological, physiological and biochemical properties of m onion and evaluation of the results

At harvest, the height, diameter, fresh-dry weight and number of leaves of each plant were determined for each trial. Membrane damage (MD) was determined by the method of Lutts et al. (1996). Carotenoid, chlorophyll values (a, b and total) in onion leaves were determined according to the method

Treatments	
S1-I1	0.3 dS/m (Control) - full irrigation (I100)
S1-I2	0.3 dS/m - 25% water deficit
S1-I3	0.3 dS/m - 50% water deficit
S2-I1	2.5 dS/m - full irrigation (I100)
S2-I2	2.5 dS/m 25% water deficit
S2-I3	2.5 dS/m - 50% water deficit
S3-I1	5.0 dS/m - full irrigation (I100)
S3-I2	5.0 dS/m - 25% water deficit
S3-I3	5.0 dS/m - 50% water deficit
S4-I1	7.5 dS/m - full irrigation (I100)
S4-I2	7.5 dS/m - 25% water deficit
S4-I3	7.5 dS/m - 50% water deficit
S5-I1	10.0 dS/m - full irrigation (I100)
S5-I2	10.0 dS/m - 25% water deficit
S5-I3	10.0 dS/m - 50% water deficit



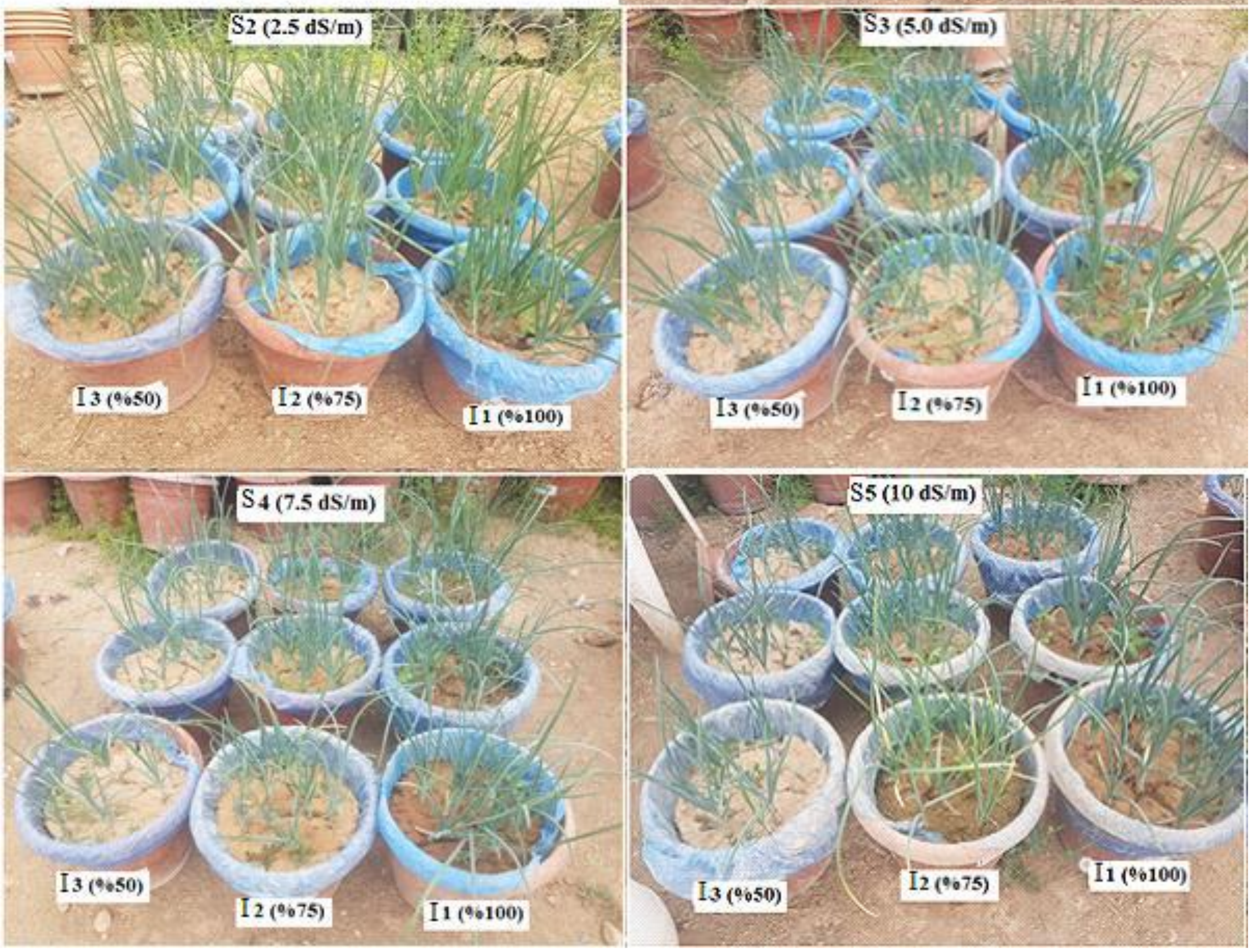


Figure 2. Plant appearances and treatments

reported by Lichtenthaler (1987). Proline contents were determined according to the methods reported by Bates et al. (1973), and protein contents according to the methods reported by Bradford (1976). Antioxidant enzymes were determined; SOD enzyme according to Beyer Jr and Fridovich (1987), POD enzyme according to Bergmeyer et al. (1983) and CAT enzyme according to the method suggested by Havir and McHale (1987). CAT, SOD and POD values were determined as enzyme units (EU/g/leaf) per unit weight (g) of a leaf (Öztürk et al., 2022). The results obtained from the experiment were subjected to ANOVA test in the SPSS-22 program, and statistically significant parameters were grouped based on the Duncan test (P<0.05).

Results

Irrigation water, actual evapotranspiration (ETa) and yield traits

Deficit irrigation started 9 days after seed sowing and ended 38 days later. During the experiment, 9.25, 7.66 and 6.07 L/pot (185, 153.2 and 121.4 mm) irrigation water was applied to I_1, I_2 and I_3 treatments, respectively. In this experiment, firstly plant water consumption in pots was calculated and then ETa values per plant were determined considering the number of plants. The highest ETa was found in S_1I_1 with 869 mL/plant and this was followed by S_2I_1 with 844 mL/plant. The lowest ETa was calculated in S_3I_3 treatment with 505 mL/plant, which was applied 5.0 dS/m salinity and 50% water stress (Table 1). In general, both water stress and salinity stress decreased ETa. The results regarding above-ground fresh and dry weights, under-ground fresh and dry weights, leaf number, stem diameter and plant height values are presented in Table 2. Except for the stem diameter, irrigation water level was found to be statistically non-significant while the effect of salinity was found to be significant. In general, the data obtained from the control group were in a different Duncan group compared to the data obtained from salinity levels. As the salinity level increased, decreases were observed in agro-morphological parameters. Similar results were obtained from S_2, S_3 and S_4 treatments in some yield components, however, 10 dS/m (S_5) salinity level had destructive effects on onion. Onion yield decreased by 17.3% in S_2, 30.3% in S_3, 37.5% in S_4 and 56.2% in S_5 compared to the control group (S_1) according to salinity levels. The highest value in onion yield according to water levels was obtained in S_1 treatment with an average of 14.1 g/plant, but the differences between the treatments did not show statistical significance. Salinity had a much more negative effect on onion yield than water stress, and it was revealed that significant yield losses would occur when onion was irrigated with saline irrigation water. When both stress conditions were evaluated together, the yield value obtained from the control (I_1S_1) group was approximately three times higher than the yield value obtained from 50% water stress and 10 dS/m salinity stress application (I_3S_5).

Table 1. The total amount of irrigation water applied to the treatments, changes in the soil moisture content at the time of seed sowing and harvest (*Δ*S), and actual evapotranspiration (ETa).

Treatments	Irrigation water amounts (mL)	ΔS (mL)	ETa per pot (mL/10plants)	ETa per plant (mL/plant)
S_1I_1	9250	565	8685	869
S_1I_2	7663	471	7192	719
S_1I_3	6075	758	5317	532
S_2I_1	9250	815	8435	844
S_2I_2	7663	883	6780	678
S_2I_3	6075	942	5133	513
S_3I_1	9250	916	8334	833
S_3I_2	7663	925	6738	674
S_3I_3	6075	1026	5049	505
S_4I_1	9250	955	8295	830
S_4I_2	7663	960	6703	670
S_4I_3	6075	965	5110	511
S_5I_1	9250	978	8272	827
S_5I_2	7663	965	6698	670
S_5I_3	6075	954	5121	512

Table 2. Changes in yield and yield components of onion under salinity and water stress conditions

Treatments		Aboveground Fresh weight (g/plant)	Aboveground Dry weight (g/plant)	Underground Fresh weight (g/plant)	Underground Dry weight (g/plant)	Leaf number (No/plant)	Plant stem diameter (mm)	Plant height (cm)
Irrigation level (I)								
I_1		14.1	1.29	1.22	0.14	6.13	6.64^{a}	38.8
I_2		13.2	1.31	1.14	0.12	5.95	6.67^{a}	38.4
I_3		13.1	1.27	1.08	0.13	5.71	6.14^{b}	37.3
Salinity (S)								
S_1		18.8^{a}	1.80^{a}	1.88^{a}	0.18^{a}	6.45^{a}	7.75^{a}	43.1^{a}
S_2		15.5^{b}	1.46^{b}	1.12^{b}	0.13^{b}	6.19^{ab}	7.06^{b}	39.4^{b}
S_3		13.1^{c}	1.22^{c}	1.01^{b}	0.12^{b}	6.07^{ab}	6.29^{c}	38.5^{b}
S_4		11.7^{c}	1.16^{c}	1.06^{b}	0.12^{b}	5.63^{bc}	6.18^{c}	38.0^{b}
S_5		8.2^{d}	0.80^{d}	0.68^{c}	0.09^{c}	5.33^{c}	5.28^{d}	32.0^{c}
I x S (Interactions)								
I_1	S_1	19.3^{a}	1.80^{a}	2.18^{a}	0.21^{a}	7.22^{a}	8.18^{ab}	41.2^{abc}
	S_2	12.5^{bc}	1.08^{de}	0.74^{ef}	0.11^{cde}	6.22^{abc}	6.59^{de}	39.4^{bcd}
	S_3	18.0^{a}	1.68^{ab}	1.58^{bc}	0.19^{ab}	5.89^{bc}	7.75^{abc}	41.3^{abc}
	S_4	9.9^{cde}	0.93^{ef}	0.60^{f}	0.08^{e}	5.45^{bc}	5.11^{g}	36.6^{c-f}
	S_5	10.8^{bcd}	0.97^{ef}	1.03^{def}	0.11^{cde}	5.89^{bc}	5.55^{efg}	35.5^{def}
I_2	S_1	19.1^{a}	1.83^{a}	2.12^{a}	0.19^{ab}	6.11^{bc}	8.31^{a}	45.1^{a}
	S_2	13.8^{b}	1.44^{bc}	0.72^{ef}	0.09^{de}	5.78^{bc}	6.68^{cde}	35.2^{def}
	S_3	12.6^{bc}	1.25^{cde}	0.95^{def}	0.11^{cde}	6.67^{ab}	6.45^{de}	39.8^{bcd}
	S_4	13.0^{bc}	1.31^{cd}	1.40^{cd}	0.14^{cd}	5.55^{bc}	7.16^{bcd}	39.8^{bcd}
	S_5	7.5^{ef}	0.73^{f}	0.50^{f}	0.08^{e}	5.67^{bc}	5.21^{fg}	32.3^{fg}
I_3	S_1	17.9^{a}	1.77^{a}	1.35^{cd}	0.15^{c}	6.00^{bc}	6.76^{cd}	42.9^{ab}
	S_2	20.3^{a}	1.85^{a}	1.90^{ab}	0.20^{a}	6.56^{abc}	7.92^{ab}	43.6^{ab}
	S_3	8.6^{def}	0.74^{f}	0.48^{f}	0.07^{e}	5.67^{bc}	4.68^{g}	34.3^{ef}
	S_4	12.4^{bc}	1.25^{cde}	1.18^{cde}	0.14^{cd}	5.89^{bc}	6.28^{def}	37.6^{cde}
	S_5	6.4^{f}	0.71^{f}	0.50^{f}	0.08^{e}	4.44^{d}	5.06^{g}	28.2^{g}
Significance								
Irrigation level (I)		ns	ns	ns	ns	ns	*	ns
Salinity (S)		**	**	**	**	**	**	**
I x S		**	**	**	**	*	**	**

ns: Not significant; *, P<0.05; **, P<0.01. Lowercase letters, uppercase letters, and italics indicate Duncan groups for Salinity (S), irrigation level (I), and *I x S* interaction, respectively.

Effect of salinity and drought stress on chlorophyll, carotenoid, membrane damage, H_2O_2

The effect of irrigation salinity levels (S), water stress (I) and IxS on chlorophyll values was statistically significant (Table 3). Chlorophyll values increased linearly with increasing water stress. The highest values were observed in I_3 treatments with severe water stress. In general, chlorophyll values increased up to salinity level S_4 (7.5 dS/m) but decreased at salinity level S_5 (10.0 dS/m). For this reason, it is seen that 7.5 dS/m water salinity is the threshold value for chlorophyll content in onion and that salinity stress higher than this level has negative effects on onion. A decrease of approximately 57% was observed between the applications with the highest chlorophyll-a value (I_3S_3) and with the lowest value (I_2S_5). Similarly, there was a decrease of more than 50% in chlorophyll-b and total chlorophyll values.

Table 3. Changes in chlorophyll, carotenoid, membrane damage and H2O2 of onion under salinity and water stress conditions

Treatments		Chl. a (mg/g)	Chl. b (mg/g)	Total chl. (mg/g)	Carotenoids content(mg/g)	Membrane damage (%)	H_2O_2 (µmol/g TA)
Irrigation level (I)							
I_1		17.5^{B}	5.64^{C}	26.8^{B}	4.51^{B}	54.9^{B}	573
I_2		16.9^{B}	6.20^{B}	26.9^{B}	4.36^{B}	54.1^{B}	526
I_3		19.8^{A}	6.79^{A}	31.2^{A}	4.94^{A}	58.4^{A}	503
Salinity (S)							
S_1		18.3^{b}	6.22^{a}	28.8^{b}	4.59^{b}	51.0^{c}	472
S_2		18.7^{ab}	6.65^{a}	29.9^{a}	4.67^{ab}	62.3^{a}	549
S_3		19.7^{a}	6.51^{a}	30.6^{a}	5.11^{a}	55.4^{bc}	494
S_4		19.5^{ab}	6.54^{a}	30.4^{a}	4.96^{ab}	55.9^{b}	544
S_5		14.0^{c}	5.12^{b}	21.8^{c}	3.69^{c}	54.3^{bc}	611
I x S (Interactions)							
I_1	S_1	21.5^{ab}	$6.84^{b\text{-}e}$	33.0^{bc}	5.61^{ab}	43.1^{f}	493
	S_2	20.6^{abc}	6.58^{cde}	31.8^{c}	5.31^{abc}	67.6^{ab}	664
	S_3	18.7^{cde}	5.87^{def}	28.6^{d}	4.53^{cd}	48.3^{ef}	548
	S_4	16.6^{ef}	5.07^{fg}	25.0^{e}	4.51^{cd}	62.3^{bc}	510
	S_5	10.2^{g}	3.83^{h}	15.7^{f}	2.58^{e}	52.9^{de}	649
I_2	S_1	16.5^{ef}	5.03^{fg}	24.9^{e}	4.17^{d}	47.8^{ef}	408
	S_2	19.8^{bcd}	8.08^{a}	33.5^{bc}	4.80^{bcd}	60.1^{bcd}	571
	S_3	17.6^{def}	5.77^{ef}	27.1^{d}	4.72^{bcd}	46.4^{ef}	462
	S_4	20.9^{abc}	7.59^{abc}	33.5^{bc}	5.22^{abc}	63.1^{bc}	547
	S_5	9.9^{g}	4.55^{gh}	15.8^{f}	2.91^{e}	53.0^{de}	642
I_3	S_1	17.1^{ef}	$6.80^{b\text{-}e}$	28.5^{d}	3.99^{d}	62.1^{bc}	516
	S_2	15.8^{f}	5.30^{fg}	24.6^{e}	3.90^{d}	59.2^{bcd}	412
	S_3	22.9^{a}	7.90^{ab}	36.3^{a}	6.08^{a}	71.4^{a}	472
	S_4	21.1^{abc}	6.98^{bcd}	32.8^{bc}	5.16^{abc}	42.3^{f}	574
	S_5	21.9^{ab}	6.97^{bcd}	33.9^{b}	5.58^{ab}	56.9^{cd}	543
Significance							
Irrigation level (I)		**	**	**	**	*	ns
Salinity (S)		**	**	**	**	**	ns
I x S		**	**	**	**	**	ns

ns: Not significant; *, P<0.05; **, P<0.01. Lowercase letters, uppercase letters, and italics indicate Duncan groups for Salinity (S), irrigation level (I), and *I x S* interaction, respectively.

It was observed that the content of carotenoid pigments, which play an active role in photosynthesis, increased in onion leaves compared to control group plants at all salinity levels except S_5 (Table 3). On the other hand, among the different water stress levels applied, the highest carotenoid content was in 50% stressed I_3 treatments. In other words, 50% water stress increased the carotenoid content in onion leaves. When both stress conditions were evaluated together, the highest carotenoid content was obtained in S_3I_3 treatment.

Both abiotic stress treatments and the interaction between them showed statistically significant effects on membrane damage (Table 3). As expected, the lowest membrane damage occurred in the control group (S_1) plants. When the effect of irrigation levels is examined, it is seen that the highest membrane damage (58.4%) occurred in the severe water stress treatment. In the evaluation made in terms of irrigation water salinity according to hydrogen peroxide (H_2O_2) concentrations, the highest H_2O_2 concentration was found in S_5, which is the highest salt stress treatment. Compared to the control group plants, H_2O_2 concentration increased between 4.6% and 29.4% in irrigation water salinity treatments, but these increases did not create a statistical difference.

Table 4. Changes in protein, proline, CAT, POD and SOD of onion under salinity and water stress conditions

Treatments		Protein content(µg/g)	Proline content(µg/g)	CAT (EU/g leaf)	POD (EU/g leaf)	SOD (EU/g leaf)
Irrigation level (I)						
I_1		45.7^{B}	34.9	1396^{B}	1044^{A}	2016^{A}
I_2		47.1^{B}	33.6	1824^{A}	651^{C}	1717^{C}
I_3		54.2^{A}	33.8	1414^{B}	794^{B}	1852^{B}
Salinity (S)						
S_1		46.6^{cd}	54.1^{a}	904^{c}	785^{b}	1876^{bc}
S_2		55.1^{a}	32.8^{b}	1386^{b}	588^{c}	1987^{ab}
S_3		49.1^{bc}	28.8^{b}	1611^{b}	773^{b}	1796^{c}
S_4		50.7^{b}	27.8^{b}	1413^{b}	776^{b}	1618^{d}
S_5		43.7^{d}	26.9^{b}	2408^{a}	1226^{a}	2031^{a}
I x S (Interactions)						
I_1	S_1	40.8^{f}	57.2	486^{ef}	885^{cde}	2503^{a}
	S_2	66.8^{b}	42.9	2040^{bc}	1008^{cd}	1661^{efg}
	S_3	43.3^{f}	31.1	1613^{bc}	1360^{b}	2420^{ab}
	S_4	26.6^{g}	24.1	1453^{bc}	501^{ghi}	1557^{g}
	S_5	51.2^{de}	19.0	1386^{bc}	1466^{b}	1938^{de}
I_2	S_1	48.8^{e}	46.1	1040^{de}	810^{def}	1596^{fg}
	S_2	37.9^{f}	31.6	1840^{bcd}	453^{ghi}	2162^{bcd}
	S_3	49.9^{e}	36.5	1553^{cd}	560^{fgh}	1441^{g}
	S_4	42.5^{f}	37.1	1340^{cd}	1104^{c}	1464^{g}
	S_5	56.7^{cd}	16.8	3346^{a}	330^{h}	1923^{de}
I_3	S_1	50.2^{e}	59.1	1186^{de}	661^{e-h}	1529^{g}
	S_2	60.6^{c}	23.8	280^{f}	305^{h}	2136^{cd}
	S_3	54.2^{de}	18.7	1666^{cd}	400^{hi}	1526^{g}
	S_4	83.1^{a}	22.3	1446^{cd}	725^{efg}	1835^{ef}
	S_5	23.2^{g}	45.0	2493^{b}	1882^{a}	2233^{bc}
Significance						
Irrigation level (I)		**	ns	*	**	**
Salinity (S)		**	*	**	**	**
I x S		**	ns	**	**	**

ns: Not significant; *, P<0.05; **, P<0.01. Lowercase letters, uppercase letters, and italics indicate Duncan groups for Salinity (S), Irrigation level (I), and *I x S* interaction, respectively.

Results regarding protein, proline and antioxidant enzyme activities

According to salinity levels, the highest protein content was determined in S_2 and the lowest in S_5 (Table 4). The physiological drought that the plant was exposed to due to the increasing salinity intensity had a destructive effect on the protein structure. When considered in terms of different irrigation levels, the protein content reached the highest value in I_3 subjects applied 50% severe drought stress. When the effects of salinity and deficient irrigation were evaluated together, the highest protein content was determined in I_3S_4 treatment (83.1 µg/g). It is thought that proteins increased as a function of the defense mechanism provided to the plant by stress physiology as a result of the increase in the interactive effect of stress. In the experiment, salinity stress had a significant effect on proline contents (Table 4). The highest proline content (54.1 µg/g) was found in the control group and up to 50% decreases in proline content were observed with the increase in salinity.

Antioxidant enzymes play an important role under stress conditions and protect plants from the negative effects of ROS. Increased salinity stress caused significant increases in CAT enzyme (Table 4). Compared to the control group plants (904 EU/g), CAT activity increased approximately 2.5 times in S_5 treatment (2408 EU/g). Among water levels, the highest CAT activity was determined in subjects with I_2

irrigation level and was statistically differentiated from the others. When the results related to POD activity were examined, the highest POD activity was found in S_5, which was the highest salt stress application, in terms of irrigation water salinity. When the effects of both stress factors were evaluated together, it was seen that the highest POD activity occurred in I_3S_5 (1882 EU/g), which was exposed to the most severe salt and water stress. When the activity of SOD enzyme was examined, it could be said that it increased with salinity stress, similar to POD, but the increase rate was not as significant as in CAT.

Water-Yield Relationships in Onion

According to salinity levels, the highest WP was calculated in S_1 application with an average of 30.2 g/L, and this application was in a different Duncan group than the others (Table 5). WP decreased by 11.1% in S_2, 31.8% in S_3, 33.0% in S_4 and 56.1% in S_5 compared to S_1. Salinity stress had a devastating effect by reducing water productivity by more than 50%, especially in S_5 application applied with 10 dS/m. The highest WP value calculated among water level applications belonged to 50% water stress application (I_3) with an average of 27.5 g/L. Significant increases in WP occurred as the irrigation water deficit rate increased. The relationships between different irrigation water salinity levels and onion yield are given in Figure 3. By evaluating the effect of salinity levels on yield, linear relationships were found between irrigation water salinity and yield with 99% confidence (R^2=0.97) as formulated. Relationships between irrigation water amount and yield are given in Figure 4. As can be seen from the figure, strong polynomial relationships were found between irrigation water amount and yield. Onion yield was negatively affected by salinity stress much more than water stress.

Table 5. Average values (g/L) and importance groups for water use efficiencies (WUE)

Salinity (S)	Irrigation level (I)			Average**	Relative WUE (%)	Difference (%)
	I_1 (%100)	I_2 (%75)	I_3 (%50)			
S_1 (Control)	24.7^{de}	29.6^{c}	36.2^{b}	30.2^{a}	100.0	0.0
S_2 (2.5 dS/m)	15.6^{gh}	21.4^{ef}	43.3^{a}	26.8^{b}	88.9	-11.1
S_3 (5.0 dS/m)	23.5^{de}	20.2^{ef}	18.0^{fg}	20.6^{c}	68.2	-31.8
S_4 (7.5 dS/m)	12.6^{h}	21.4^{ef}	26.5^{cd}	20.2^{c}	67.0	-33.0
S_5 (10.0 dS/m)	14.3^{gh}	11.9^{h}	13.5^{gh}	13.2^{d}	43.9	-56.1
Average **	18.2^{C}	20.9^{B}	27.5^{A}			
Relative WUE (%)	100.0	115.0	151.4			
Difference (%)	0.0	15.0	51.4			

ns: Not significant; *, P<0.05; **, P<0.01. Lowercase letters, uppercase letters, and italics indicate Duncan groups for Salinity (S), irrigation level (I), and *I x S* interaction, respectively. Significant: IxS: **

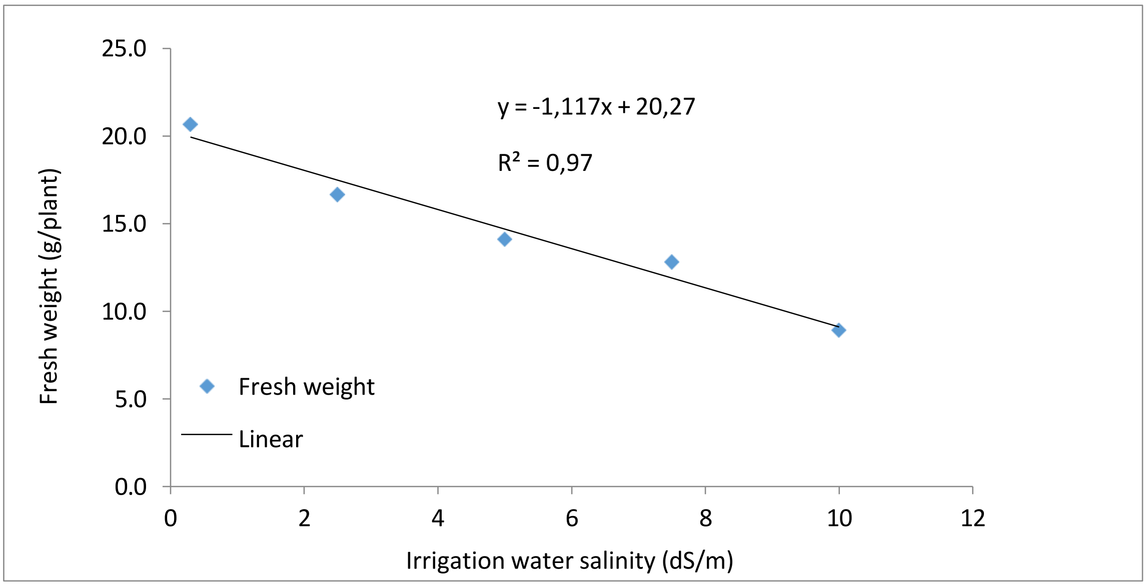


Figure 3. Relationship between irrigation water salinity and yield

Discussion

Drought and salinity, which are the main abiotic stress factors, cause significant losses in plant production (Yavuz et al., 2023). It has been reported that drought stress affects not only yield but also seed quality (El Balla et al., 2013), but salinity stress is a determinant in yield and yield parameters (Al-Harbi et al., 2002; Bekheet et al., 2006; Krasensky and Jonak, 2012). Similar to our findings, some researchers have stated that water stress causes decreases in yield in vegetable species. It has been reported that drought stress causes decreases in aboveground fresh and dry weight in lettuce (Oh et al., 2009), tomato (Çebi et al., 2018), Chinese cabbage (Shawon et al., 2020) and spinach (Yavuz et al., 2022). In the evaluation of water-yield relationships, it is expected that plants have high WP values, that is, the highest yield in return for the water consumed by the plant. Researchers evaluate plants under different stress conditions to determine these threshold values (Munoz-Perea et al., 2007; Blum, 2009; Liu et al., 2016; Yavuz et al., 2023; Seymen et al., 2024). Similarly, in our study, water productivity was determined, and the highest WP (43.3 g/L) was obtained from plants without salt stress under deficit irrigation conditions.

Some researchers think that fluctuations in chlorophyll contents occur as a result of inhibition of chlorophyll biosynthesis and enzymes, and pigments resulting from increased chloroplast membrane permeability, which are broken down to a degree that disrupts metabolic functions (Foyer and Shigeoka, 2011). Basahi et al. (2014) reported that drought stress increased chlorophyll a content in lettuce, but had no effect on chlorophyll b content. In our study, chlorophyll values increased with drought stress but were not statistically different from each other at low salinity stress levels. Carotenoids are pigments that protect chlorophyll from excessive light intensity in leaves, have antioxidant properties, and are auxiliary to photosynthesis (Smirnoff, 2005; Keyvan, 2010). In parallel with our findings, abiotic stress conditions and severity increased the carotenoid content of spinach (Yavuz et al., 2022) and lettuce (Basahi et al., 2014).

The combined effect of stress factors increased membrane damage, but this increase did not occur regularly and significantly. In a research on salinity stress in blackberry, it was reported that salinity increased membrane damage (Arıkan et al., 2018). Similarly, in a study on spinach, it was reported that increasing the salt content of irrigation water increased membrane damage (Yavuz et al., 2022). In this study, among water stress levels, the highest membrane damage was found in the treatment applied with 50% water deficit. It was reported that membrane damage increased significantly in treatments applied with severe water stress (80% water deficit) in spinach (Seymen, 2021). Similarly, it was reported that water stress increased membrane damage in lettuce during the last harvest period (Yavuz et al., 2021).

H_2O_2, an important ROS species, is a compound that causes oxidative stress (Cruz et al., 2013). There was an increase in H_2O_2 content in onion leaves in salinity applications, but this increase was not found to be statistically significant. In the study, it is thought that the reason why H_2O_2 concentration did not increase under water stress conditions is due to the increase in enzyme activities that clean ROS triggered by oxidative stress. These enzymes play important roles in determining the level of effectiveness of each stage of plant responses to stress (Chen et al., 2017). Plants have a magnificent antioxidant defense system consisting of enzymatic and non-enzymatic components that keep ROS under control in order to

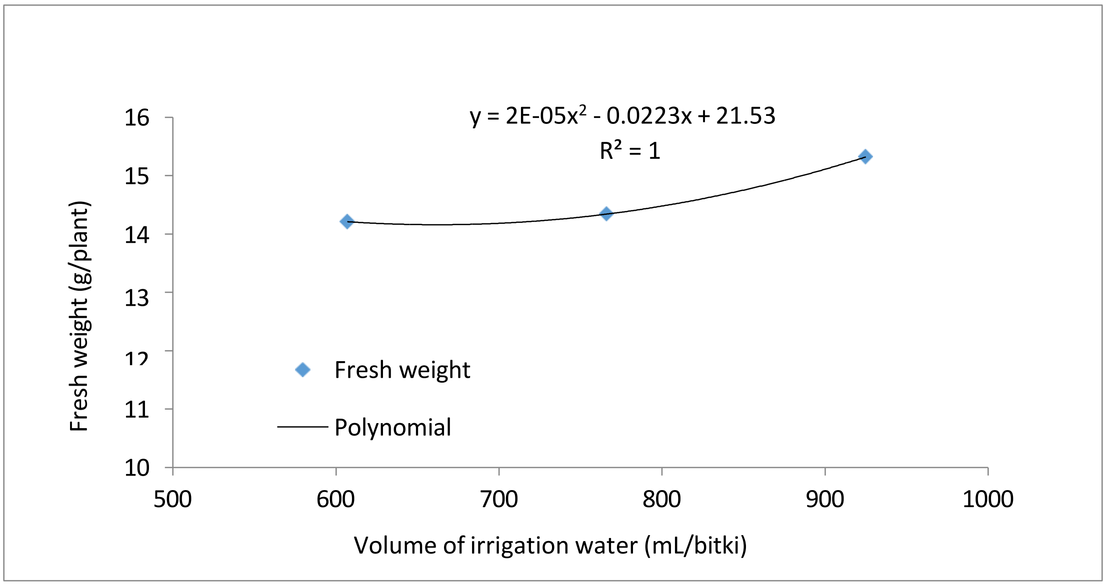


Figure 4. Correlation between irrigation water amount and yield

avoid stress or to continue with minimal damage under oxidative stress (Reddy et al., 2004). The most important ROS-cleaning mechanisms of plants are superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). The functioning and timing of these enzymes in plant metabolism are vital in maintaining the balance of superoxide radical and H_2O_2, which cause damage in plant cells under stress conditions. SOD is responsible for the defense system by catalyzing the conversion of superoxide to H_2O_2 and O_2, POD leads in stress signaling, and CAT is highly effective in scavenging ROS that increase during stress (Mittler, 2002; Mishra and Sharma, 2019; Zulfiqar and Ashraf, 2021). The results obtained from the present study, in line with the literature, show that CAT, POD and SOD fulfill their duties under stress conditions applied to onion.

Conclusions

Onion showed more negative responses to salinity stress than water stress, and it was observed that saline irrigation water would cause significant yield losses in onion. In general, both increasing water salinity and increasing water stress decreased the water consumption of the onion. When the joint effect of irrigation water salinity and irrigation levels (T x S) was evaluated, there were significant yield reductions (about 66.8%) at full and restricted irrigation levels in subjects treated with 10 dS m^{-1} irrigation water salinity. Although chlorophyll values (chlorophyll-a, chlorophyll-b, and total chlorophyll) increased with salinity stress (at T1, T2, T3, and T4 levels), no statistically significant difference was found. Enzymatic (CAT, SOD, POD) and non-enzymatic (protein, proline) responses of onion under stress conditions were evaluated as statistically significant. The metabolism of onion, which reacts to different salt levels, increased enzyme activities significantly to avoid stress. Here, CAT enzyme responded with the highest increase to the signals created by the applied stress conditions. Based on membrane damage values, both stressors were found to be statistically significant. Although the effect of salinity levels fluctuated, the highest membrane damage values were observed at 50% water stress, as expected. Abiotic stress factors such as salinity and drought are important factors affecting plant production in arid and semi-arid areas. Determining the responses of plants to irrigation water salinity under deficit irrigation conditions in such areas is very important for sustainable agricultural production. When the data obtained from the study are evaluated together, it shows that the use of water with an electrical conductivity above 2.5 dS/m in irrigation will cause significant yield decreases in onion. It is obvious that irrigation waters containing very high salt content, especially 7.5 and 10 dS/m, will cause serious yield reductions in onion. On the other hand, it is clearly understood from the results of this study that farmers who have clean water resources and do not have sufficient water can save irrigation water by applying 25% water stress in green onion cultivation in greenhouse.

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Author Contribution

E.A.: Data curation, Investigation (Master's student). **D.Y.:** Methodology, Investigation, Formal analysis, Data curation, Validation (Thesis advisor). **S.K.:** Writing- Original draft, Data curation, Investigation. **N.Y.:** Writing- Original draft, Investigation. **M.S.C.:** Writing- Original draft.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

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